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Short communication

Effect of dexamethasone on bacteriostatic activity of turkey monocytes and implications for food safety☆

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ABSTRACT

Stress has been shown to affect the immune system of turkeys making them more susceptible to bacterial infections. Five-week-old male and female turkeys were treated with 3 intra-muscular injections of dexamethasone (Dex) at 0, 0.5 and 2.0 mg/kg body weight. Twenty-four hours after the third injection birds were bled and white blood cell (WBC) differentials and bacteriostatic activity of monocytes were measured. Dex at both 0.5 and 2.0 mg/kg decreased phagocytic activity in females only. Bacteriostatic activity was decreased at both concentrations of Dex at 8 and 16 h post-infection in both sexes and was lower in males as compared to females. Total WBC counts were increased in females at both concentrations of Dex whereas male total WBC counts were unaffected. Both males and females had an increase in the heterophil to lymphocyte ratio. Within the same study, replicate pens of turkeys were challenged with intra-air sac inoculation of 100 cfu of *Escherichia coli*. Isolation of *E. coli* was significantly increased by both Dex and *E. coli* challenge, but there were no differences between sexes. These results suggest that stress can compromise the bacteriostatic activity of turkey monocytes and increase bacterial colonization of blood and tissues, potentially affecting food safety.

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1. Introduction

Opportunistic bacteria are emerging pathogens that can affect the safety of food animal products but which generally do not cause disease in healthy individuals, and are commensals in animals with little or no effect on health or production (Taube, 2002). Common stressors present in farm animal production systems have been shown to affect both the colonization and virulence of such opportunistic bacteria, thus potentially affecting food safety, however the mechanisms are poorly understood (Rostagno, 2009).

Females in general have been shown to be more resistant to bacterial infection than males (Homo-Delarche et al., 1991) and this protection appears to be related to the stress response (Mason et al., 1990; Mason, 1991; Yamamoto and Friedman, 1996). In recent human studies, males were shown to have increased risk of severe sepsis as compared to females (Mayr et al., 2014) and females were shown to have a better response to stress due to the effects of estrogen (Wei et al., 2014).

We have established an experimental model for the reproduction of two important production diseases of turkeys that appear to be affected by both sex and environmental stressors: turkey osteomyelitis complex (TOC, Huff et al., 1998, 2000) and clostridial dermatitis of turkeys (CD, Huff et al., 2013, 2014). Both of these diseases are more commonly a problem in adolescent male turkeys as compared to females (Nairn, 1973; Clark et al., 1991, 2010; Bayyari et al., 1994; Carr et al., 1996; Mutalib et al., 1996). In this experimental model turkeys are immunosuppressed with the synthetic steroid, dexamethasone (Dex), followed by respiratory challenge with low levels of *Escherichia coli* to simulate the effects of extreme stress which can occur during turkey production (Huff et al., 1998, 2000). Avian pathogenic *E. coli* (APEC), which are ubiquitous in poultry litter, are transmitted through the respiratory system, and can result in the systemic infection called colibacillosis. The objective of the following study was to determine the effects of severe stress, as modeled using Dex treatment, on phagocytosis and bacteriostatic activity of adherent monocytes from peripheral blood of both male and female turkeys and to determine if monocyte bacteriostatic activity is related to the sexual dichotomy reported in these turkey diseases.

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2. Materials and methods

2.1. Animals

Male ($N = 240$) and female ($N = 240$) commercial Nicholas turkeys from the same hatch were transported from a local hatchery (Cobb-Vantress, Siloam Springs, AR) to the University of Arkansas farm at day-of-age. They were weighed by pen and wing-banded and each sex was randomly assigned to one of 24 floor pens with duplicate pens for each treatment in a 2 (Sex) × 3 (Dex-treatment) × 2 (bacterial challenge) experimental design. They were provided ad libitum access to water and a standard turkey starter diet that met or exceeded NRC nutrient requirements (NRC, 1994) and were brooded on pine shavings under standard production temperatures with a 23 h light/1 h dark photoperiod. All experimental conditions and animal protocols were approved by the University of Arkansas Institutional Animal Care and Use Committee.

2.2. Dexamethasone/E. coli challenge

At 5 weeks of age birds were treated with three injections of dexamethasone (Dex, Sigma–Aldrich, St. Louis, MO) into the thigh muscle at either 2 mg/kg BW (high dose), 0.5 mg/kg BW (low dose) or 0 mg/kg BW (Control). Dexamethasone was diluted in normal sterile saline and injections were given on alternating days (Monday, Wednesday, Friday) as previously described (Huff et al., 1998). On the day of the third Dex injection, half of the pens from each treatment were challenged with an air sac injection containing approximately100 cfu of a non-motile, lactose negative strain of *E. coli* serotype 02 in tryptose phosphate broth (TPB) while the other half were inoculated with sterile TPB (Huff et al., 1998).

2.3. Hematology and bacterial isolation

Twenty-four hours after the third Dex injection and challenge, four birds from each pen were bled (8 birds/treatment) and blood was placed into EDTA-treated glass tubes. Each tube was sampled for bacterial culture using a sterile transport swab (Bacti-Swab® (Remel, Inc., Lenexa, KS) and they were taken to the laboratory where they were plated on MacConkey agar for 24 h at 37 °C. Representative lactose-negative colonies were identified using API 20-E test kits (Bio-Mérieux Vitek Inc., Hazelwood, MO) and were compared with the challenge strain. Blood samples having isolation of lactose-negative colonies with the same API 20-E profile as the challenge strain were considered positive.

Total leukocyte counts and differentials were determined for each bird using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL). Heterophil/lymphocyte ratios, which are a standard measure of the stress response of birds (Gross and Siegel, 1983), were determined.

2.4. Monocyte bacteriostatic assay

An additional 5 ml of whole blood from each bird in the pens not challenged with *E. coli* was also sampled, and was layered onto Histopaque 1077 (Sigma–Aldrich, St. Louis, MO) and centrifuged at 250 × *g* for 45 min. Cells at the phase interface were transferred to cold siliconized glass tubes and washed 2 times with cold Medium 199 with Earles' salts and L-glutamine (Sigma–Aldrich, St. Louis, MO). Cells were then pooled by pen, counted, pelleted and diluted in 3 ml to approximately 1.5×10^7 cells/ml in Medium 199 and then 200 μl was transferred to triplicate wells on each of 4 Nunc Lab-Tec 16-well glass chamber slides (Thermo Scientific, Waltham, MA). Cells were allowed to attach for 1 h at 37 °C and were washed four times with warm Gey's balanced salt solution (GBSS) (Sigma–Aldrich, St. Louis, MO). Monolayers were grown for 5 days in 200 μl of Medium 199 with 25% turkey serum after which media was removed and cells were challenged with 100 μl of TPB containing $2–5 \times 10^7$ cfu of the same serotype O_2 strain of *E. coli* used in the air sac challenge of turkeys.

Bacteriostatic activity was determined using a modification of methods reported by Schaffner (1985). Briefly, after 1 h to allow for phagocytosis, cells were washed four times in GBSS and then incubated for 8, 16, or 24 h in Medium 199 with 25% turkey serum and 200 μg/ml Gentamycin (Sigma–Aldrich, St. Louis, MO), which is used to prevent continuous infection by extracellular bacteria. At 0, 8, 16, and 24 h medium was flicked off of the wells, and slides were dried and stained with Diff-Quik stain kit (Dade Behring Inc., Newark, NJ). The percentage of cells with intracellular bacteria was counted from 200 cells in triplicate for each pen. Discrimination was noted for cells with 1–10 bacteria/cell and for cells with more than 10 bacteria/cell. These numbers were then combined for analysis.

2.5. Bacterial isolation from tissues

At two weeks post challenge with *E. coli* all birds were necropsied. Sterile transport swabs were used to culture the air sac and liver of all mortalities and necropsied birds and the challenge strain of *E. coli* was isolated as described above for blood samples.

2.6. Data analysis

Differences between pen means were analyzed using the General Linear Models and Least Square Means procedures of SAS as either a 2 (Sex) × 3 (Dex) factorial for blood parameters or as a 2 (Sex) × 3 (Dex) × 2 (*E. coli*) factorial for tissue bacterial isolation data. (SAS Institute Inc., 2008). A *P*-value of less than 0.05 was considered significant unless otherwise stated.

3. Results and discussion

Treatment of turkeys with 2 mg of Dex/kg BW significantly decreased phagocytosis of *E. coli* (percent cells infected at Time = 0; $P \leq 0.05$) by turkey adherent monocytes (Fig. 1a) and this decrease was due only to a decrease in the female response (Fig. 1b). The percentage of infected cells of combined males and females was significantly increased by both levels of Dex at 8, 16, and 24 h PI (Fig. 1a; $P \leq 0.05$). This indicates a decrease in bacteriostatic activity relative to the control, and suggests bacterial growth within these cells, since they contained many more bacteria than were initially phagocytosed (data not shown). The percentage of infected cells peaked at 8 h PI. At 24 h PI untreated females had significantly less infected cells than did males, indicating that monocytes from female turkeys may have intrinsically higher bacteriostatic activity than males (Fig. 2a; $P \leq 0.05$). There were no differences between males and females given the low dose of Dex (Fig. 2b), however males treated with the high dosage had a significantly higher percentage of infected cells at 8 and 16 h PI (Fig. 2c; $P \leq 0.05$). This suggests that the male response to the high level of Dex was more likely to decrease bacteriostatic activity of adherent monocytes than was the female response.

Dexamethasone treatment increased the main effect mean (MEM) number and percentage of monocytes ($P = 0.01$) and heterophils ($P \leq 0.0004$) and decreased the MEM number and percentage of lymphocytes ($P = 0.003$ and $P = 0.0001$, respectively), thus increasing the H/L ratio ($P = 0.02$) (Table 1). The H/L ratio was increased in males given the low dosage of Dex and in females given the high dosage of Dex, indicating differences between the male and female response to stress (Fig. 3b). The female response to the high level of Dex resulted in higher WBC and heterophil counts