Influence of Stressors on Normal Intestinal Microbiota, Intestinal Morphology, and Susceptibility to Salmonella Enteritidis Colonization in Broilers

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ABSTRACT In modern poultry production systems, environmental stressors may influence bird performance and susceptibility to pathogens such as Salmonella Enteritidis. Experiments were conducted to determine the influence of 24-h feed withdrawal and 24-h exposure to high temperature (30°C) on intestinal characteristics of broilers. Attachment of Salmonella Enteritidis to ileal tissue was determined using an in vitro ileal loop assay. Changes in commensal intestinal microbial populations were determined using denaturing gradient gel electrophoresis, and alterations in ileal morphology were determined histologically. Ex vivo attachment of Salmonella Enteritidis to ileal tissues increased by 1.5 logs (9.05 log_{10} vs. 7.59 log_{10} Salmonella Enteritidis/g of ileal tissue; \( P = 0.0006 \)) in broilers fasted for 24 h. Similarly, ileal tissues from birds subjected to 30°C for 24 h had increased ex vivo attachment of Salmonella Enteritidis (8.77 log_{10} vs. 8.50 log_{10} Salmonella Enteritidis/g of ileum; \( P = 0.01 \)) compared with birds held at 23°C. Exposure to 30°C for 24 h also altered microbial community structure in the ileum and cecum. Subjecting birds to 30°C for 24 h reduced crypt depth (6.0 vs. 7.8 μm, respectively; \( P = 0.002 \)), but had no effect on villus height or villus:crypt ratio. This research shows that acute stressors in poultry production systems can cause changes in the normal intestinal microbiota and epithelial structure, which may lead to increased attachment of Salmonella Enteritidis.

INTRODUCTION

Salmonella is the leading cause of bacterial foodborne infection in the United States and causes approximately 1.2 million cases of human Salmonellosis each year (Centers for Disease Control and Prevention, 2007). The most commonly implicated source of foodborne Salmonellosis is consumption and handling of undercooked poultry products (Smith et al., 1984; Bryan and Doyle, 1995; Antunes et al., 2003). The correlation between poultry and foodborne illness has focused efforts toward identification of factors contributing to Salmonella infection in poultry, with the goal of decreasing carcass contamination and reducing animal morbidity and mortality. Environmental stress has been shown to be a factor that may induce colonization of food animals by enteric pathogens, facilitate horizontal transmission of pathogens between animals, increase pathogen shedding, and contribute to carcass contamination during processing (Rigby and Pettit, 1980; Mulder, 1995; Isaacson et al., 1999; Poppe, 1999; Jones et al., 2001).

Stress is an important consideration in poultry production systems, because birds are routinely subjected to stressors such as feed withdrawal, temperature fluctuations, and confinement during transportation (Abeyesinghe et al., 2001; St-Pierre et al., 2003; Humphrey, 2006). Broilers are subjected to fasting to reduce the volume of intestinal contents before slaughter, and thus minimize the risk of carcass contamination by rupture of the intestinal tract during processing (May and Deaton, 1989). However, feed withdrawal has been associated with increased Salmonella colonization of the crop (Humphrey et al., 1993; Ramirez et al., 1997) and intestine (Bierer and Eleazer, 1965; Ramirez et al., 1997). Exposure to extreme temperature is an additional stressor encountered in seasonal environments, particularly during the summer months, and is also associated with increased intestinal colonization and fecal shedding of pathogens in poultry (Bailey, 1988). Stress can have a profound effect on overall physiology, animal health, and productivity. The gastrointestinal tract is particularly responsive to stressors, which can cause a variety of changes including alteration of the normal, protective microbiota (Tannock and Savage, 1974; Bailey and Coe, 1999, 2004) and decreased integrity of the intestinal epithelium (Saunders et al., 1994; Meddings and Swan, 2000; Soderholm et al., 2002). The commensal intestinal bacterial popula-
tions can protect the host from pathogen colonization by competing for epithelial binding sites and nutrients, strengthening the intestinal immune response, and by producing antimicrobial bacteriocins (Guaner and Malagelada, 2003; Sansonetti, 2004; MacDonald and Monteleone, 2005). In addition, the intestinal epithelium serves as a barrier between luminal pathogens and the underlying blood supply (Sansonetti, 2004). Therefore, stress-induced perturbation of the normal intestinal microbiota or the integrity of the gut epithelium reduces innate protective mechanisms and may increase the potential for pathogens such as Salmonella to bind to and colonize the intestinal epithelium. Such colonization in poultry will increase the risk of carcass contamination during processing, and will increase the potential for Salmonella to translocate to the reproductive tract, where it can contaminate eggs during formation.

The objective of the following study was to determine the influence of feed withdrawal and heat stress on intestinal microbial populations, small intestinal morphology, and intestinal susceptibility to Salmonella Enteritidis colonization in broilers. It was expected that stressors alone would induce changes in microbial community structure of the intestine and decrease intestinal integrity, and would increase intestinal susceptibility to Salmonella Enteritidis attachment.

MATERIALS AND METHODS

Birds

For feed withdrawal and heat stress experiments, birds were obtained from a larger study (Thompson and Applegate, 2006) in which 960 male Ross 308 broilers were allocated to 24 floor pens with 40 birds/pen. Birds were raised to 42 or 44 d of age on a standard corn-soybean meal diet that met or exceeded National Research Council (1994) nutrient requirements and had ad libitum access to feed and water. Initial ambient temperature was held at 35°C for newly hatched chicks, and was gradually decreased to 23°C by 21 d and held at 23°C for the duration of the 6-wk experiment. Animal procedures were approved for these studies by the Purdue Animal Care and Use Committee.

Stress Conditions

Feed Withdrawal Study. At d 42, 10 birds were randomly chosen, killed by carbon dioxide asphyxiation, and sampled. Feed was withdrawn (0 h) from the remaining flock, and birds were kept on litter and given access to water for 4 h before being placed in transport crates for 20 h. After 24 h of feed withdrawal, 10 additional birds were killed by CO₂ asphyxiation and tissues were sampled.

Heat Stress Study. At d 44, 20 birds were randomly chosen and 10 were immediately sacrificed and sampled, whereas the remaining 10 were subjected to 30°C ambient temperature in floor pens for 24 h, with full access to feed and water, before euthanasia with CO₂ overdose and sampling.

Intestinal Sampling

Feed Withdrawal Study. A 10-cm section of the ileum was taken, starting 3 cm proximal to the ileo-cecal junction, from each bird for an ex vivo ileal challenge assay and was gently flushed with 0.05 M PBS (pH 7.2). Tissue sections were immediately placed in ice-cold Dulbecco’s Modified Eagle Medium + l-glutamine (DMEM, Mediatech, Herndon, VA) and kept on ice until used for an in vitro Salmonella challenge assay as described below.

Heat Stress Study. Intestinal tissue and contents were obtained immediately after euthanasia, starting 3 cm proximal to the ileo-cecal junction and were collected as follows: 1) a 10-cm section of the ileum was taken for an in vitro Salmonella challenge assay as described below; 2) a 4-cm ileal section, 13 cm from the ileo-cecal junction, was collected for denaturing gradient gel electrophoresis (DGGE) analysis of microbial community structure. Ileal tissues were opened and contents were gently removed, placed in microfuge tubes, and immediately frozen at −20°C. Ileal tissues were gently flushed with 0.05 M PBS (pH 7.2), and were frozen at −20°C; 3) a 2-cm section of ileal tissue, 17 cm from the ileo-cecal junction, was collected for analysis of intestinal morphology, flushed with PBS and fixed in 10% neutral buffered formalin for 48 h; and 4) a 4-cm tissue section of the center of the cecum and cecal contents were obtained for DGGE analysis and processed as described above.

Ileal Loop Assay for Attachment of Salmonella Enteritidis

Challenge Microorganism. Salmonella enterica serovar Enteritidis (NVSL 7759) was a gift from Tom Stable (National Animal Disease Center, Ames, IA) and was transfected with a kanamycin-resistance plasmid to allow selection in the presence of kanamycin (courtesy of Bruce Applegate, Purdue University, Lafayette, IN). The stock culture was grown in Luria Bertani (LB) broth containing 50 µg of kanamycin/mL (LB-kan) and stored with 20% (vol/vol) added glycerol. Fresh cultures were grown statically overnight in LB-kan broth, transferred to fresh LB-kan broth, and grown overnight for the challenge study. Bacterial cells were harvested by centrifugation at 6,000 × g at 4°C for 15 min, and were washed 3 times in equal volumes of sterile PBS. Cells were resuspended in DMEM to an optical density (OD)₆₀₀ of 0.4 (approximately 1 × 10⁶ cells/mL) as determined by a prior standard curve. The inoculum was serially diluted and plated on LB broth to obtain the actual number of cells in the inoculum.
Ileal Loop Assay. The organ culture procedure of Naughton et al. (2001) was used. Briefly, the ileal sections were removed from DMEM, sealed at one end with 35-mm dialysis clamps, and inoculated with approximately 6 mL of Salmonella Enteritidis culture suspended in DMEM. The open end of the ileal section was sealed with dialysis clamps, the exterior was rinsed with PBS, and the ileal loops were incubated in 100 mL of DMEM for 1 h at 37°C in a rotary water bath in a 10% CO₂ atmosphere. After incubation, ileal contents were removed, the interior and exterior of each section was rinsed with PBS, tissues were homogenized (T-25 basic homogenizer, Ultra-Turrax, IKA-Werke, Wilmington, NC), serially diluted in buffered peptone broth, and plated on LB agar plates containing 50 µg/mL kanamycin. Plates were incubated at 37°C for 24 h and were enumerated for Salmonella Enteritidis. Differences due to feed withdrawal (0 vs. 24 h) or heat stress were determined and analyzed using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC).

Intestinal Morphology

After fixation in 10% neutral buffered formalin, a single 0.5-cm sample was cut from each ileal section, dehydrated with increasing concentrations (70, 80, 95, and 100%) of ethanol, cleared with xylene (Sub-X, Surgipath Medical Industries, Richmond, IL), and placed into polyethylen embedding wax (Polysciences, War- rington, PA). Tissue sections (5 µm) were cut, floated onto slides, stained with hematoxylin (Gill #2, Sigma, St. Louis, MO) and eosin (Sigma), and measured for villus height and crypt depth using light microscopy and a micrometer. Measurements for villi length were taken from the tip of the villus to the valley between individual villi and measurements for crypt depth were taken from the valley between individual villi to the basolateral membrane. Eight villi and villus-associated crypts were measured for each sample. Morphology data were analyzed using the GLM procedure in SAS.

DGGE

Genomic DNA was isolated from intestinal digesta and tissue samples using the Ultraclean Fecal DNA kit (MoBio, Solana Beach, CA), according to the manufacturer's protocol. Samples were diluted 1:1 with sterile distilled water, and 0.25 g of the diluted sample was added to a bead beating tube containing beads, bead solution, and lysis solution. Cells were lysed by a combination of detergent and mechanical action using a MoBio Vortex Adapter (MoBio) on a standard vortex. From the lysed cells, the released DNA was bound to a silica spin filter. The filter was washed and DNA was eluted using DNase-free Tris buffer.

The DGGE was performed according to previously described methods with modification (Muyzer et al., 1993; Muyzer and Smalla, 1998), using bacteria-specific PCR primers to conserved regions flanking the variable V3 region of 16S rDNA. Each PCR reaction mixture contained 0.02 nmol of reverse primer (534r): 5'-ATT ACC GCG GCT GCT GG-3' and 0.02 nmol of forward primer with a GC clamp (341FGC): 5'CGC CCG CGC CGG GCG GGG GCG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3' (Inte grated DNA Technologies, Skokie, IL). 3.75 units of Taq DNA Polymerase (Promega, Madison, WI), 5 to 10 ng of template DNA, 10× DNA Polymerase Buffer (containing 10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton X-100), and 25 mM MgCl₂. Amplifications were performed using a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA) using the following program: 1) denaturation at 94°C for 3 min; 2) subsequent denaturation at 94°C for 1 min; 3) annealing at 65°C for 1 min, −0.5°C per cycle; 4) extension at 72°C for 1 min; 5) steps 2 to 4 repeated for 30 cycles; 6) denaturation at 94°C for 1 min; 7) annealing at 55°C for 1 min; 8) extension at 72°C for 1 min; steps 6 to 8 repeated for 7 cycles; 9) extension at 72°C for 7 min; 10) 4.0°C final holding temperature.

Polyacrylamide gels (8% acrylamide-bisacrylamide ratio 37:5:1) were cast with a 40-60% urea:deionized formamide gradient. The 100% denaturing acrylamide contained 7 M urea and 40% deionized formamide. Amplified DNA was mixed with a 20% volume of 5× loading buffer [0.025% (wt/vol) bromophenol blue, 0.025% (wt/vol) xylene cyanol, 47% (vol/vol) 0.1 M EDTA and 47% (vol/vol) glycerol], and 20 µL was loaded into each sample well (20-well comb). Gels were placed in a DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA) and electrophoresed in 0.5× Tris-acetate-EDTA buffer at 60 for 10 min at 200 V, followed by 16 h at 70 V. Gels were silver stained as described by Sambrook et al. (1989), using the following procedure: fixation solution for 3 min (77% ethanol, 4% glacial acetic acid, 16% distilled water), silver stain for 10 min (0.2% AgNO₃, 99.8% fixation solution), developer for 15 to 20 min (0.001% NaBH₄, 25% 1.5 M NaOH, 75% formaldehyde), and preservation solution for 7 min (27% etha nol, 12% glycerol, 61% distilled water).

Fragment pattern relatedness was determined using Bionumerics Software (version 2.5; Applied Maths, Austin, TX), which determined the number of bands per sample and similarity coefficients for banding patterns between pairs of samples. A distance matrix was calculated using the DICE function and dendrograms were constructed from this matrix using the unweight ed pair group means average (UPGMA) function. The degree of similarity of banding patterns between pairs of samples was represented as a similarity coefficient. All DGGE data were analyzed using the Mixed Model of SAS (SAS Inc., Cary, NC). Similarity coefficients between pairs of samples were segregated by treatment and similarity coefficients across treatments were used as an estimate of similarity assuming no treatment effect. Tukey-Kramer means separation was used to identify treatment differences. Significance was determined using P-value < 0.05.
RESULTS

Effect of 24-h Feed Withdrawal on Ileal Susceptibility to Salmonella Enteritidis Attachment

Intestinal tissues from fasted birds were more susceptible to pathogen attachment than tissues from control birds, with a 1.5 log increase ($P = 0.01$) in Salmonella Enteritidis associated with the ileal tissue of fasted birds compared with nonfasted controls (Figure 1).

Influence of an Acute High Temperature on Intestinal Susceptibility to Salmonella Enteritidis Adhesion, Commensal Microbial Populations, and Morphology of the Small Intestine of Broilers

Broilers placed in the 30°C room showed behavioral signs of heat stress such as panting and spreading of wings. In the ileal loop assay, numbers of Salmonella Enteritidis associated with the ileum were greater ($P = 0.0006$) in heat-stressed birds (8.77 log$_{10}$ cfu/g) compared with nonstressed birds (8.50 log$_{10}$ cfu/g of ileum; Figure 1). Amplicon profiles for bacteria in the intestinal contents and tissues revealed differences in banding patterns between heat-stressed and nonstressed birds. Birds held at 30°C exhibited lower ($P = 0.0001$) similarity coefficients for microbial communities in ileal contents (Table 1) than did birds at 23°C. In all intestinal samples (Table 1), the similarity coefficients for microbial communities in ileal contents (Table 1) than did birds at 23°C. In all

<table>
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<th>Item</th>
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<td>44.5$^b$</td>
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$^a$–$^c$Means within rows with different superscript letters are significantly different ($P < 0.05$).

$^2$Results represent the mean similarity coefficients for 10 birds/treatment.

DISCUSSION

Salmonella Enteritidis and Salmonella Typhimurium are widely recognized as causative agents in poultry-related foodborne illness outbreaks. Since 1996, the incidence of Salmonella Typhimurium-induced Salmonellosis has declined in the United States, but the rate of Salmonella Enteritidis foodborne infection has remained steady (Centers for Disease Control and Prevention, 2006). Therefore, identification of on-farm risk factors for Salmonella colonization in poultry is necessary to develop strategies for reducing Salmonella load in animals at time of slaughter and processing. Because stressors such as feed withdrawal and high environmental temperature have been linked to increased incidence of enteric disease in poultry and other food-producing animals, our study aimed to identify ways in which these stressors affect normal intestinal conditions, such as the protective commensal microbiota and the integrity of the intestinal epithelium (Abeyesinghe et al., 2001; St-Pierre et al., 2003; Humphrey, 2006). We also examined how these stressors influenced the binding of Salmonella Enteritidis to intestinal tissue.

Adhesion of Salmonella Enteritidis to Intestinal Tissue Following Stress Exposure

The ileal loop assays demonstrated that stress due to 24-h feed withdrawal and exposure to high temperatures is associated with increased susceptibility of intestinal tissues to Salmonella Enteritidis colonization. Intestinal tissues from fasted birds had significantly greater attachment of Salmonella than did tissues from control birds (Figure 1). These results are in agreement with others who have observed increases in enteric pathogen colonization and shedding in birds subjected to feed withdrawal (Bierer and Eleazer, 1965; Humphrey et al., 1993; Ramirez et al., 1997). Less is known about the effect of heat stress on pathogen colonization of the intestinal mucosa. In the study reported here, numbers of Salmonella Enteritidis associated with the ileum were greater in heat-stressed birds (8.77 log$_{10}$ cfu/g) compared with nonstressed birds (8.50 log$_{10}$ cfu/g
ileum; Figure 2), indicating that stress may contribute to increased intestinal colonization by Salmonella. This is supported by previous findings that heat stress may have damaging effects on mucosal structure (Sengupta and Sharma, 1993). In addition, heat shock proteins, whose expression can be induced by high ambient temperature and other environmental stressors (Lindquist, 1986; Lindquist and Craig, 1988), may act as epithelial surface receptors for pathogen binding (Dziewanowska et al., 2000; Wampler et al., 2004).

Changes in Intestinal Microbial Populations Influenced by Stressors

The normal gut microbiota is a complex ecosystem that can benefit the host by serving as a barrier to pathogen colonization (Van der Waaij, 1989). Alteration of this protective barrier may leave the host more susceptible to colonization by enteric pathogens (Durant et al., 1999). There is a linear reduction in the mucus lining the intestinal tract over a 24-h fast, as well as changes in intestinal morphology (Thompson and Applegate, 2006). Hinton et al. (2000) showed that there were increases in intestinal Enterobacteriaceae and cecal aerobes with a concurrent decrease in lacto acid bacteria in broilers subjected to a 24-h feed withdrawal. Neurohormones associated with stress can increase growth and virulence factor expression in microbes including Escherichia coli, Yersinia enterocolitica, and Pseudomonas aeruginosa in vitro (Lyte and Ernst, 1992; Lyte et al., 1997b; Rahman et al., 2000). Release of norepinephrine in the intestinal tract increased the number of gram-negative bacteria within the lumen (Lyte and Bailey, 1997a). Most studies to date used traditional microbiological culturing techniques to examine the influence of stressors on a few specific bacterial populations. In our study, we used DGGE to evaluate stress-induced changes in the overall intestinal microbial profile, rather than focusing on a few specific microorganisms. One possible limitation of analyzing similarity coefficients within treatments is that each treatment analysis is separate; thus, one may end up with numerically comparable similarity coefficients, but banding patterns within treatment may differ, as the data from ileal tissue (Table 1 and Figure 3b) demonstrate. The dendrogram shows that banding patterns were highly similar within each treatment, with similarity coefficients of 73.7 and 72.5 for birds at 23 and 30°C, respectively. However, the banding patterns are obviously different between treatments. Thus, we used across-treatment pairwise comparisons (cross-product) to determine differences between treatments when similarity coefficients were not significantly different. In essence, the cross-products represent the similarity coefficient of the population as a whole. If treatment similarity coefficients are significantly different than the cross-product, even if they are numerically similar to each other, then the microbial community structure between treatments is different.

The DGGE data show changes in the commensal intestinal bacterial populations of birds subjected to heat-stress, as indicated by changes in similarity coefficients in intestinal tissue and contents and the decreased number of bands in the ileal mucosa of birds subjected to 24-h heat stress (Tables 1 and 2, Figure 3). Bacterial communities in the ileal contents from birds at 30°C had lower similarity coefficients than in the ileal contents from birds at 23°C. The decrease in similarity of bacterial communities in heat-stressed birds does not necessarily mean that there is an increase in the number of bacterial species (diversity) within individual birds, but that there is increased variation in the composition of intestinal bacteria between birds. In contrast, bacterial communities in the cecal tissue of birds at 30°C had greater similarity values than those

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<td>Villuscrypt</td>
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<td>6.9</td>
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Table 2. Influence of heat stress on number of bands present in various intestinal ecosystems of broilers as determined by denaturing gradient gel electrophoresis analysis.

Table 3. Influence of heat stress on small intestinal morphological characteristics, including villus height, crypt depth, and the villus:crypt ratio.

![Figure 2. Influence of heat stress on ileal susceptibility to Salmonella Enteritidis attachment; n = 10 birds/time point. Error bars represent SEM.](https://academic.oup.com/ps/article-abstract/87/9/1734/1548479)
in birds at 23°C, suggesting that the bacterial communities between birds were more similar. Figure 3a shows a slightly more complex dendrogram. It is difficult with complex dendrograms to visually determine treatment differences; thus, the use of the cross-product helps differentiate treatment differences. In all intestinal samples, the similarity coefficients across treatments were lower than the similarity coefficients within individual treatments, indicating that the intestinal microbial community structure was significantly changed when birds were exposed to high temperatures for 24 h. The ileal tissue from birds held at 30°C contained fewer amplicon fragments than the ileal tissue from birds at 23°C (Table 2), indicating that exposing birds to high temperatures for 24 h caused a reduction in microbial species associated with the ileal wall. These data show that greater changes occur in the ileum than the cecum when birds are subjected to elevated temperatures for 24 h, indicating that microbial populations in the ileum may be more sensitive to short-term changes than those in the cecum.

**Influence of Stress on Intestinal Morphology**

Maintenance of normal morphology and structural integrity of the small intestine are imperative for preventing bacterial translocation from the intestinal tract. Extensive research has been conducted on the influence of stress due to fasting or weaning on small intestinal morphology. These nutritional stressors have deleterious effects on the absorptive epithelium of the intestine, resulting in reduction in villus height and crypt depth (Mayhew, 1987; Yamauchi et al., 1995; Yamauchi et al., 1996). Less is known about the effect of high environmental temperature on intestinal morphology. Birds subjected to 30°C for 24 h had reduced crypt depth compared with birds at 23°C (Table 3). Villus height and the villus: crypt ratio were unchanged in birds exposed to 30°C. There are several possible reasons why ileal villus height was unchanged in response to elevated temperature, including the short duration of the stressor and the resistance of the ileum to structural change compared with other regions of the small intestine (Yamauchi et al., 1995). It is likely that changes in cell proliferation would be observed first in the stem cells of the crypt rather than the villus because of the high proliferative activity of the crypt (Yamauchi et al., 1995). Morphological changes in response to fasting occur more rapidly in the proximal two-thirds of the small intestine than in the ileum. In feed withdrawal studies with chickens, the structure of the duodenum and jejunum mucosa changes rapidly, often within 36 h of the onset of stress, whereas the ileum maintains its normal morphology longer and requires extended periods of stress to influence its structure (Yamauchi et al., 1995, 1996).

Data indicate that acute (24-h) stressors can invoke significant changes in the normal intestinal microbiota, intestinal morphology, and in vitro susceptibility.

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**Figure 3.** Dendrogram representing similarity of microbial banding patterns in ileal contents (a) and ileal tissue (b) of heat stressed (30°C) and control (23°C) birds. Relative similarity of band patterns is indicated by their grouping on the dendrogram. Each line on the dendrogram represents an individual bird. The percentage similarity coefficient is indicated on the graduated bar at the top of each panel.
for *Salmonella* Enteritidis attachment to the ileum in broilers. The findings from the 24-h heat stress study suggest that stressors could act in several ways to increase intestinal susceptibility to in vitro *Salmonella* Enteritidis attachment. By disrupting the normal protective microbiota and altering intestinal morphology, stressors create an opportunity for pathogens to colonize the intestine. Exposure to stressors may also cause subtle changes in epithelial mucus production and mucus composition, which may occur before gross anatomical changes in the intestinal morphology. Alterations in the mucus layer could change the attachment capabilities of both commensal and pathogenic microorganisms (Deplancke and Gaskins, 2001), which could account for changes in DGGE community profiles and for changes in intestinal susceptibility to *Salmonella* Enteritidis attachment. These findings are important for the poultry industry, where broilers are routinely fasted for gastrointestinal emptying before transportation, and where birds are often exposed to high environmental temperatures during the summer months.

Future work should focus on the use of prophylactic measures to prevent or reduce the stress-induced alteration of the intestinal microbiota and changes in gut integrity. Probiotic organisms such as lactobacilli, bifidobacteria, some strains of *E. coli*, and yeast offer promise for reducing pathogen colonization when fed orally (Line et al., 1997; Patterson and Burkholder, 2003; Wehkamp et al., 2004; Parvez et al., 2006). When administered consistently, probiotic organisms can colonize and form a niche in the intestine and may be a useful dietary treatment if administered before feed withdrawal or transportation and processing. In addition, nondigestible oligosaccharides, or prebiotics, have been shown to enhance intestinal growth of probiotic or beneficial commensal organisms (Bouhnik et al., 2004; Macfarlane et al., 2008). The fermentation of prebiotic complex carbohydrates by intestinal microbes produces volatile fatty acids, which promote epithelial cell proliferation and renewal (Macfarlane et al., 2008). Such activity may enhance integrity of the intestinal epithelium. Therefore, dietary supplementation of the flock with prebiotics before periods of anticipated stress may also limit the damage to the intestinal epithelium elicited by stressors. In conclusion, understanding the mechanism by which stress alters normal intestinal characteristics and induces susceptibility to enteric infection is an important first step in designing on-farm strategies aimed at reducing pathogen contamination in poultry.

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