Differential Effects of Sex and Genetics on Behavior and Stress Response of Turkeys[1]

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ABSTRACT Three lines of turkeys were tested for response in T-maze and open-field tests during the first 8 d after hatch, and behavior was observed after catching, moving, and transport. They were also compared for corticosterone (CORT) levels and heterophil:lymphocyte ratios (H:L) at 15 wk of age in response to an *Escherichia coli* challenge followed by transport stress. Large commercial-(COMM) line birds were faster and more active in the T-maze at d 2 than egg-line birds. Male COMM-line birds were faster than male egg-line birds when tested in an open field at d 8. Egg-line birds had more sleeping behavior after moving to a new floor pen as compared with both an intermediate-sized line (F line) and the COMM line. Transport stress increased CORT levels in all 3 lines, and the increase was greater in males compared with females. The egg line had higher basal CORT levels ($P = 0.03$) and higher levels after transport ($P < 0.0001$). The H:L ratios were affected by both transport stress and line but not by sex. The H:L ratio was lower in the egg line as compared with both the F line and the COMM line ($P < 0.0001$), with the COMM line having the greatest increase in response to transport. These data, combined with those from previous studies of these lines, suggest that differences in activity of fast-growing turkeys may be used to select birds that are less susceptible to inflammatory bacterial disease and that the H:L ratio may be more useful than serum CORT in evaluating the deleterious effects of stress.

Key words: turkey, behavior, transport stress, genetics, sex, heterophil:lymphocyte ratio

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INTRODUCTION

The interactions among behavior, genetics, and the stress response of poultry have been studied for over 50 yr; however, most of this work has been focused on either egg-laying or meat-type chickens (*Gallus domesticus;* Siegel, 1984, 1989). Although the domestic turkey (*Meleagris gallopavo*) has more recently become a significant poultry product of worldwide interest (Windhorst, 2006), behavioral research dedicated to this species is sparse, and the 2 species are often assumed to be similar in behavioral and physiological responses (Hale et al., 1969).

Mauldin and Graves (1984) decried the fact that the poultry industry did not have much interest in recognizing the relationships between behavior and production, particularly the effects of moving birds to a new environment and changing the social groups. However, in recent years, the animal production industries have begun implementing programs designed to assess and improve the welfare of food animals (Hester, 2005; Seng and Laporte, 2005), making the literature relating behavior, genotype, and environmental stressors more relevant to commercial production.

We have reported that the stress of gently handling male turkeys once or twice daily for the first 10 d of life, although tending to increase BW, can also result in both an increased or decreased resistance to a bacterial respiratory challenge, depending on the number of handling events and the number of prior treatments with dexamethasone (Huff et al., 2001a,b). The results of our handling studies have suggested that response to early handling was affected by individual variability and that prediction of this variability may be useful for the genetic selection of turkeys whose physiological responses to the many stressors of commercial production do not impair their health and productivity.

It has been established that young broiler chicks that quickly traverse a T-maze to come into visual contact with their hatch mates have higher BW, decreased adrenocortical responses to a stressor, and higher levels of sociality than do slower chicks (Marin et al., 1997, 1999; Jones et al., 1999; Marin and Jones, 1999). The T-maze

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test has thus been suggested as a simple, noninvasive selection criteria for commercial breeding programs that are based on increasing BW (Marin et al., 1999).

However, we have found that 2-d-old turkey poults that have the fastest responses in the T-maze and are also stressed during the first 2 wk by environmental enrichment actually have lower BW than poults that test as slow when they are later challenged with dexamethasone and *Escherichia coli* at 5 wk of age (Huff et al., 2003). There were no differences between fast and slow nonchallenged control birds when they were exposed to stressful enrichment or between fast and slow challenged birds that had not had an enriched early environment. This study suggested that there may be subtle differences in the stress response of turkeys as compared with broiler chickens. Our observation of broiler chicken and turkey lines, both of which have been highly selected for fast growth, shows dramatic differences in the level of activity, with broiler chickens being relatively inactive except when eating and commercial meat turkeys being far more responsive to the environment and more socially interactive.

The genetic selection of poultry for increased BW has clearly been accompanied by changes in immunocompetence and decreased resistance to disease (Han and Smyth, 1972; Saif et al., 1984; Sacco et al., 1991, 1994a,b, 2000; Tsai et al., 1992; Qureshi and Havenstein, 1994; Nestor et al., 1996a,b,c, 1999a,b; Li et al., 1999, 2000a,b,c, 2001; Saif and Nestor, 2002; Cheema et al., 2003). 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 at Ohio State University. These lines include a randombred contol 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).

Both chickens and turkeys have been intensively selected for specific strains that profitably produce either meat or eggs, and correlated responses in behavior have been observed. Relative to egg strains, the chicken meat strains have been described as docile with excessive appetites, decreased motor ability, and poor immunoresponsiveness (Siegel, 1989).

We have previously reported differences in both stress response and disease susceptibility between the Ohio Agricultural Research and Development Center egg line and the F line as well as between both of these and an even faster growing commercial line using 2 stress models (Huff et al., 2005, 2006). The objective of the present study was to determine whether behaviors of male and female poults from these 3 lines are different and are associated with differences in adult corticosterone (**CORT**) levels and heterophil:lymphocyte (**H:L**) ratios after a respiratory *E. coli* challenge and transport stress.

MATERIALS AND METHODS

Birds

Male and female turkeys from 3 genetic lines differing in growth rate were compared for their behavioral responses during the first 2 wk after hatch. The turkey lines were a slow-growing line selected exclusively for increased egg production over a 250-d period (egg line), another experimental line selected for increased 16-wk BW (F line), and a fast-growing commercial meat-line (**COMM**). The birds from the egg and F lines were the progeny of a hatch of eggs obtained from the Ohio Agricultural Research and Development Center. The hatch consisted of 66 egg-line birds and 42 F-line birds of mixed sex. Fifty COMM poults of mixed sex were obtained from a commercial turkey hatchery at 1 d of age and were transported to USDA facilities and set in pens on the same day as the closed lines. All turkeys were reared in floor pens with an area of 4 m^2 on pine shavings and given ad libitum access to a standard corn and soybean turkey ration meeting or exceeding the NRC recommended allowances (NRC, 1994). They 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 pen for each line. At 2 wk of age, they were separated into 12 pens in a 3 line × 2 treatment design with 2 replicate pens for each line × treatment group. To compensate for predicted mortality, 5 or 6 birds were placed into each of the control pens, and 7 to 10 birds were placed into each of the challenge pens.

T-Maze Time and Freezing or Active Behavior Scoring

At 2 d of age, a sample of 10 poults from each line was tested for the time it took to reinstate contact with hatch mates using a T-maze procedure as previously described (Marin and Jones, 1999; Huff et al., 2003). The T-maze boxes were constructed out of the heavy cardboard transporters used to deliver chicks from the hatchery. A 13 × 9 cm mirror was fixed to the wall at the end of the T corridor. At the end of one of the perpendicular arms, a wire screen separated the T-maze from a 25 × 30 cm area containing 10 hatch mates. Hatch mates were provided with feed and water throughout the test period and were not used for behavioral testing. The T-maze boxes were kept clean by placing brown paper towels on the surface, which were changed when dirtied. A second person used an ad libitum sampling methodology (Lehner, 1992) to record relevant behaviors that included freezing, sleeping, walking, chirping, jumping, and exploring. Speaking was not allowed during the test period, and observers were blinded to the treatment group. Poults were quietly herded to 1 corner of the pen and caught and placed in a plastic basket. The baskets were carried to the testing room, and poults were acclimatized within the basket for 15 min before testing began. Each poult was placed in the center of the isolation chamber facing the back wall, and the time it took to turn around, reach the mirror, and reinstate contact with its hatch mates was measured. Individual birds were scored for freezing behavior or active behavior. Birds that were described as having freezing behavior did not move at all in the T-maze and had times >90 s. In addition to not moving from the initial position, they often tended to lie down and close their eyes. Birds that were characterized as having active behavior displayed fast times in the T-maze, appeared alert, and had recorded behaviors that included walking, chirping, exploring, and jumping to escape the T-maze. Each bird was identified by wing band number, and sex was determined at necropsy.

Behavior in an Open-Field Test

At 8 d of age, 12 birds from each group that had not been tested at d 2 were observed for their reactions in an open-field test. The open-field apparatus was constructed from an identical 1.8 × 2.1 m floor pen in which the birds were reared; however, the flooring was gray concrete and was not covered with wood shavings. The pen was divided across its width into 10 equally sized areas using duct tape, with area 1 being at the entrance and areas 9 and 10 being at the farthest end of the pen and adjacent to a wire fence separating the pen from another pen full of poults. A 0.914-m-high plywood entrance wall prevented poults from observing the tester. Thus, tested poults were in view of another pen of turkeys at the far end of the field but were unable to view the observer.

Birds were gently herded to a corner of their original brooding pen, were randomly caught and placed in a basket, and were then individually placed in box 1 of the open-field pen in which they were observed and timed for a period of 60 s. Birds were scored for the time it took to reach box 9 or 10 and for recorded freezing or active behaviors. Each bird was identified by wing band number, and sex was determined at necropsy.

Behavior After Catching and Moving to Treatment Group Floor Pens

At 2 wk of age, poults were randomly separated into treatment groups as described and moved to new floor pens. Behaviors were scored 15 to 30 min after moving and again at 24 h after moving by a single individual using a combination of all-animals and scan-sampling methodology (Lehner, 1992). An observer moved from pen to pen and counted the number of birds in each pen displaying the following predefined behaviors during a period of 60 s: sleeping (defined as having closed eyes and no movement for the entire period of observation), sitting or standing, and walking or running.

E. coli Challenge and Transport Stress

At 14 wk of age, all transport group birds were transported to a separate biosecure building where they were inoculated in the left cranial-thoracic air sac with sterile tryptose phosphate broth (**TPB**) containing approximately 5,000 to 10,000 cfu of a nonmotile strain of *E. coli* serotype O2, which had originally been isolated from chickens with colisepticemia (Huff et al., 1998). The inoculum was prepared by adding 2 loopfulls of an overnight culture on blood agar to 100 mL of TPB 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 TPB based on the standard plate count.

Challenged birds, none of which had signs of morbidity or loss of BW 8 d after challenge, were at that time subjected to the following transport stress procedure, which consisted of a total of 12 h of transport stress, including holding time in the transport vehicle. Birds were caught and 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 Department of Poultry Science Processing Plant, where the transport vehicle was parked in a covered holding area. After a total of 12 h from time of loading, birds were returned to their original pens where they had access to feed and water. Behavior of birds in each pen was recorded by a single individual using an ad libitum sampling methodology (Lehner, 1992) after they were returned to their pens.

Blood Collection, CORT Assay, and H:L Ratio

The following morning, all birds were bled by venipuncture of the brachial vein, and blood was placed into both serum collection and EDTA-coated Vacutainer tubes (BD, Franklin Lakes, NJ). All challenged, transported birds and control birds were bled at the same time, which was 12 h after the end of the transport period and 9 d after challenge with *E. coli.* Serum was collected and stored at –20°C until assayed. Corticosterone assay was conducted using a high-sensitivity competitive enzyme immunoassay kit (Corticosterone HS, IDS Inc., Fountain Hills, AZ).

The numbers of lymphocytes and heterophils were measured using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL), which employs both electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Heterophil:lymphocyte ratios, 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.

Statistics

Behavioral tests on d 2 and 8 posthatch were analyzed as pen means in a 3 × 2 factorial design (line × sex) using the GLM procedure of SAS software (SAS Institute, 1988). Percentage data for behavior on d 14 and 15 were analyzed as pen means for line only. Corticosterone data were analyzed as a 3 × 2 × 2 factorial design (line × treatment × sex) using the GLM procedure of SAS software. Percentage data for H:L ratio were subjected to arc sine square root of the percentage transformation for analysis. Means were separated using Duncan's multiple range test. Differences between the inoculated, transported treatment relative to untreated controls and between lines within treatments were separated using the least square means procedure of SAS software. A P-value of less than or equal to 0.05 was considered significant unless otherwise stated.

RESULTS

T-Maze and Freezing or Active Behavior

The time it took poults to traverse a T-maze at 2 d of age was significantly lower in the COMM line as compared with both the egg line and the F line (Figure 1, panel A). Significantly more COMM-line birds were scored as active as compared with egg-line birds (Figure 1, panel B), and no COMM-line birds were scored as freezing (Figure 1, panel C). There were no significant differences between males and females at 2 d of age, and there were no line × sex interactions.

Behavior in an Open-Field Test

The time it took 8-d-old poults to reach box 9 or 10, at the far end of the open-field pen, was lower in male birds of the COMM line as compared with male birds of the egg line (P = 0.03), and all COMM-line birds tended to have faster times than did egg-line birds (P = 0.06, Figure 2, panel A). More COMM-line birds were scored active than were egg-line birds (P = 0.05, Figure 2, panel B), and males were more active than were females (P = 0.05). There were no line × sex interactions. More freezing behavior was displayed by females as compared with males (Figure 2, panel C, P = 0.05); however, there were no line differences or interactions for freezing behavior.

Behavior After Moving to Treatment Group Floor Pens

When 2-wk-old poults were scored for sleeping behavior at 15 to 30 min after being moved to new treatment group floor pens, more egg-line birds appeared to sleep as compared with both the F line (P = 0.05) and the COMM line (P = 0.009) (Figure 3, panel A). This difference persisted when the birds were scored 24 h later. The percentage of birds that were sitting but alert was higher in the F line (P = 0.05) and approached significance in the COMM line (P = 0.06) as compared with the egg line at 15 to 30 min after moving (Figure 3, panel B). At 24 h after moving, the difference between egg-line and F-line birds approached significance (P = 0.07). There were no significant difference in the percentages of poults scored as walking or running; however, there was a trend for more activity in COMM-line birds as compared with the egg line (P = 0.1, Figure 3, panel C). When the scores obtained at 15 to 30 min were combined with the scores taken 24 h later, the differences in percentage of sleeping behavior between egg line and F line (P = 0.008) and the COMM line (P = 0.0009) were increased (data not shown). When the percentage sitting scores were combined, both the F line (P = 0.006) and the COMM line (P = 0.01) displayed more sitting behavior than did the egg line (data not shown). There were no significant differences in percentage walking when scores were combined.

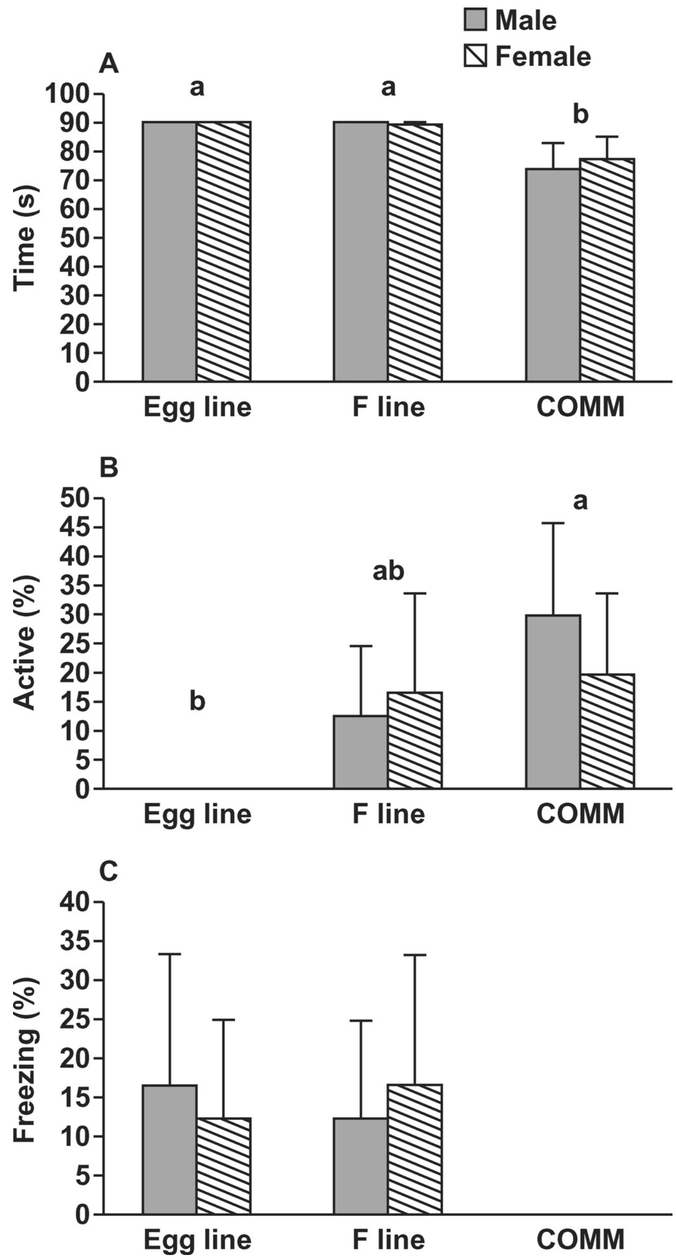

Figure 1. The effects of line on time to traverse a T-maze at 2 d posthatch (panel A) and the percentage of poults that displayed active behaviors (panel B) or freezing behavior (panel C) in the T-maze. Commercial- (COMM) line birds were faster (panel A, P = 0.02) and more active (panel B, P = 0.05) than egg-line birds. [a,b]Bars with differing letters represent significantly different main effect means for line (P ≤ 0.05).

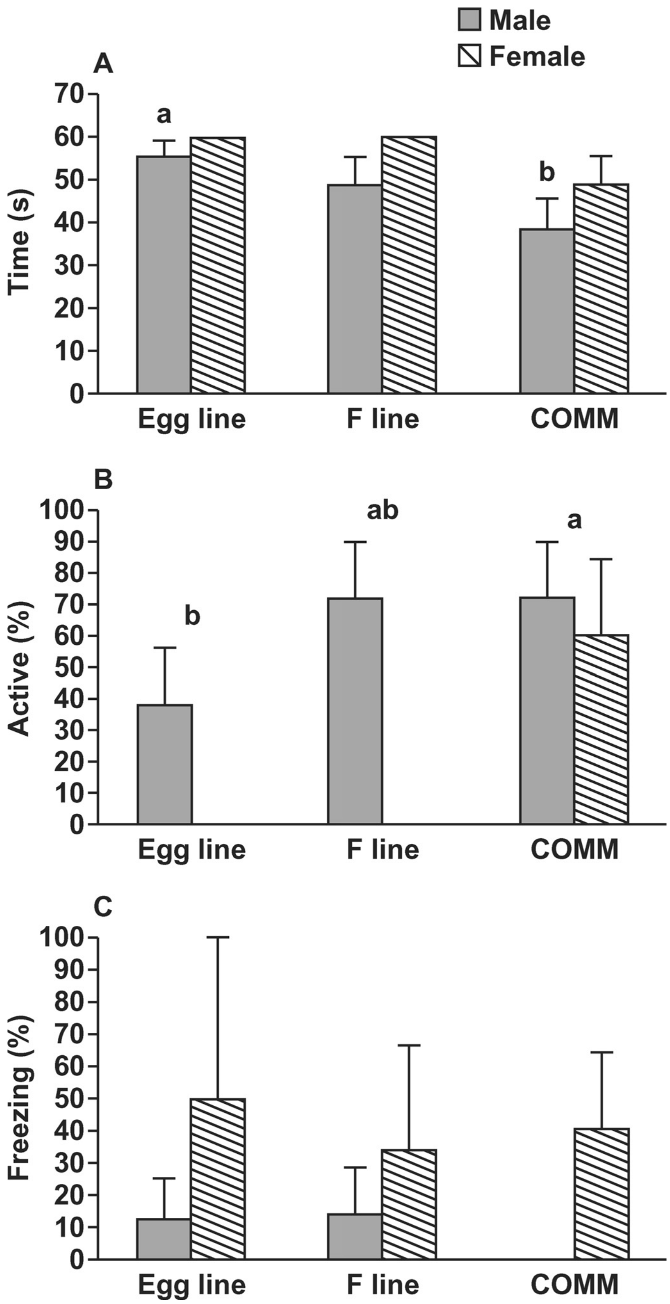

Figure 2. The effects of sex and line on time to traverse an open field at 8 d posthatch and the percentage of poults that displayed active or freezing behaviors in the open-field pen. Male commercial- (COMM) line birds were faster (panel A, $P = 0.03$) than male egg-line birds, and the COMM line was more active than the egg line (panel B, $P = 0.05$). Females had more freezing behavior than males (panel C, $P = 0.05$). Bars represent means ± SEM. [a,b]Bars with differing letters are significantly different ($P \leq 0.05$).

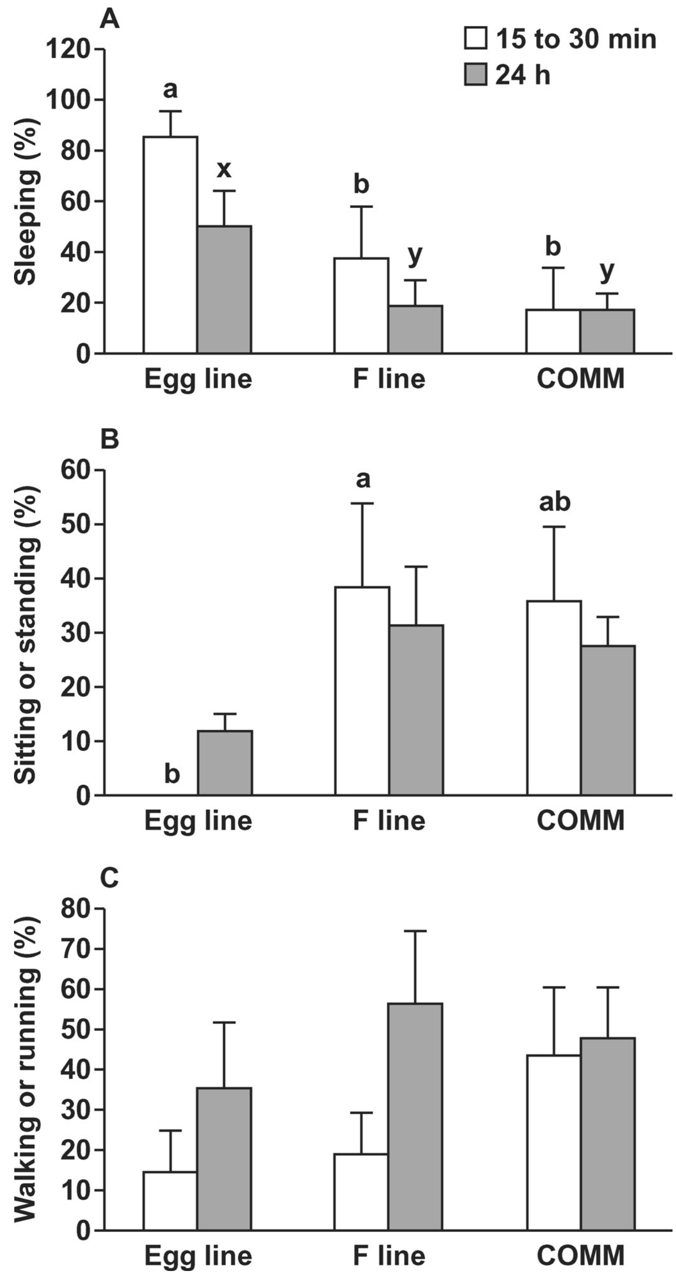

Figure 3. The effects of line on relative activity of 2-wk-old turkey poults after being moved from brooding pens to treatment group pens. Birds were scored for sleeping behavior (panel A), alert sitting or standing behavior (panel B), or walking and running behavior (panel C) at both 15 to 30 min and 24 h after moving to new pens. The egg line had more sleeping behavior at both times than either F-line ($P = 0.05$) or commercial- (COMM) line ($P = 0.009$) birds (panel A) and had less alert sitting and standing behavior than the F line at 15 to 30 min after moving (panel B). Bars represent means ± SEM. [a,b] and [x,y]Bars with differing letters are significantly different ($P \leq 0.05$).

Behavior After Transport and Holding for 12 h

Fifteen-week-old transported birds were observed within 30 min after being returned to their original pens. The F-line and COMM-line birds were universally prostrate, immobile, often panting, and none were eating or drinking, whereas the egg-line birds were standing, walking, eating, and drinking. Within 2 h, some of the large-bodied birds regained equilibrium and began walking, eating, and drinking. The next morning, about 12 h after the end of transport stress, all birds had recovered from transport stress, and there were no apparent differences between the egg line and the large-bodied lines in these behaviors.

Table 1. Effects of sex, genetic selection for egg production or BW, and *Escherichia coli* challenge followed by transport stress (transport) on levels of corticosterone (ng/mL) in serum of 15-wk-old turkeys[1]

	Egg line		F line		COMM	
Treatment	Male (n = 24)	Female (n = 20)	Male (n = 28)	Female (n = 22)	Male (n = 26)	Female (n = 26)
Control	6.9 ± 1.6^{b}	9.2 ± 1.9^{b}	3.6 ± 1.0^{b}	2.9 ± 0.3	3.6 ± 0.7^{b}	3.0 ± 0.4^{b}
Transport[2]	$39.5 \pm 1.9^{a,x}$	$30.6 \pm 1.6^{a,y}$	$18.2 \pm 2.6^{a,x}$	6.4 ± 0.6^{y}	$20.2 \pm 2.3^{a,x}$	$15.2 \pm 2.0^{a,y}$

	MEM[3]	P-value	Interactions	P-value
Control	4.30^{b}		Line × treatment	<0.0001
Transport	21.91^{a}	<0.0001	Sex × treatment	<0.0001
			Line × sex	0.3
Male	15.32^{a}		Line × treatment × sex	0.3
Female	13.41^{b}	<0.0001		
Egg line	26.01^{a}			
F line	7.20^{c}			
COMM	11.52^{b}	<0.0001		

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

[x,y]Means within a row and within a line with no common superscripts differ significantly ($P \leq 0.05$).

[1]Egg line = a slow-growing-line selected exclusively for increased egg production over a 250-d period; F line = a line selected for increased 16-wk BW; and COMM = a commercial line.

[2]Transport included injection of approximately 5,000 to 10,000 cfu of *E. coli* into the air sac 8 d before a 12-h transport and holding procedure. Blood was collected 12 h after the end of transport and 9 d after *E. coli* challenge.

[3]MEM = main effect mean

CORT Assay

Corticosterone levels were increased by transport stress ($P < 0.0001$), and the increase was greater in males as compared with females ($P < 0.0001$, Table 1). Line affected CORT level ($P < 0.0001$), and there were interactions between line and treatment ($P < 0.0001$) and between sex and treatment ($P < 0.0001$, Table 1).

There were no line or sex differences in control birds that were not challenged with *E. coli* and subjected to transport stress (Table 1). Within each line, there were no differences in CORT levels between males and females that were not subjected to both *E. coli* challenge and transport stress. However, the male birds of all 3 lines had significantly higher CORT levels compared with females after challenge and transport stress (Table 1). The basal CORT levels (control) of males and females combined were higher in the egg line ($P = 0.02$) as compared with both large-bodied lines (data not shown), and the increase in CORT levels of egg-line birds after transport was more highly significant ($P \leq 0.0001$).

H:L Ratio

Main effect mean H:L ratios were increased by *E. coli* challenge and transport stress ($P < 0.0001$) and by line ($P < 0.0001$); however, the main effect mean for sex was not significant, and there were no significant interactions (Table 2). The egg line had lower H:L ratios as compared with both the F line ($P = 0.02$) and the COMM line ($P = 0.0003$), which were not different from each other. Within the COMM line, both males and females had significantly increased H:L ratios after challenge and transport stress; however, this increase was not significant in either males or females of egg-line or F-line birds (Table 2).

DISCUSSION

This study describes behavioral differences between turkeys from a small-bodied line that was selected for increased egg laying as compared with larger turkey lines selected for fast growth. As shown in both a T-maze test at 2 d of age and in an open-field test at 8 d of age, the egg-line birds were less active than the COMM line, and the F-line birds, which were intermediate in size, had an intermediate level of activity. This response was also seen at 2 wk of age, when egg-line birds had more sleeping behavior after being moved to a new pen, whereas the large lines remained more alert. This behavioral response changed dramatically in mature birds as reflected in the observation that, universally, the egg-line birds had little behavioral response to a 12-h transport stress at 15 wk of age and were immediately eating and drinking, walking, and appearing normally active after this severe stressor, whereas the birds in both large-bodied lines were prostrate and unwilling to move, eat, or drink for several hours, even though they had endured a 12-h fast.

This dramatic difference in the response to transport stress was reflected in higher CORT levels and lower mean H:L ratios in transport-stressed egg-line birds as compared with both large-bodied turkey lines. We have previously reported that the smaller egg line had the least degree of inflammatory respiratory disease, lower mortality, and higher BW after transport stress, with the COMM line having significantly more adverse response to transport stress and the F line being intermediate in response (Huff et al., 2005, 2006). Thus, it is possible that the higher basal and reactive CORT levels of the egg-line birds may have been protective in this disease challenge and may have prevented both the increase in H:L ratio

Table 2. Effects of sex, genetic selection for egg production or BW, and *Escherichia coli* challenge followed by transport stress (transport) on the heterophil:lymphocyte ratios of 15-wk-old turkeys[1]

	Egg line		F line		COMM	
Treatment	Male (n = 17)	Female (n = 170)	Male (n = 12)	Female (n = 13)	Male (n = 18)	Female (n = 19)
Control	0.6 ± 0.1	0.7 ± 0.2	1.9 ± 0.5	2.3 ± 0.8	1.9 ± 0.5[b]	1.7 ± 0.7[b]
Transport[2]	2.1 ± 0.4	1.6 ± 0.2	1.7 ± 0.2	4.1 ± 1.1	5.5 ± 1.9[a]	4.2 ± 0.8[a]

	MEM[3]	*P*-value	Interactions	*P*-value
Control	1.53[b]		Line × treatment	0.3
Transport	3.38[a]	<0.0001	Sex × treatment	0.2
			Line × sex	1.0
Male	3.52		Line × treatment × sex	0.4
Female	3.11	<0.3215		
Egg line	1.67[a]			
F line	3.66[b]			
COMM	4.58[b]	<0.0001		

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Egg line = a slow-growing line selected exclusively for increased egg production over a 250-d period; F line = a line selected for increased 16-wk BW; COMM = a commercial line.

[2]Transport included injection of approximately 5,000 to 10,000 cfu of *E. coli* into the air sac 8 d before a 12-h transport and holding procedure. Blood was collected 12 h after the end of the transport period and 9 d after *E. coli* challenge.

[3]MEM = main effect mean.

experienced by the larger lines and the concurrent increase in respiratory inflammation and mortality.

It is known that glucocorticoids can protect the host from the deleterious effects of its own immune response, because cytokines stimulated during challenge not only coordinate the fight against infection but can also result in autoimmunity, hyperinflammation, and death of the host if not regulated (Kapcala et al., 1995; Sternberg, 1995, 2001; Raberg et al., 1998;). These data suggest that the immunosuppressive effects of transport stress may be more severe in turkeys that have been selected for fast growth because of their blunted hypothalamic-pituitary-adrenal (**HPA**) axis as evidenced by low CORT levels relative to turkeys selected for egg production. Thus, using changes in CORT to measure stress as an indicator of turkey welfare may overlook the fact that a blunted CORT response may be characteristic in highly selected commercial meat-line turkeys, and thus may not accurately reflect the deleterious effects of production stressors.

A blunted HPA axis is also characteristic of fast-growing broiler chickens as compared with chicks from an egg-laying line (Saito et al., 2005). However, in the study by Saito et al. (2005), the larger-bodied meat-line chicks were less active in an open-field test than were the egg-line chicks, which were also more vocal and had higher plasma CORT levels after both isolation stress and intracerebrovetricular injection of corticotrophin-releasing factor. Again, it appears that behavioral response in chickens may not be the same as in turkeys.

Over the past 13 yr, the H:L ratio, a recognized measure of stress in birds (Davison et al., 1983; Gross and Siegel, 1983; Maxwell, 1993; Al-Murrani et al., 1997, 2002; Post et al., 2003), has been used as the primary stress indicator in studying the etiology of turkey osteomyelitis complex (**TOC**), a disease in which healthy-appearing processed male turkeys have chronic and unapparent muscle, joint, and bone infections that are only detected when the carcasses are cut open during a mandatory US Food Safety Inspection Service screening process (Huff et al., 2000). This research has suggested that individual differences in the stress response of fast-growing male turkeys may be responsible for the immunosupression leading to the development of these bacterial lesions and support the hypothesis 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.

In the present study, egg-line birds had higher basal CORT levels as compared with both large lines and higher CORT levels 12 h after transport stress. Because these birds were also shown to have more adaptive behavior immediately after transport stress and also had less disease and mortality due to *E. coli* challenge and stress (Huff et al., 2006), it may be argued that higher serum CORT levels may sometimes be an indication of well-being. This might explain the lack of correlation between CORT levels and H:L ratios seen in a study designed to determine if broiler chickens that were feed restricted as compared with those fed ad libitum had decreased welfare (de Jong et al., 2002). The feed-restricted birds had higher CORT levels but no difference in H:L ratio and also had less heart failure, reproductive problems, and fat deposition.

Kowalski et al. (2002) compared males from 2 large-bodied meat turkey lines, the BUT-9 line and the Big-6 line, for their behavior in an open-field test at 7 to 8 wk of age and their physiological and immunological responses to crowding and transport stress. They found that the lighter and slower-growing BUT-9 line was more active in the open-field test and had a lower increase in

CORT after a 1-h transport stress than the faster-growing Big-6 line. They concluded that the lighter BUT-9 turkeys were less sensitive to environmental stress than the larger birds and may be better suited to commercial production conditions than the larger lines. It is difficult to compare our results with this study because of the age differences at the time of behavioral testing and the difference in degree of transport stress. Albentosa et al. (2003) reported that there is a decrease in fearfulness and an increase in exploratory behaviors in laying hens as they age. The dramatic change in activity of the turkey egg-line birds that occurred between the neonatal tests in which they were inactive relative to the larger lines and their higher level of activity after transport stress suggests that this difference may also be age-related. The duration of transport stress used in the Kowalski et al. (2002) study (1 h) was far less severe than the 12-h total holding time used in the present study, which was designed to model the circumstances that result in commercial turkeys being held on transport vehicles at the processing plant for extended periods of time due to unforeseen delays, high levels of condemnation, or both.

In the present study, males tended to be more active than females, and this difference was significant when comparing egg-line and COMM-line males in the open-field test at 8 d of age. Males also had higher CORT levels than females. Marin et al. (2002) have shown that male broiler chickens have a greater response to stress than females. We have previously reported that the male transported birds were also more susceptible to inflammatory disease than females, because they had twice the incidence of the inflammatory disease TOC and significantly higher isolation of the challenge strain of *E. coli* from the TOC lesions (Huff et al., 2006). The immunosuppressive effects of stress appear to be greater in male birds than in females (Redig et al., 1985; Huff et al., 1999, 2006), and female turkeys are more resistant to a dexamethasone-*E. coli* challenge than are males (Huff et al., 1999). Turkey osteomyelitis complex is a disease that is mainly a problem of male turkeys from the ages of 9 to 20 wk that are beginning to develop secondary sexual characteristics and is not considered to be a disease problem in females (Nairn, 1973; Clark et al., 1991; Mutalib et al., 1996).

This research suggests that using CORT levels alone to measure the deleterious effects of stress in turkeys may be inappropriate, because this index overlooks the protective effects that CORT can have on modulation of the inflammatory response. The H:L ratio may be a more direct end point for measuring the effects of stress, because it is an actual indicator of the degree of inflammatory response rather than the measurement of a modulator of inflammation.

There are many environmental, social, and management stressors that can result in a deleterious stress response during turkey production. The stress response, although generally protective to homeostasis, has clearly been shown to affect immunity, to reduce resistance to bacterial diseases, and to have a remarkable degree of individual variability in the many animal species that have been studied (Kelley, 1980, 2004; Siegel, 1980, 1995; Gross and Siegel, 1988; Khansari et al., 1990; Meaney et al., 1993; Mormede et al., 2002). It is important to understand individual variation in the turkey stress response, how this individual variation can be modified and exploited, and how the turkey immune system can be protected from insult due to stress.

The genetic selection for rapid growth and increased BW in poultry, particularly in males, and the well-documented correlation of this selection with decreased resistance to disease provides a valuable model for dissecting the mechanisms that must interact in determining the individual responses to stress. The behavior of young poults may be used to select the most active individuals, which due to their excessive growth and blunted HPA axis, may be more susceptible to opportunistic bacterial infections and chronic inflammatory disease such as TOC. In this study, the higher basal and reactive CORT levels of the smaller egg-line birds may have been protective to the stress challenge, preventing both the increase in H:L ratio experienced by the larger-bodied lines and the concurrent susceptibility to respiratory infection and mortality seen in the male commercial-line birds.

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