Effect of Early Handling of Turkey Poults on Later Responses to a Dexamethasone-*Escherichia coli* Challenge. 1. Production Values and Physiological Response[1]

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ABSTRACT The stress responses of mice and rats has been shown to be permanently altered by brief, gentle handling during the first 10 d of life, resulting in increased BW and resistance to stress-induced immunosuppression. The purpose of this study was to determine whether early handling of turkey poults could permanently affect production values and physiology of adult turkeys. Turkey poults were handled 0, 1 (1×), or 2 (2×) times daily for the first 10 d after hatch. Handling consisted of gently catching each poult and holding it for 10 s. On Day 11 after hatch, half of the birds from each handling treatment were treated with three injections of 2 mg dexamethasone (DEX)/kg BW on alternating days. On the day of the third DEX injection, duplicate pens of birds were also inoculated in the airsac with 0 or 50 cfu of *Escherichia coli*. The same birds were treated with a second series of DEX injections at 5 wk of age. Two weeks later, all birds were weighed, and 3 wk later four birds per pen were bled and 10 birds per pen were necropsied; relative organ weights were then determined. Surviving birds were treated with a third series of DEX injections at 10 wk of age; 2 wk later, all surviving turkeys were bled, weighed, and necropsied. Feed consumption was determined weekly.

There were no differences due to handling treatment on the body weights or on the relative organ weights of birds that died after the first DEX treatment. Birds treated with a second DEX injection at 5 wk of age and handled 1× daily had decreased BW. Those handled 1× or 2× daily had higher feed conversion ratios. Surviving birds that were given a third DEX treatment had higher BW and no difference in feed conversion when handled 1× or 2× daily.

Relative liver, heart, and spleen weights were affected by handling of DEX-*E. coli*-treated birds, as were serum chemistry values for calcium, iron, glucose, total protein, blood urea nitogen, uric acid, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and γ-glutamyltransferase. Handling also affected the numbers of white blood cells of DEX-treated birds. These results indicate that brief and gentle handling of turkey poults during the first 10 d after hatch has lasting effects on production values and physiology of adult turkeys and that these effects can be positive or negative. These results suggest a genetic divergence in the response to stress and its effect on production values and physiology of commercial turkey populations.

(*Key words*: turkey, osteomyelitis, stress, handling, *Escherichia coli*)

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INTRODUCTION

Neonatal handling has been shown to permanently affect the development of the hypothalamic-pituitary-adrenal axis responses to a wide variety of stressors in mice and rats. Merely removing an animal from its environment or holding the animal for a few minutes can increase the number of glucocorticoid receptors in the hypothalamus, and this has been related to a more efficient and stable hormonal response to stress in the adult animal (Meaney et al., 1993). Increased BW of rats has been observed as the result of various early handling or stimulation practices (Ruegamer et al., 1954; McClelland, 1956; Weininger, 1956).

This type of study was applied to broiler chicks by Thompson (1976), who removed chicks from their hatchmates and placed them in solitary cages for 3 min/d for the first 5 d after hatch or the first 15 d after hatch. Birds thus handled for 5 d had significantly increased BW

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Abbreviation Key: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; DEX = dexamethasone; GABA = γ-aminobutyric acid; GGT = γ-glutamyltransferase; H/L = heterophil to lymphocyte ratio; LDH = lactate dehydrogenase; TPB = tryptose phosphate broth; UA = uric acid; WBC = white blood cells.

relative to unhandled controls, whereas those handled for the first 15 d had less improvement than those in the 5-d group. This weight improvement was achieved without increased feed consumption. Handling of chicks has been shown to reduce their fear of humans, lower the duration and number of inductions required to induce tonic immobility, and result in decreases in forebrain γ-aminobutyric acid ($GABA_A$) receptors and in vitro GABA release from brain tissue (Jones and Waddington, 1992, 1993; Jones, 1993; Fluck et al., 1997). A negative effect of early handling on growth rate has also been observed. In an experiment of Freeman and Manning (1979) chicks were removed from brooders twice daily, 5 d/wk for 3 wk. They were removed individually and placed in a crate, left as a group for 5 min, and then returned individually to their brooders. At the end of 3 wk, the handled birds as well as unhandled controls were subjected to the same handling procedure and then bled. Besides a decrease in growth rate, the handled birds showed decreased serum glucose and corticosterone levels and increased free fatty acid levels as compared to the unhandled controls. Recently, studies in swine have indicated that neonatal handling (removal from the farrowing crate for 10 min/d for the first 14 d after birth) resulted in permanently altered functions of the hypothalamic-pituitary-adrenal axis and significantly decreased BW at 7 mo of age (Weaver et al., 2000).

Adaptation of older chickens to human contact by slow and deliberate attention and stroking for 90 to 120 s/d results in increased BW and disease resistance (Gross and Siegel, 1979). Jones and Hughes (1981) also reported increases in growth and feed conversion of handled adult broilers and female layers but no effects in males of a layer strain. Nicol (1992) reported no effect or a positive effect on BW of transported adult broilers in four handling experiments. Other studies have reported that handling of older birds had no effect on performance (McPherson et al., 1961; Reichman et al., 1978).

We have developed an experimental model that allows us to reproduce air sacculitis and osteomyelitis in turkeys by injecting them with 2 mg/kg of synthetic glucocorticoid, dexamethasone (DEX), followed by air sac inoculation with low levels of *Escherichia coli* (Huff et al., 1998, 1999b). This model has suggested that repeated elevation of glucocorticoid levels may undermine the immunocompetence of some turkeys, resulting in susceptibility to opportunistic bacterial infections. One of the strongest effects of this treatment is a decrease in BW and increase in feed conversion ratio. The purpose of the present study was to determine whether early handling could affect production parameters and physiological functions of turkey poults and alter their response to treatment with pharmacological levels of glucocorticoid and air sac inoculation with *E. coli*.

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.

MATERIALS AND METHODS

Seven hundred twenty day-old male poults were wing-banded and divided into 24 randomly assigned floor pens. Untreated control birds and birds challenged with *E. coli* only were housed at 15 poults per pen. Those to be treated with only DEX were housed at a density of 30 poults per pen, and those challenged with DEX and *E. coli* were housed at 60 poults per pen. This variation was allowed to compensate for predicted mortality, and the number of birds per pen was relative to the degree of challenge stress. The number of birds assigned to each handling treatment remained consistent. Duplicate pens of each DEX and *E. coli* challenge group were assigned to each of three handling treatments that consisted of gentle handling of individual birds 0, 1 (1×), or 2 (2×) times daily for the first 10 d after hatch. The handling procedure was standardized, performed in silence, and consisted of gently catching each individual poult, holding it in both hands for 10 s, and then placing it into a basket with the other poults. When all of the poults had been handled, they were returned to the floor pens sequentially, starting with the first pen handled. Handling was performed by nine individuals, who were randomly assigned to pens according to a pre-arranged schedule. Birds were exposed to 23 h incandescent lighting per day and were provided access ad libitum to a standard turkey starter diet that met or exceeded the nutrient requirements established by the NRC (1994). Feed consumption was determined weekly.

DEX 1

On the eleventh day, half of the birds were given three intramuscular injections of approximately 2 mg of DEX[3]/kg BW into a thigh muscle on three alternating days. On the day of the third DEX injection, all birds were inoculated in the left cranial-thoracic air sac with 200 μL of sterile tryptose phosphate broth (TPB) or with 200 μL of TPB containing approximately 50 cfu of a nonmotile strain of *E. coli* serotype O2. The inoculum was prepared by adding two loopfuls of an overnight culture on blood agar to 100 mL of TPB and then 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. The dilution was made and verified with a second plate count.

DEX 2

Surviving birds were raised until 5 wk of age, at which time all birds previously treated with DEX were given another set of three DEX injections as previously described. Two weeks later all birds were weighed; 3 wk later, four birds per pen were bled, and 10 birds per pen were necropsied. Body and organ weights were determined at necropsy. Serum was collected, and we used clinical chemistry analysis to measure serum levels of calcium, phosporus, total protein, albumin, glucose, triglycerides, cholesterol, uric acid (UA), blood urea nitro-

gen (BUN), and iron and enzyme activities of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase by using an automated clinical chemistry analyzer according to the manufacturer's directions.[4] Peripheral blood total leukocyte counts (WBC) and the numbers and proportions (%) of various leukocytes were determined using a Cell-Dyn 3500[5] blood analysis system that was standardized for analysis of turkey blood. Heterophil/lymphocyte ratios (H/L), an indicator of stress in birds (Gross and Siegel, 1983), were calculated.

DEX 3

Surviving birds were maintained until 10 wk of age, at which time remaining DEX-treated birds were given a third series of DEX injections. Two weeks later four birds per pen were bled; all surviving birds were weighed and necropsied, and relative organ weights were determined.

All percentage data were subjected to arcsine transformation for analysis. Pen means for the effects of DEX, *E. coli*, and handling were analyzed for ANOVA as a 2 × 2 × 3 factorial arrangement (two DEX treatments, two *E. coli* treatments, and three handling treatments) by using the general linear models and least-squares means procedures of SAS software (SAS Institute, 1988). Significance was considered at $P \leq 0.05$, unless otherwise noted.

RESULTS

BW and Feed Conversion

DEX 1. There were no significant differences due to handling treatment on BW of mortalities after the first DEX treatment.

DEX 2. Body weights of birds treated with a second DEX injection at 5 wk of age were significantly decreased in those groups that were handled 1×/day. Weights of birds handled 2×/day were not affected. The DEX treatment and *E. coli* challenge decreased BW. There was a significant interaction between handling and DEX treatment toward lowering BW of birds handled 1×/day (Table 1).

Feed conversion was determined at 51 d of age (2 wk after the second DEX treatment). At that time, birds handled 1× or 2× daily had significantly higher feed conversion ratios compared with unhandled controls. The DEX treatment and *E. coli* challenge increased feed conversion ratios (Table 2).

DEX 3. Birds handled 1× or 2× daily had significantly higher BW ($P \leq 0.05$) after the third DEX treatment as compared with unhandled birds (Table 3). Body weights were decreased by DEX treatment, and there was a significant interaction between handling, DEX, and *E. coli*. There were no differences in average feed conversion ratios due to handling at the conclusion of the 12-wk study (Table 4). DEX treatment, but not *E. coli* challenge, significantly increased final feed conversion ratios, and there was an interaction between DEX and *E. coli* (Table 4).

Relative Organ Weights

DEX 1. There were no significant differences due to handling among relative organ weights of mortalites after the first DEX treatment.

DEX 2. Differences in relative organ weights between handling treatments occurred mainly in birds treated with DEX and *E. coli*; therefore, Table 5 displays these data only. Liver weights were higher and bursa/spleen ratios were lower in birds handled 1× and challenged with DEX and *E. coli* as compared with unhandled birds (Table 5). DEX-*E. coli* treatment increased liver and heart weights and decreased bursal weights and bursa/spleen ratio, regardless of handling treatment. Spleen weights were increased only in birds handled 1× and treated with DEX-*E. coli* (Table 5).

DEX 3. Relative liver and heart weights were increased and spleen weights were decreased in birds handled 2× as compared with unhandled controls in birds treated with DEX and challenged with *E. coli*. Treatment with DEX-*E. coli* resulted in increased liver and heart weights and decreased bursa weights and bursa/spleen ratios, regardless of handling treatment (Table 5).

Clinical Chemistry

DEX 2. Clinical chemstry values were affected by handling treatment mainly in birds both treated with DEX and challenged with *E. coli*, therefore Tables 6 and 7 display only these data. Serum calcium levels were increased in control birds which had been handled 2× relative to non-handled birds. Calcium levels were increased in birds challenged with DEX-*E. coli* and handled 1× relative to those of unhandled controls (Table 6). Iron levels of DEX-*E. coli*-challenged birds were increased only in birds handled 1× relative to unhandled controls. Serum glucose was decreased and protein, BUN, and UA levels were increased in DEX-*E. coli*-challenged birds handled 1×. Treatment with DEX-*E. coli* resulted in increased calcium, protein, BUN, and UA and decreased glucose only in 1× handled birds, but not in unhandled or 2× handled birds. Treatment with DEX-*E. coli* resulted in decreased iron levels only in unhandled birds (Table 6). Serum levels of AST were increased in unchallenged controls handled 2× relative to unhandled control birds. Serum levels of AST as well as lactate dehydrogenase (LDH) and γ-glutamyltransferase (GGT) were increased in DEX-*E. coli*-challenged birds that were handled 1× relative to unhandled controls. ALT and AST levels were higher in DEX and *E. coli* challenged birds handled 1× relative to 2× handled birds (Table 7). Challenge with DEX-*E. coli* resulted in increased levels of AST, ALT, LDH, and GGT only in birds handled 1× (Table 7).

[4]Express Plus, Ciba-Corning Diagnostics Corp., Medfield, MA 02052.
[5]Abbott Diagnostics, Abbott Park, IL 60064.

TABLE 1. The effect of handling poults one time (1×) or two times (2×) daily on body weights of 7-wk-old turkeys that were challenged by air sac inoculation with 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk and again at 5 wk of age (Dex 2)[1]

	No *E. coli*		*E. coli*		Handled
	No DEX	DEX	No DEX	DEX	MEM[2]
Not Handled	5,212 ± 245	2,166 ± 212[ab]	4,892 ± 456	2,223 ± 199[a]	2,891[a]
Handled 1×	5,321 ± 154	1,886 ± 168[b]	5,254 ± 249	1,692 ± 128[b]	2,427[b]
Handled 2×	5,504 ± 130	2,687 ± 226[a]	4,365 ± 521	2,216 ± 191[a]	3,017[a]
MEM					
No *E. coli* 2,965[a]	No DEX 5,088[a]				
E. coli 2,573[b]	DEX 2,094[b]				
Probability values					
Handled	0.0004				
DEX	0.0001				
E. coli	0.0298				
Handled × DEX	0.0412				
Handled × *E. coli*	0.1717				
DEX × *E. coli*	0.3454				
Handled × DEX × *E. coli*	0.5827				

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

[1]Values indicate the mean ± SE of all mortalities and 20 necropsied birds per treatment.

[2]MEM = Main effect means.

DEX 3. Calcium levels were decreased in challenged birds handled 1× relative to unhandled controls (Table 6). Calcium levels were increased by the DEX-*E. coli* challenge only in unhandled birds (Table 6). Iron levels were decreased by the DEX-*E. coli* challenge only in birds handled 1×. Handling of birds 1× resulted in decreased protein levels of challenged birds relative to unhandled controls. Protein levels were increased by the DEX-*E. coli* challenge in unhandled and 2× handled birds but not in those handled 1×. Handling birds 2× increased UA levels of control birds, and handling 1× decreased UA levels of challenged birds. There was a high level of variability in UA data of challenged birds handled 2×, resulting in lack of significance. Uric acid levels were increased by the DEX-*E. coli* challenge only in the unhandled birds (Table 6). Unchallenged birds handled 2× had higher levels of LDH relative to birds handled 1× (Table 7). The only serum chemistry differences induced by DEX-*E. coli* challenge were increased GGT in birds handled 2× (Table 7).

Peripheral Blood Leukocyte Counts

DEX 2. Handling resulted in significant differences in peripheral blood leukocyte numbers mainly in birds treated with DEX but not challenged with *E. coli*; therefore, Table 8 displays only these data. Birds handled 1×/d and treated twice with DEX, regardless of *E. coli* challenge, had significantly higher total WBC counts, numbers of

TABLE 2. The effect of handling poults one time (1×) or two times (2×) daily on average feed conversion of 7-wk-old turkeys that were challenged by air sac inoculation with 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk and again at 5 wk of age (DEX 2)[1]

	No *E. coli*		*E. coli*		Handled
	No DEX	DEX	No DEX	DEX	MEM[2]
Not Handled	1.67 ± 0.001	2.84 ± 0.210	1.68 ± 0.205[a]	2.67 ± 0.024[a]	2.21[b]
Handled 1×	1.77 ± 0.157	3.08 ± 0.211	2.47 ± 0.131[b]	3.65 ± 0.356[b]	2.74[a]
Handled 2×	1.81 ± 0.126	2.84 ± 0.331	2.27 ± 0.295[ab]	3.29 ± 0.225[ab]	2.55[a]
MEM					
No *E. coli* 2.33[b]	No DEX 1.94[b]				
E. coli 2.67[a]	DEX 3.06[a]				
Probability values					
Handled	0.0146				
DEX	0.0001				
E. coli	0.0199				
Handled × DEX	0.7633				
Handled × *E. coli*	0.0878				
DEX × *E. coli*	0.6686				
Handled × DEX × *E. coli*	0.9551				

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

[1]Values indicate the mean ± SE of all mortalities and 20 necropsied birds per treatment.

[2]MEM = Main effect means.

TABLE 3. The effect of handling poults one time (1×) or two times (2×) daily on body weights of 12-wk-old turkeys that were challenged by air sac inoculation with 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3)[1]

	No *E. coli*		*E. coli*		Handled
	No DEX	DEX	No DEX	DEX	MEM[2]
Not Handled	7,669 ± 292	3,503 ± 217	8,757 ± 291	3,101 ± 89	4,182[b]
Handled 1×	8,097 ± 397	3,056 ± 139	7,974 ± 548	3,605 ± 121	5,161[a]
Handled 2×	8,345 ± 518	3,585 ± 119	8,009 ± 617	3,008 ± 96	4,845[a]
MEM					
No *E. coli* 5,328	No DEX 8,156[a]				
E. coli 4,178	DEX 3,257[b]				
Probability values					
Handled	0.0001				
DEX	0.0001				
E. coli	0.5202				
Handled × DEX	0.6146				
Handled × *E. coli*	0.0653				
DEX × *E. coli*	0.2409				
Handled × DEX × *E. coli*	0.0146				

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

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]MEM = Main effect means.

heterophils, monocytes, and basophils, and H/L compared with unhandled birds (Table 8). There were no differences in differential WBC percentages affected by handling. The DEX treatment increased the numbers of WBC, heterophils, monocytes, and basophils only in birds handled 1×. The H/L ratio was increased by DEX only in birds handled 1× or 2× daily (Table 8).

DEX 3. Handling 2×/d increased total WBC counts and heterophil, basophil, and eosinophil numbers of DEX-treated birds (Table 8). Percentages of heterophils and monocytes were increased by both handling treatments, and percentages of lymphocytes were decreased by both handling treatments in DEX-treated birds (data not shown). The H/L ratio of DEX-treated birds was increased in the birds handled 2× daily relative to unhandled and 1× handled birds.

Treatment with DEX resulted in increases in WBC counts, heterophil, and monocyte numbers in all birds, regardless of handling treatment and basophil and eosinophil numbers were increased in birds handled 2× (Table 8). H/L ratios were increased by DEX treatment only in birds handled 1× or 2× daily (Table 8).

DISCUSSION

There have been contradictory reports regarding the effects of handling of neonatal animals. Handling has been shown to increase, decrease, or to have no effect on

TABLE 4. The effect of handling poults one time (1×) or two times (2×) daily on average feed conversion through Week 12 of turkeys that were challenged by air sac inoculation with 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3)[1]

	No *E. coli*		*E. coli*		Handled
	No DEX	DEX	No DEX	DEX	MEM[2]
Not Handled	1.93 ± 0.452	3.58 ± 0.303	2.06 ± 0.050	3.33 ± 0.131	2.72
Handled 1×	1.93 ± 0.420	4.02 ± 0.228	2.50 ± 0.086	3.07 ± 0.225	2.88
Handled 2×	2.02 ± 0.525	3.77 ± 0.017	2.29 ± 0.341	3.31 ± 0.037	2.85
MEM					
No *E. coli* 2.88	No DEX 2.12[b]				
E. coli 2.76	DEX 3.51[a]				
Probability values					
Handled	0.7238				
DEX	0.0001				
E. coli	0.5072				
Handled × DEX	0.9464				
Handled × *E. coli*	0.9405				
DEX × *E. coli*	0.0218				
Handled × DEX × *E. coli*	0.3838				

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

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]MEM = Main effect means.

TABLE 5. The effect of handling poults one time (1×) or two times (2×) daily for the first 10 d after hatch on relative organ weights of turkeys 3 wk after the second dexamethasone treatment (DEX 2) and 2 wk after the third DEX treatment (DEX 3)[1]

	Liver		Heart		Spleen		Bursa		Bursa/spleen ratio	
Treatment	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*
DEX 2										
Handling										
0	2.03 ± 0.06^{y}	3.46 ± 0.19^{b,x}	0.47 ± 0.04^{y}	0.75 ± 0.07^{x}	0.11 ± 0.01	0.14 ± 0.01	0.11 ± 0.01^{x}	0.08 ± 0.01^{y}	101.32 ± 4.25^{x}	66.35 ± 4.69^{a,y}
1×	2.10 ± 0.07^{y}	4.10 ± 0.19^{a,x}	0.43 ± 0.02^{y}	0.87 ± 0.05^{x}	0.11 ± 0.01^{y}	0.16 ± 0.01^{x}	0.10 ± 0.01^{x}	0.07 ± 0.00^{y}	95.95 ± 8.03^{x}	53.83 ± 4.22^{b,y}
2×	2.23 ± 0.10^{y}	3.60 ± 0.20^{ab,x}	0.48 ± 0.03^{y}	0.75 ± 0.06^{x}	0.13 ± 0.01	0.14 ± 0.01	0.11 ± 0.01^{x}	0.07 ± 0.01^{y}	88.16 ± 10.74^{x}	54.78 ± 3.43^{ab,y}
DEX 3										
Handling										
0	1.95 ± 0.10^{y}	4.08 ± 0.11^{b,x}	0.36 ± 0.01^{y}	0.68 ± 0.03^{b,x}	0.09 ± 0.01	0.13 ± 0.01^{a}	0.06 ± 0.00^{x}	0.04 ± 0.00^{y}	73.71 ± 10.4^{x}	34.30 ± 3.20^{y}
1×	1.86 ± 0.14^{y}	3.99 ± 0.22^{ab,x}	0.44 ± 0.04^{y}	0.71 ± 0.02^{ab,x}	0.09 ± 0.01	0.13 ± 0.01^{a}	0.06 ± 0.00^{x}	0.04 ± 0.00^{y}	69.74 ± 6.69^{x}	32.45 ± 3.77^{y}
2×	1.76 ± 0.08^{y}	4.23 ± 0.15^{a,x}	0.37 ± 0.01^{y}	0.78 ± 0.03^{a,x}	0.10 ± 0.01	0.10 ± 0.01^{b}	0.07 ± 0.00^{x}	0.03 ± 0.00^{y}	72.60 ± 6.02^{x}	30.91 ± 2.41^{y}

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

[x,y]For each tissue, means within rows with no common superscript differ significantly ($P \leq 0.05$).

[1]Data include only birds challenged with DEX and *Escherichia coli* and untreated controls.

TABLE 6. The effect of handling poults one time (1×) or two times (2×) daily for the first 10 d after hatch on serum levels of calcium, iron, glucose, total protein, blood urea nitrogen (BUN) and uric acid (UA) of turkeys bled 3 wk after the second dexamethasone treatment (DEX 2) and 2 wk after the third DEX treatment (DEX 3)[1]

	Calcium (mg/dL)		Iron (μg/dL)		Glucose (mg/dL)		Protein (g/dL)		BUN (mg/dL)		UA (mg/dL)	
Treatment	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*
DEX 2												
Handling												
0	8.3 ± 1.0^{b}	9.3 ± 0.6^{b}	136 ± 12^{x}	87 ± 11^{b,y}	226 ± 32	264 ± 6^{a}	2.8 ± 0.3	3.2 ± 0.2^{b}	1.3 ± 0.2	1.6 ± 0.2^{b}	6.2 ± 0.9	6.8 ± 0.7^{b}
1×	8.9 ± 0.2^{ab,y}	11.1 ± 0.7^{a,x}	139 ± 12	163 ± 33^{a}	257 ± 7^{x}	178 ± 32^{b,y}	3.3 ± 0.1^{y}	4.9 ± 0.6^{a,x}	1.2 ± 0.1^{y}	2.8 ± 0.8^{a,x}	5.7 ± 0.6^{y}	9.3 ± 1.2^{a,x}
2×	10.0 ± 0.2^{a}	10.1 ± 0.6^{ab}	137 ± 16	119 ± 16^{ab}	255 ± 16	262 ± 14^{a}	3.0 ± 0.4	3.6 ± 0.4^{b}	1.2 ± 0.1	1.8 ± 0.2^{b}	6.0 ± 0.5	7.9 ± 0.5^{ab}
DEX 3												
Handling												
0	10.6 ± 0.3^{y}	13.1 ± 0.5^{a,x}	230 ± 30	183 ± 50	257 ± 8	252 ± 19	3.2 ± 0.2^{y}	5.6 ± 0.2^{a,x}	1.0 ± 0.1	2.0 ± 0.4	7.0 ± 0.8^{b,y}	11.5 ± 1.0^{a,x}
1×	10.6 ± 0.4	10.4 ± 0.8^{b}	209 ± 39^{x}	128 ± 34^{y}	265 ± 8	221 ± 21	3.5 ± 0.2	4.0 ± 0.3^{b}	1.2 ± 0.1	2.1 ± 0.3	8.9 ± 0.8^{ab}	9.3 ± 0.7^{b}
2×	9.9 ± 0.2	11.4 ± 0.9^{ab}	162 ± 22	139 ± 114	271 ± 7	243 ± 42	3.1 ± 0.1^{y}	4.6 ± 0.2^{ab,x}	1.4 ± 0.2	2.5 ± 1.1	10.0 ± 0.9^{a}	9.0 ± 1.4^{ab}

[ab]Means within columns with no common superscript differ significantly ($P \leq 0.05$).

[x,y]For each parameter, means within rows with no common superscript differ significantly ($P \leq 0.05$).

[1]Data include only birds challenged with DEX and *Escherichia coli* and untreated controls.

TABLE 7. The effect of handling poults one time (1×) or two times (2×) daily for the first 10 d after hatch on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and γ-glutamyltransferase (GGT) in the blood of turkeys 3 wk after the second dexamethasone treatment (DEX 2) and 2 wk after the third dexamethasone treatment (DEX 3)[1]

	AST (U/L)		ALT (U/L)		LDH (U/L)		GGT (U/L)	
Treatment	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*	No DEX No *E. coli*	DEX *E. coli*
DEX 2								
Handling								
0	214.8 ± 17[b]	238.5 ± 14[b]	7.75 ± 1.1	7.68 ± 1.0[ab]	487.9 ± 54	478.4 ± 24[b]	1.67 ± 0.3	1.14 ± 0.3[b]
1×	258.4 ± 11[ab,y]	345.3 ± 32[a,x]	6.20 ± 0.4[y]	8.81 ± 1.6[a,x]	436.2 ± 21[y]	684.8 ± 145[a,x]	1.29 ± 0.3[y]	3.40 ± 2.0[a,x]
2×	270.8 ± 10[a]	270.7 ± 13[b]	6.04 ± 0.5	5.91 ± 0.6[b]	489.5 ± 15	531.6 ± 62[ab]	1.50 ± 0.27	1.14 ± 0.3[b]
DEX 3								
Handling								
0	294.8 ± 39	337.9 ± 83	9.64 ± 1.2	8.75 ± 1.0	575.0 ± 80[ab]	520.2 ± 130	1.88 ± 0.5	3.88 ± 0.8
1×	372.2 ± 51	326.9 ± 52	8.84 ± 0.9	7.93 ± 1.0	514.8 ± 33[b]	642.9 ± 96	1.75 ± 0.3	3.75 ± 0.7
2×	354.6 ± 25	518.8 ± 117	10.13 ± 0.8	10.15 ± 0.6	798.0 ± 86[a]	674.5 ± 373	2.57 ± 0.5[y]	7.50 ± 4.5[x]

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

[x,y]For each enzyme, means within rows with no common superscript differ significantly ($P \leq 0.05$).

[1]Data include only birds challenged with DEX and *Escherichia coli* and untreated controls.

production values of the adult animal. These data present all three of these outcomes, depending on the number of handlings, the number of DEX injections, and the age of the animals. Although the results were not clear cut, it is apparent that handling during the first 10 d after hatch does have a lasting effect on the growth and physiology of birds subsequently treated with DEX or challenged with *E. coli*, influencing not only production values but also WBC numbers and serum biochemical profiles.

In this study, handling had very little effect on the control animals. The only variables that were affected in birds not challenged with DEX or *E. coli* were calcium and AST, which were increased in the birds handled 2× at 7 wk, and UA, which was decreased in the birds handled 2× at 12 wk. Far more effect was observed for birds challenged with DEX and *E. coli*. In these birds, nearly all tested parameters were affected. A particularly strong pattern was that shown in birds handled 1× and given two DEX treatments. These birds had decreased BW and increased feed conversion at 7 wk; increased liver relative weights and decreased bursa/spleen ratio; and increased serum calcium, iron, protein, BUN, UA, AST, LDH, GGT and decreased glucose relative to unhandled controls also challenged with DEX-*E. coli*. Their total WBC counts and numbers of heterophils, monocytes, and basophils were increased, as were their H/L ratios.

These changes may reflect a greater susceptibility to disease, as these same birds also had higher mortality incidence and higher air sacculitis scores (Huff et al., 2001). This negative effect of handling 1× daily of 7-wk-old birds, but not in those handled 2× daily, suggests that birds handled twice daily might have become conditioned to the handling experience. This result may also be explained by assuming that the DEX challenges selected for

TABLE 8. The effect of handling poults one time (1×) or two times (2×) daily for the first 10 d after hatch on total leukocyte counts (WBC × 10^3) and numbers (×10^3) of heterophils (HET), lymphocytes (LYM), monocytes (MONO), and basophils (BASO) in the blood of turkeys 3 wk after the second dexamethasone treatment (DEX 2) and 2 wk after the third dexamethasone treatment (DEX 3)[1]

	WBC		HET		LYM		MONO		BASO		EOS		H/L Ratio	
	No DEX	DEX	No DEX	DEX	No DEX	DEX	No DEX	DEX	No DEX	DEX	No DEX	DEX	No DEX	DEX
	(×10^3/μl)													
DEX 2														
Handling														
0	20.1	21.7[b]	11.1	14.7[b]	6.5	4.2	2.5	3.5[b]	0.06	0.08[b]	0.02	0.01	2.70	4.23[b]
1×	20.9[y]	40.3[a,x]	12.0[y]	28.0[a,x]	5.9	4.3	2.9[y]	7.5[a,x]	0.10[y]	0.29[a,x]	0.01	0.03	3.62[y]	8.46[a,x]
2×	20.6	31.3[ab]	11.9[y]	21.6[ab,x]	6.3	4.9	2.6	5.1[ab]	0.08	0.12[b]	0.02	0.02	2.61[y]	6.15[ab,x]
DEX 3														
Handling														
0	27.6[y]	59.2[b,x]	17.5[y]	41.1[b,x]	8.0	9.0	2.3[y]	9.0[x]	0.15	0.3[b]	0.01	0.01[b]	2.52	5.29[b]
1×	29.2[y]	73.8[ab,x]	18.2[y]	51.5[ab,x]	7.9	10.1	2.9[y]	11.9[x]	0.19	0.26[b]	0.02	0.02[ab]	2.76[y]	7.69[b,x]
2×	28.9[y]	80.4[a,x]	15.6[y]	58.9[a,x]	10.6	8.4	2.5[y]	12.7[x]	0.19[y]	0.43[a,x]	0.02[y]	0.04[a,x]	2.02[y]	16.07[a,x]

[a,b]For each DEX treatment, means within columns with no common superscript differ significantly ($P \leq 0.05$).

[x,y]For each white cell type, means within rows with no common superscript differ significantly ($P \leq 0.05$).

[1]Data for birds treated with DEX only and for untreated controls.

a different, more resistant population in each successive DEX treatment. The birds that died after the first DEX treatment might have been more sensitive to the immunosuppressive effects of glucocorticoids, leaving survivors better adapted to endure high levels of glucocorticoid. Although not significant, a higher percentage of birds died in the group handled 2× daily after the first DEX challenge (34 vs. 26%) (Huff et al., 2001). The positive effects of handling on mean BW, which occurred after three DEX treatments, were within a population of birds that had already survived two previous treatments. The birds that were challenged with DEX and or *E. coli* contributed little to this effect, with the main difference being in the untreated control birds. The handled control birds also had numerically higher BW than the unhandled control birds at 7 wk.

It has been suggested that handling could have potential benefits for poultry welfare and performance because it reduces a chick's fear of humans, allowing more physiological resources to be used for responding to environmental stressors or for growth (Jones, 1993). The young turkey, however, may be deleteriously affected by such treatment. Fluck et al. (1997) reported basic differences in the neurochemical responses to handling between rats and chickens. Handled rats were reported to be less anxious in a maze and to have increased cortical benzodiazepine and $GABA_A$ receptors and increased $GABA_A$ uptake. In contrast, handled chicks had decreased forebrain $GABA_A$ receptors, decreased $GABA_A$ release, and no change in benzodiazepine binding. These data suggest that care must be taken in such an approach when considering turkey poults, because it appears that early handling can have deleterious effects on production and disease resistance when turkeys are stressed at a young age. The positive effects of handling occurred later, in populations already selected for resistance to the DEX treatment.

Because commercial turkeys are generally submitted to a number of successive and unpredictable "stresses" during a typical growout, handling, or other stimulation of the newly hatched poult may be damaging to a segment of the population. These "stresses" may include production practices that can be perceived as frightening, such as catching, vaccination, transportation, litter tilling, repairs and maintenance during brooding, or running a tractor outside of a turkey house. On the other hand, early stimulation appears to be beneficial for some of the poults, because after 12 wk, the surviving population of DEX-treated, handled birds had higher BW than did unhandled birds. This effect may be important when considering the higher economic loss of losing birds at the end of production.

These data suggest that there is divergence in the effect that early handling can have on later exposure to stress and infection. We have previously shown divergence in response to DEX injection. Male turkeys are significantly more sensitive to DEX treatment than are females and also have a much higher field incidence of opportunistic infection and turkey osteomyelitis complex (Huff et al., 1999a). Coturnix quail that are selected for fast early growth are more sensitive to DEX treatment than random-bred controls (unpublished data). Such divergence suggests the potential to select populations of turkeys better able to withstand the stresses of commercial production.

In summary, this research suggests there may be genetic divergence within the turkey population affecting the response to stress and its effects on production variables and physiology. Handling or stimulation of young poults may be detrimental to some individuals but may increase survivability of others. It is hoped that this research will draw attention to the effects of the stressors imposed on young poults because they may have an effect that persists throughout the life of the bird.

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