

## Stress Response Differences and Disease Susceptibility Reflected by Heterophil to Lymphocyte Ratio in Turkeys Selected for Increased Body Weight<sup>1</sup>

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**ABSTRACT** Three genetic lines of turkeys were compared for their responses to *Escherichia coli* challenge following dexamethasone injection (Dex) or *E. coli* challenge preceding transport stress (TS). The turkey lines were a slow growing line selected for increased egg production (Egg line), a fast growing line selected for increased 16-wk BW (F line), and a commercial line (Comm line). At 14 wk of age, the Dex group was treated with 3 injections of 2 mg of Dex/kg of BW followed by airsac challenge with 100 cfu of *E. coli*. The TS group was given the same *E. coli* challenge at  $1 \times 10^4$  cfu/bird without Dex treatment, and was subjected to transport stress, including 12 h of holding time in a transport vehicle, 8 d after the challenge. All treated birds and untreated control birds were bled at the same time, which was 1 d after transport and 9 d after challenge with *E. coli*. The main effect mean (MEM) total leukocyte counts (WBC) and the percentages of eosinophils (Eos) and basophils (Baso) were the same for all 3 lines; however, the MEM percentages of heterophils (Het) and monocytes (Mono) and the heterophil/lymphocyte ratio (H/L) were lower and the percentage of lym-

phocytes (Lym) was higher in the Egg line compared with the 2 fast-growing lines. Both stress treatments increased WBC, Het, and H/L and decreased Lym in all 3 lines; however, these effects were significantly greater in both fast growing lines compared with the Egg line. Sixteen-week BW was unaffected by either treatment in the Egg line and was decreased by both treatments in the Comm line and by the Dex treatment in the F line. Main effect mean airsacculitis score (AS) was not affected by line and was significantly increased by TS and Dex treatments. Neither treatment affected AS of the Egg line birds, whereas Dex treatment increased AS of the F line, and both Dex and TS increased AS of the Comm line. Mortality was significantly higher in the Comm line compared with the Egg line and was intermediate in the F line. The differences between these lines in their disease resistance and physiological response to stress in 2 stress models suggests that increasing selection for BW of turkeys is accompanied by changes in the stress response resulting in increased susceptibility to opportunistic bacterial infection.

(Key words: turkey, transport stress, dexamethasone, genetics, *Escherichia coli*)

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### INTRODUCTION

Over the past 50 yr, the commercial poultry industry has made monumental gains in production through a combination of improved breeding, nutrition, and advances in disease control. The genetic selection of poultry for superior growth rate has arguably been the primary method for increasing productivity; however, many studies have shown that such selection may be coincidentally

accompanied by decreased resistance to disease or changes in immunological response (Han and Smyth, 1972; Saif et al., 1984; Saif and Nestor, 2002; Sacco et al., 1991, 1994a, 2000; Tsai et al., 1992; Miller et al., 1992; Qureshi and Havenstein, 1994; Nestor et al., 1996a,b,c, 1999a,b; Li et al., 1999, 2000a,b,c, 2001; Cheema et al., 2003).

The reciprocal situation, selection for improved immunological response, has also been shown to result in de-

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**Abbreviation Key:** AS = airsacculitis score; Baso = basophils; Comm = commercial line; Dex = dexamethasone injection; Egg line = slow-growing line selected for increased egg production; Eos = eosinophils; F line = a fast-growing line selected for increased 16-wk BW; Het = heterophils; H/L = heterophil/lymphocyte ratio; Lym = lymphocytes; MEM = main effect mean; Mono = monocytes; OARDC = Ohio Agricultural Research and Development Center; TS = transport stress group; WBC = total leukocytes.

creased BW (Siegel and Gross, 1980; Siegel et al., 1982; van der Zijpp, 1983; Okada et al., 1988; Martin et al., 1990; Afraz et al., 1994; Parmentier et al., 1996; Gross et al., 2002). It has been suggested that genetic variation in the responses to varied environmental and social stressors greatly complicates the development of poultry lines with high levels of immunocompetence (Gross and Siegel, 1988; Siegel, 1995).

Research comparing the immune responses of slow growing broilers from a 1957 randombred strain with faster growing commercial strains from 1991 (Qureshi and Havenstein, 1994; Cheema et al., 2003) suggests that genetic selection for improved broiler performance resulted in decreased antibody response and increased cell-mediated and inflammatory responses. The 2001 commercial strain also had more leg-problem associated mortality at 84 d (Havenstein et al., 2003).

In Israel, broiler chickens selected for fast growth were shown to have increased mortality relative to slower growing lines under commercial conditions (Yunis et al., 2000). However, a line that was specifically selected at a young age for high antibody responses to *Escherichia coli* has been shown to have higher levels of disease resistance without compromising BW (Leitner et al., 1992; Yunis et al., 2002a,b).

In turkeys, the association between fast growth and decreased disease resistance has come primarily through the study of 4 closed genetic turkey lines developed at the Ohio Agricultural Research and Development Center (OARDC) at The Ohio State University. These lines include a randombred control line (RBC1) and its subline (Egg line) selected exclusively for increased egg production over a 250-d period and another randombred control (RBC2) and its subline selected for increased 16-wk BW (F line). Further information regarding the history of these lines is available in the following references: McCartney (1964), McCartney et al., (1968), Nestor, (1977, 1984), and Nestor et al., (2000).

The F line was observed to have higher mortality due to natural outbreaks of erysipelas and fowl cholera than its parent line, RBC2, and the Egg line had higher mortality than its parent line, RBC1, in an outbreak of fowl cholera (Saif et al., 1984). The Egg line had lower antibody responses to both Newcastle disease virus and *Pasteurella multocida* than did its parent line, RBC1 (Sharaf et al., 1988a), and had higher mortality in 1 of 3 challenges with the same organism (Sharaf et al., 1988b). All 4 lines were studied in separate *P. multocida* (Sacco et al., 1991) and Newcastle disease virus challenges (Tsai et al., 1992). The F line had earlier clinical signs and mortality than the other 3 lines in the *P. multocida* challenge and had the highest mortality in both challenges. The F line had higher antibody titers to Newcastle disease virus at 9 and 15 wk after vaccination than did RBC2, but had lower antibody titers to *P. multocida* at 15 wk (Sacco et al., 1994b). These studies implicate selection for increased BW, and to a lesser extent, selection for egg production, as being correlated with increases in nonprotective antibody and an increase in susceptibility to disease. Lymphocyte num-

bers and the hypersensitivity response to phytohemagglutinin-P were shown to be significantly decreased in the F line compared with RBC2 but there were no such differences in the Egg line relative to its randombred control (Bayyari et al., 1997). The compromise of disease resistance for populations under heavy selection for production traits is consistent with genetic homeostasis as described by Lerner (1954).

Recently, Kowalski et al. (2002) compared 2 lines of commercial European turkeys, a faster-growing heavy line and a slower growing medium line from the same breeder, for their physiological responses to the stressors of transport, crowding, and overheating. They reported that the faster growing line was more sensitive to adverse environmental factors, and had a much larger increase in corticosterone when exposed to transport stress than the slower-growing line.

They suggested that lighter and slower growing lines may be more suitable for certain commercial production situations due to their adaptability to stress. The purpose of the following study was to compare the effects of 2 different stress models, transport stress and dexamethasone treatment, on changes in stress response as measured by heterophil/lymphocyte ratio and resistance to *Escherichia coli* infection in 3 genetic lines of turkeys that differed in their rate of growth.

## MATERIALS AND METHODS

Three genetic lines of turkeys were compared for their responses to stress and *E. coli* challenge. The turkey lines were a slow growing line selected exclusively for increased egg production over a 250-d period (Egg), another line selected for increased 16-wk BW (F), and a commercial line (Comm). The birds from the Egg and F lines were the progeny of a hatch of eggs obtained from the OARDC. The hatch consisted of 66 Egg line birds and 42 F line birds of mixed sex. Fifty Comm poult of mixed sex were obtained from a commercial turkey hatchery at day of age and were transported to our facilities and set in pens on the same day as the closed lines. All turkeys were reared in floor pens on pine shavings, given ad libitum access to a standard corn and soybean turkey ration that met or exceeded the NRC recommended allowances (National Research Council, 1994), and were kept under incandescent lighting on a light schedule consisting of 23L:1D. For the first 2 wk, the birds were brooded under heat lamps in a single 3.9-m<sup>2</sup> pen for each line. At 2 wk of age they were separated into eighteen 3.9-m<sup>2</sup> pens in a 3 line × 3 treatment design with 2 replicate pens for each line × treatment group. Duplicate pens each held the following numbers of birds adjusted by treatment for predicted mortality: control Egg line = 6, 5; control F line = 5, 5; control Comm line = 6, 6; transport Egg line = 8, 8; transport F line = 8, 6; transport Comm line = 10, 9; Dex Egg line = 8, 6; Dex F line = 8, 6; and Dex Comm line = 10, 9.

## Dexamethasone and *E. coli* Challenge

At 13 wk of age, 1 group was immunosuppressed with 3 injections of the synthetic glucocorticoid, dexamethasone (Dex)<sup>3</sup> into a thigh muscle at a dosage of approximately 2 mg/kg of BW as previously described (Huff et al., 1998). A 200 mg/mL stock solution of Dex was prepared in absolute ethanol. This solution was suspended in sterile normal saline and an average volume of 1.0 mL was inoculated into each bird, based on a mean BW of 2.73 kg for the Egg line, and 6.49 kg for the 2 large-bodied lines. Birds were weighed the day before the first Dex injection and the amount each bird received was determined using a running scale correlating BW and volume. On the day of the third Dex injection (14 wk of age), all Dex-treated birds were inoculated in the left cranial-thoracic air-sac with sterile tryptose phosphate broth containing approximately 100 to 200 cfu of a nonmotile strain of *E. coli* serotype O2, which had originally been isolated from chickens with colisepticemia. The inoculum was prepared by adding 2 loopfulls of an overnight culture on blood agar to 100 mL of tryptose phosphate broth and incubating for 2.5 h in a 37 C shaking water bath. The culture was held overnight at 4 C while a standard plate count was made. Ten-fold dilutions were then made in tryptose phosphate broth based on the standard plate count.

## Transport Stress

Birds from each line were similarly challenged at 13 wk with approximately 5,000 to 10,000 cfu of the same *E. coli* culture but without Dex treatment. These birds were subjected to the following transport stress (TS) procedure, which occurred 8 d after the bacterial challenge and included 12 h of holding time in the transport vehicle. Birds were loaded into an open-fenced trailer covered with a tarp. The Egg line birds were separated from the other 2 lines by a fence to protect them from the larger birds. The temperature ranged from 18 to 21 C and there was a slight drizzle. The birds were driven around the University farm facilities for 3 h with occasional stops. They were then driven to the University Pilot Processing Plant, where the transport vehicle was parked in a covered holding area. After 12 h from the time of loading, birds were returned to their original pens where they had access to feed and water.

## Bleeding

The following morning all birds were weighed and bled by venipuncture into EDTA-coated tubes. All treated birds and untreated control birds were bled at the same time, which was 1 d after transport and 9 d after challenge with *E. coli*. Total leukocyte counts (WBC) and the num-

bers and proportions (percentage) of heterophils (Het), lymphocytes (Lym), monocytes (Mono), eosinophils (Eos), and basophils (Baso) were determined using a Cell-Dyn 3500 blood analysis system<sup>4</sup> which uses electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Heterophil/lymphocyte ratios (H/L), an indicator of stress in birds (Gross and Siegel, 1983), were calculated by dividing the number of heterophils in 1 mL of peripheral blood by the number of lymphocytes.

## Necropsy

Mortalities were collected twice daily postchallenge and were weighed and examined for lesions of airsacculitis. The following key, modified from that described by Piercy and West (1976), was used to score lesions of airsacculitis/pericarditis observed in mortalities and at necropsy: 0 = no inflammation; 1 = opacity and thickening of the inoculated air sac; 2 = mild airsacculitis and mild pericarditis; 3 = moderate airsacculitis/pericarditis with spread to liver and/or abdominal cavity (perihepatitis/peritonitis); 4 = severe fibrinous airsacculitis and severe pericarditis; 5 = severe airsacculitis/pericarditis with spread to liver or abdominal cavity.

Four days after bleeding, all surviving birds were weighed, euthanized, and necropsied (sex was recorded at necropsy). The birds were 109 d of age.

## Statistics

Pen means were analyzed as a 3 × 3 factorial arrangement for line and treatment and as a 3 × 3 × 2 factorial arrangement for line, treatment, and sex using the GLM procedure of SAS software (SAS Institute, 1988). All percentage data were subjected to arcsine transformation for analysis. Means were separated using Duncan's multiple range test (SAS Institute, 1988). Differences between each treatment relative to untreated controls and between sex and lines within treatments were separated using the least square means procedure of SAS software. A *P* value of less than 0.05 was considered significant for all treatment effects and their interactions unless otherwise stated.

## RESULTS

Determination of sex at necropsy indicated a 1.1 ratio of males to females in the total population. There was a significant difference in BW between males and females (*P* < 0.0001) and an interaction between line, treatment, and sex on BW (*P* = 0.04). There were no significant differences by sex or interactions between sex and line or treatment in any other variable, so sex was not included in the following analyses.

The line main effect mean (MEM) for WBC and for the percentages of Eos and Baso was the same for all 3 lines, however the MEM for percentage of Het and Mono was lower and the MEM for percentage of Lym was higher in the Egg line compared with both fast-growing lines

<sup>3</sup>Sigma Chemical Company, St. Louis, MO.

<sup>4</sup>Abbott Diagnostics, Abbott Park, IL.

TABLE 1. Line<sup>1</sup> and treatment<sup>2</sup> main effect means (MEM) for total leukocyte counts (WBC), and percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils in peripheral blood of 15-wk-old turkeys

Variable	MEM Line				MEM Treatment			
	Egg	F	Comm	P-value	Control	TS	Dex	P-value
WBC ( $\times 10^3/\mu\text{L}$ )	48.0	48.7	45.2	0.61	28.9 <sup>b</sup>	54.7 <sup>a</sup>	56.4 <sup>a</sup>	<0.0001
Heterophils (%)	52.3 <sup>b</sup>	62.2 <sup>a</sup>	64.7 <sup>a</sup>	<0.0001	46.5	65.1 <sup>a</sup>	66.5 <sup>a</sup>	<0.0001
Lymphocytes (%)	41.7 <sup>a</sup>	27.4 <sup>b</sup>	24.9 <sup>b</sup>	<0.0001	43.0 <sup>a</sup>	27.7 <sup>b</sup>	24.2 <sup>b</sup>	<0.0001
Monocytes (%)	4.9 <sup>b</sup>	9.4 <sup>a</sup>	9.4 <sup>a</sup>	<0.0001	9.8 <sup>a</sup>	6.2 <sup>b</sup>	7.9 <sup>a</sup>	0.0005
Eosinophils (%)	0.02	0.02	0.01	0.37	0.04	0.03	0.04	0.49
Basophils (%)	1.0	0.89	0.97	0.86	0.62	0.97	1.32	0.07

<sup>1</sup>A slow growing line selected exclusively for increased egg production over a 250-d period (Egg), a line selected for increased 16-wk BW (F), and a commercial line (Comm).

<sup>2</sup>Transport stress (TS) consisted of injection of approximately 5,000 to 10,000 cfu of *Escherichia coli* into the airsac, 8 d before a 12-h transport and holding procedure. Dexamethasone (Dex) treatment consisted of 3 intramuscular injections of 2 mg/kg BW Dex on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

(Table 1). The treatment MEM for WBC and Het was increased and for Lym was decreased by both TS and Dex relative to untreated controls. The treatment MEM for Mono was increased by TS relative to untreated controls and Dex treatment. There were no significant interactions for any of these variables.

Comparison of the 2 fast-growing lines relative to the Egg line, within each treatment, indicates that untreated control birds of the Egg line had WBC counts similar to controls of the 2 fast-growing lines; however, the percentages of Het and Mono were significantly lower and the percentage of Lym was significantly higher than in the control treatment of either of the 2 large-bodied lines (Table 2). The percentage of Het in TS birds was higher in the Comm line relative to the Egg line. The percentage of Lym was lower and the percentage of Mono was higher in both large-bodied lines compared with the Egg line. There were no significant differences in percentage Eos or Baso of TS birds in any line. The Dex treatment increased WBC of the F line relative to both the Egg line and the Comm line. The Dex treatment increased percentage Het and Mono and decreased percentage Lym of both the F line and Comm line, relative to the Egg line. There were no significant differences in percentage Eos affected by Dex; however, there was an increase in percentage of Baso of the Comm line relative to the Egg line ( $P = 0.01$ ). There were no significant interactions for any of these variables.

Evaluation of treatment effects for each of the 3 lines indicated that TS increased total WBC counts in all 3 lines relative to untreated controls; however, the increase due to TS was greater in the Comm line ( $P < 0.0001$ ) than in the F line ( $P < 0.001$ ) and the Egg line ( $P = 0.004$ ) (Table 2). Transport stress increased percentage Het relative to controls in the Egg line ( $P < 0.0001$ ) and the Comm line ( $P < 0.0001$ ) but not in the F line ( $P = 0.08$ ). Transport stress decreased percentage Lym in both Egg ( $P = 0.0003$ ) and Comm ( $P < 0.0001$ ) relative to untreated controls, but had no effect on percentage Lym in the F line. Transport stress also decreased percentage Mono in the Egg line ( $P = 0.04$ ) and in the Comm line ( $P = 0.004$ ) relative to

untreated controls, but there were no effects of TS on percentage Mono of the F line. There were no significant interactions for any of these variables.

The Dex treatment increased WBC in all 3 lines relative to untreated controls with the Egg line having less increase ( $P = 0.01$ ) than both the F line and the Comm line ( $P < 0.0001$ ) (Table 2). The Dex treatment had a greater effect on increase of percentage Het relative to untreated controls in the Egg line and Comm line ( $P < 0.0001$ ) than in the F line ( $P = 0.007$ ). The decrease in percentage Lym due to Dex treatment was greater in the Egg line and the Comm line ( $P < 0.0001$ ) than in the F line ( $P = 0.01$ ). The Dex treatment had no effect on percentage Mono in any line. There were no significant interactions for any of these variables.

Heterophil/lymphocyte ratios were increased by the Dex treatment in all 3 lines relative to untreated controls; however, TS increased H/L only in the Comm line (Figure 1a). The MEM Het to Lym ratio (H/L) was significantly increased by both TS and Dex treatment, with Dex treatment higher than TS (Figure 1b). The MEM H/L ratio was lower in the Egg line compared with both the F line and the Comm line (Figure 1b). There were no significant interactions.

Sixteen-week BW was unaffected by either treatment in the Egg line and was decreased by Dex treatment in the F line and by both transport and Dex treatment in the Comm line (Figure 2a). Main effect mean BW was higher in both large-bodied lines compared with the Egg line and was significantly decreased by both TS and Dex treatment (Figure 2b). There was an interaction between line and treatment affecting BW ( $P = 0.01$ ).

Neither treatment affected airsacculitis scores (AS) of the Egg line birds, whereas Dex treatment increased AS of the F line and both Dex and TS increased AS of the Comm line (Figure 3a). Main effect mean AS was not affected by line and was significantly increased by both TS and Dex treatments (Figure 3b). There was no increase in percentage mortality postchallenge within the Egg line or the F line by either TS or Dex treatment relative to untreated controls; however, mortality was significantly

TABLE 2. Effect of 2 stress models on total leukocyte counts (WBC), and percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils of peripheral blood of 15-wk-old turkeys from 3 genetic lines<sup>1</sup>

Variable	Untreated control			<i>E. coli</i> challenge + transport stress			Dex + <i>E. coli</i> challenge		
	Egg line n = 9	F line n = 9	Comm line n = 12	Egg line n = 13	F line n = 10	Comm line n = 16	Egg line n = 9	F line n = 6	Comm line n = 9
WBC ( $\times 10^3/\mu\text{L}$ )	34.5 $\pm$ 3	29.2 $\pm$ 3	24.6 $\pm$ 3	54.2 $\pm$ 4	52.6 $\pm$ 3.6	56.3 $\pm$ 4	51.5 $\pm$ 4	71.6 $\pm$ 9*	52.8 $\pm$ 10
Heterophils (%)	34.3 $\pm$ 2	55.9 $\pm$ 3***	48.6 $\pm$ 4**	57.9 $\pm$ 2	63.5 $\pm$ 3.5	71.9 $\pm$ 2***	59.8 $\pm$ 3	69.6 $\pm$ 4*	73.4 $\pm$ 3**
Lymphocytes (%)	58.9 $\pm$ 3	33.0 $\pm$ 4†	38.7 $\pm$ 5***	37.7 $\pm$ 3	27.7 $\pm$ 3.8*	19.6 $\pm$ 2†	33.3 $\pm$ 3	18.5 $\pm$ 5**	15.9 $\pm$ 3***
Monocytes (%)	6.1 $\pm$ 0.1	10.4 $\pm$ 2*	12.1 $\pm$ 2***	3.5 $\pm$ 0.3	8.0 $\pm$ 2.0**	7.5 $\pm$ 1**	5.7 $\pm$ 1	10.4 $\pm$ 2*	9.2 $\pm$ 1*
Eosinophils (%)	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.04 $\pm$ 0.03	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02	0.03 $\pm$ 0.01
Basophils (%)	0.74 $\pm$ 0.1	0.65 $\pm$ 0.1	0.51 $\pm$ 0.05	1.07 $\pm$ 0.3	0.79 $\pm$ 0.25	1.01 $\pm$ 0.1	1.11 $\pm$ 0.4	1.42 $\pm$ 0.5	1.53 $\pm$ 0.4*

<sup>1</sup>A slow growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm).

<sup>2</sup>Transport stress consisted of injection of approximately 5,000 to 10,000 cfu of *Escherichia coli* into the airsac, 8 d before a 12-h transport and holding procedure.

<sup>3</sup>Dexamethasone (Dex) treatment consisted of 3 intramuscular injections of 2 mg/kg BW Dex on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; † $P \leq 0.0001$  (within each treatment and relative only to Egg line).

increased by Dex treatment of the Comm line (Figure 4a). Main effect mean percentage mortality was significantly increased by Dex treatment and was significantly higher in the Comm line as compared with the Egg line (Figure 4b). There were no significant interactions between line and treatment of AS or mortality.

## DISCUSSION

Genetic selection for increased BW and feed conversion has resulted in dramatic increases in the production po-

tential of commercial turkeys. These gains have not been without cost, as the increases seen in a number of metabolic diseases of turkeys have been attributed to rapid growth and heavy BW, including ruptured aorta, spontaneous turkey cardiomyopathy and sudden death, and tibial dyschondroplasia (Julian, 1998). Rapid growth in turkeys has also been correlated with a decreased ability to control body temperature, which could result in increased mortality and stress-related pathology (Mills et al., 1999). Rapidly growing commercial turkeys have been shown to have increased incidence of muscle fiber abnor-

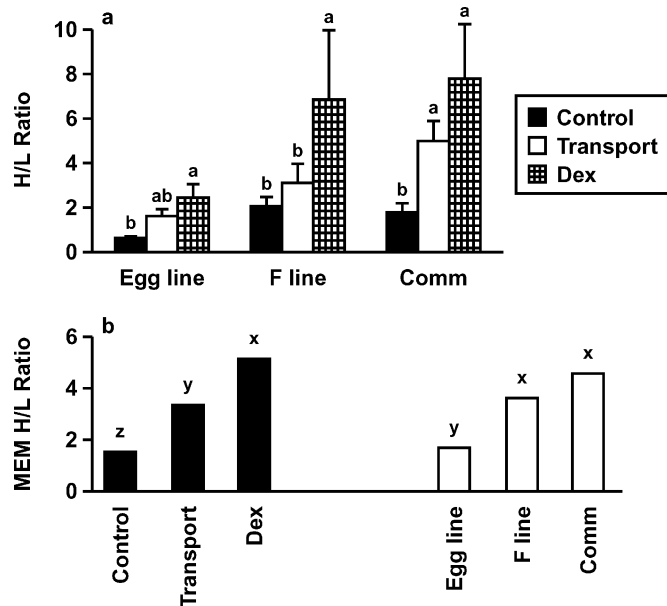


FIGURE 1. a) Effect of transport stress following *Escherichia coli* challenge (Transport) and dexamethasone (Dex) injection preceding *E. coli* challenge (Dex) on heterophil/lymphocyte (H/L) ratios in peripheral blood of 15-wk-old turkeys from 3 genetic lines: a slow growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm). b) Main effect means (MEM) of H/L ratio for treatment and line. Bars represent means  $\pm$  SEM and bars within the same line or treatment group with differing superscripts are significantly different ( $P \leq 0.05$ ).

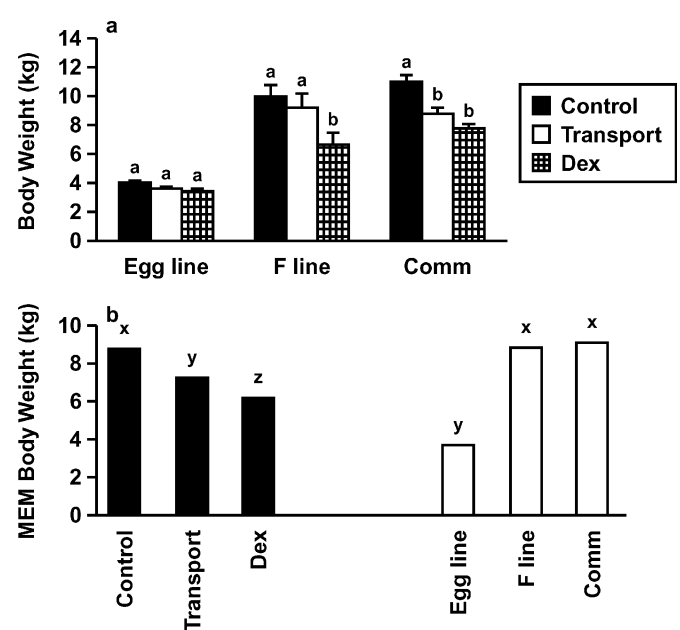


FIGURE 2. a) Effect of transport stress following *Escherichia coli* challenge (Transport) and dexamethasone (Dex) injection preceding *E. coli* challenge (Dex) on BW of 15-wk-old turkeys from 3 genetic lines: a slow growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm). b) Main effect Mean BW for treatments and lines. Bars represent means  $\pm$  SEM and bars within the same line or treatment group with differing superscripts are significantly different ( $P \leq 0.05$ ).

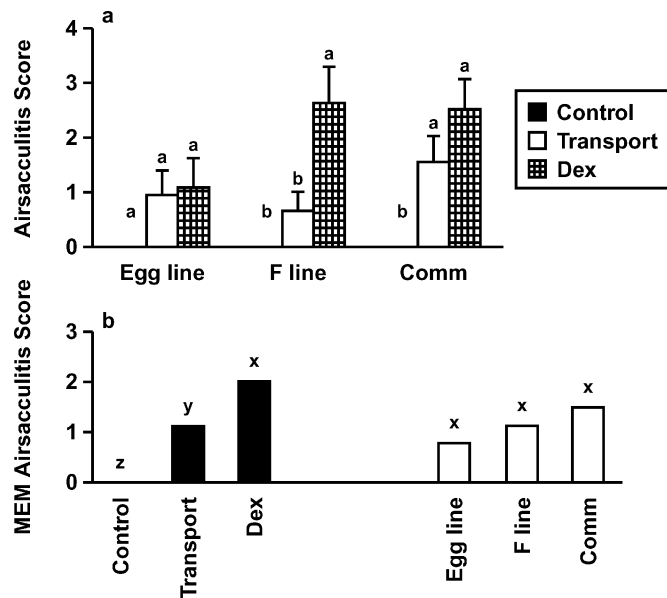


FIGURE 3. a) Effect of transport stress following *Escherichia coli* challenge (Transport) and dexamethasone (Dex) injection preceding *E. coli* challenge (Dex) on airsacculitis (AS) scores of 15-wk-old turkeys from 3 genetic lines: a slow growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm). b) Main effect mean AS scores for treatment and line. Bars represent means  $\pm$  SEM and bars within the same line or treatment group with differing superscripts are significantly different ( $P \leq 0.05$ ).

malities compared with a smaller, traditional line, which may be related to meat quality problems (Mills et al., 1998). The OARDC F line, which was selected for increased BW, and a commercial sire line were both shown to have degenerative muscle fiber changes compared with the smaller RBC2 line (Velleman et al., 2003). Such muscle abnormalities resulting from rapid growth and stress may be related to the development of pale, soft, and exudative meat in turkeys (Owens et al., 2000). The interaction between genotype and stress on meat quality has been cited as an area in need of further research (Le Bihan-Duval, 2004).

A decrease in resistance to bacterial infection has also been characteristic of the selection of the F line turkeys, and male sire lines from the 3 primary turkey breeders have been shown to be more susceptible to *P. multocida* infection compared with the RBC2 line (Nestor et al., 1996b; 1999b). Studies of immune parameters that might be involved in this susceptibility suggest that the humoral response of the F line is higher than that of RBC2 but that these antibodies are not associated with disease resistance (Li et al., 2000a,c). In another study, the F line was shown to have decreased phagocytic activity but no difference in antibody responses to either *P. multocida* or Newcastle disease virus (Li et al., 2001). Peripheral blood mononuclear cells of the F line were shown to have lower mitogenic responses to concanavalin A (Li et al., 1999), and flow cytometric analyses of T lymphocyte subpopulations suggest that the F line has a larger CD4<sup>+</sup>CD8<sup>-</sup> T cell subpopulation than the RBC2 line (Li et al., 2000b). In

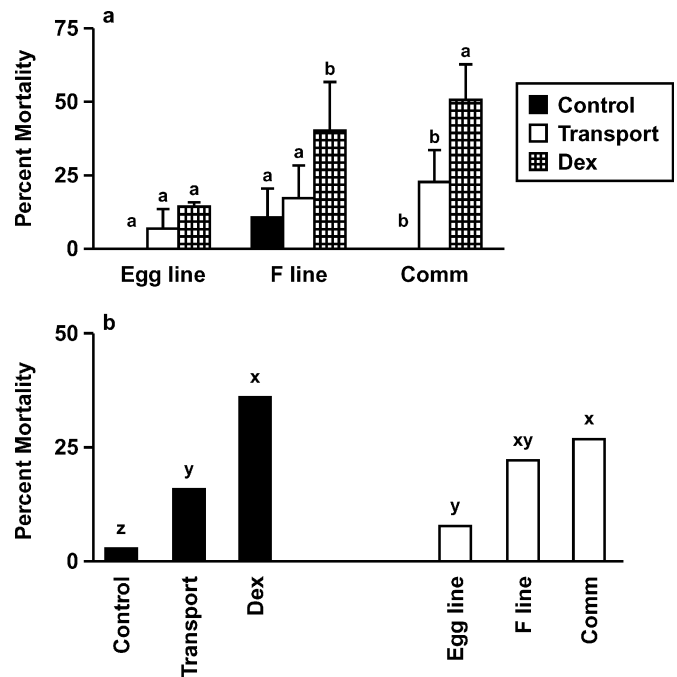


FIGURE 4. a) Effect of transport stress following *Escherichia coli* challenge (Transport) and dexamethasone (Dex) injection preceding *E. coli* challenge (Dex) on percentage postchallenge mortality of 15-wk-old turkeys from 3 genetic lines: a slow growing line selected exclusively for increased egg production over a 250-period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm). b) Main effect mean percentage mortality for treatment and line. Bars represent means  $\pm$  SEM and bars within the same line or treatment group with differing superscripts are significantly different ( $P \leq 0.05$ ).

spite of these differences, the decreased disease resistance of the F line cannot be explained by known changes in the frequency of MHC haplotypes (Nestor et al., 1996c).

In a previous study of the OARDC lines in the authors' laboratory, both lymphocyte numbers and the hypersensitivity response to phytohemagglutinin-P were significantly decreased in the F line compared with RBC2, but there were no such differences in the Egg line relative to its randombred control (Bayyari et al., 1997). Large-bodied birds, which included 2 commercial lines and the F line, were compared with small birds, which included the 2 OARDC RBC lines and the Egg line. The large turkeys had significantly lower Lym numbers and higher Het and Mono numbers than did the small birds and the H/L ratio of the large birds was much higher than that of the small birds ( $P \leq 0.0001$ ) (Bayyari et al., 1997).

In the present study, both of the large-bodied lines (F line and Comm line) had a more severe reaction to the experimental stressors than did the small-bodied Egg line as evidenced by increased H/L ratios. The H/L ratio is a recognized measure of stress in birds (Davison et al., 1983; Gross and Siegel, 1983; Maxwell, 1993; Al-Murrani et al., 1997, 2002) that has become a valuable tool in stress research especially when combined with the convenience and repeatability of automated blood cell counts (Post et al., 2003). The H/L ratio has been suggested as a selection criteria for general stress resistance in broiler chickens by Al-Murrani et al. (1997) who showed that light-bodied

Iraqi fowl had significantly lower H/L ratios compared with a heavy Iraqi broiler line. The broiler line was a mixed-sex population with only 10% males, and the male population had significantly higher H/L ratios compared with females, suggesting that the additional stress of higher BW in males accounted for the increase in H/L ratio (Al-Murrani et al., 1997). In another experiment, male broilers of a heavy line with high H/L ratios were not significantly heavier than those with lower ratios; however, their weights were numerically higher (Al-Murrani et al., 2002). The birds with the lowest H/L ratios were designated stress-resistant using a 99% confidence limit, and were shown to exceed birds with the highest H/L ratios in many aspects of immune response to *Salmonella typhimurium* challenge.

Over the past 12 yr, the authors' laboratory has used the H/L ratio as the primary stress indicator in studying the etiology of turkey osteomyelitis complex, a disease in which healthy-appearing processed turkeys have chronic soft tissue and bone lesions that are only detected when the carcasses are cut open during a mandatory US Food Safety Inspection Service screening process (Huff et al., 2000). The research suggests that individual differences in the stress response of fast-growing male turkeys can be responsible for the immunosuppression leading to the development of these bacterial lesions (Huff et al., 2003). These data support the proposal that the genetic selection for fast growth in modern turkey lines has been accompanied by selection for individuals, particularly males, with a stress response that may be incompatible with the severe stressors that sometimes occur during commercial poultry production.

The smaller Egg line had the least mortality and loss of BW after stress and *E. coli* challenge with the Comm line having significantly more adverse response to both Dex injection and transport stress, and the F line being intermediate in response. These data support the hypothesis that the genetic selection of turkeys for increased BW has been accompanied by changes in the stress response that can lead to decreased resistance to opportunistic bacterial infection (Huff et al., 2000). Furthermore, the data suggest that growers may be able to improve both disease resistance and the safety of their poultry products by choosing slower growing commercial lines, especially when management conditions, heat or cold stress, or other predictable disease stressors indicate that the flock will be at risk.

Unintentional selection for reduced immune response in large-bodied lines may lead to decreased resistance to disease as well as an increase in metabolic defects in pathways dependent on the immune response and its products (Cook, 1994). Specific defects in the immune response may even enhance performance, as immune stimulation has been shown to have a negative effect on productivity (Klasing et al., 1987; Chamblee et al., 1992; Cook et al., 1993). The necessity of maintaining breeding stock in relatively clean and biosecure facilities may also contribute to the selection of birds with relatively low ability to respond to the intensity and diversity of anti-

genic challenge and stressors present in field conditions (Gavora, 1990). Although this practice will select for fast-growing lines with low mortality under optimum conditions, the ability to withstand severe or cumulative stress and resolve chronic or latent infections may be diminished.

The T-cell mediated immune response of chickens has significant variation among birds of different genetic lineages (Miggiano et al., 1976; Lassila et al., 1979; Morrow and Abplanalp, 1981; Fredericksen and Gilmour, 1983; Lamont and Smyth, 1984; Cheng and Lamont, 1988). Successful divergent selection of chickens for various T cell functions suggests that many of these functions are highly heritable, and are often negatively correlated with BW (Sengar et al., 1970; Okada and Mikami, 1974; Yamamoto and Okada, 1990; Afraz et al., 1994).

It is interesting that in the present study, the large-bodied lines had the same total WBC counts as did the Egg line, but had a much higher percentage of both Het and Mono. This might reflect a greater tendency toward the inflammatory response in the larger birds that underwent either Dex treatment or transport stress.

The current study supported the conclusions of Kowalski et al. (2002), who found that the lighter and slower-growing BUT-9 line was more resistant to stress than the faster-growing Big-6 line and suggested that the lighter line would be more suitable for commercial poultry production because of its better response to stress. Much of the progress seen in intensive poultry production over the past 50 yr has been possible due to the availability of very effective and inexpensive antibiotics which have prevented stress-induced opportunistic bacterial disease and allowed efficient production even as density and growth rate increased. The current political climate in the United States and in Europe is unfavorable toward the use of antibiotics in food animal production because of the potential for the direct selection of antibiotic-resistant bacteria that could affect human health and the transfer of antibiotic resistance genes to human pathogens. Should antibiotic usage be curtailed, and present management conditions and rates of growth are maintained, such restriction might increase bacterial disease and colonization with organisms of both production and food safety importance. This situation is forcing the industry to look for alternatives to antibiotics. Most turkey breeders offer several product lines that differ in the time taken to reach market weights. One such alternative may be the simple choice of which line of bird to use in a specific production situation. It is apparent that the response to stress has been altered by selection for increased BW, suggesting that incorporation of stress models into the selection process may be needed to further develop high performance lines that are more stress tolerant and disease resistant.

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