

The feathering gene is linked to degranulation and oxidative burst not cytokine/chemokine mRNA expression levels or *Salmonella enteritidis* organ invasion in broilers

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In the past, we showed differences in heterophil function between parental broilers (A [fast feathering] > B [slow feathering]) and their F1 reciprocal crosses (D [fast feathering] > C [slow feathering]). In the present study, we evaluated the linkage of the feathering gene to heterophil function, pro-inflammatory cytokine/chemokine mRNA expression levels, and resistance to *Salmonella enteritidis* organ invasion. Heterophils were isolated from 2-day-old chickens (C and D) separated into males and females—slow males and females (SM and SF), and fast males and females (FM and FF). Heterophil functions of degranulation and oxidative burst were measured. Heterophils from FF chickens (183 ± 8.9) released more ($P < 0.05$) β -d-glucuronidase (μ M) than heterophils from SF chickens (149 ± 3.7); FF heterophils (4.6×10^4) generated a significantly ($P < 0.05$) greater oxidative burst (mean relative fluorescent units) compared with SF heterophils (4.2×10^4). Interleukin-6, CXCLi2, and interferon- α mRNA expression levels were quantitated by real-time quantitative reverse transcriptase-polymerase chain reaction. No differences were observed between SM and FM or between SF and FF heterophils. Finally, 1-day-old chickens were administered *S. enteritidis* and liver/spleen organ invasion was quantitated. No differences were observed between the number of *S. enteritidis*-positive FF and SF chickens, but FM were significantly ($P < 0.05$) more resistant to *S. enteritidis* organ invasion than SM chickens. The data indicate degranulation and oxidative burst were linked with the feathering gene; however, interleukin-6, CXCLi2, and interferon- α mRNA expression levels were not. Furthermore, susceptibility to *in vitro* *S. enteritidis* organ invasion was not linked to the feathering gene.

Introduction

The poultry industry suffers major economic losses from both mortality due to infectious diseases and potential contamination of food products due to transfer of microorganisms. Our laboratories have been evaluating the innate immune system of pedigree broilers in order to assess their capacity to protect against pathogen invasion, including *Salmonella enteritidis*. We have extensively characterized the innate immune response of two parental broiler lines—A (fast feathering) and B (slow feathering). To date, we have shown that an increased *in vitro* heterophil function (Swaggerty *et al.*, 2003a,b) corresponds with an increased *in vivo* resistance to organ invasion by Gram-positive (Swaggerty *et al.*, 2005b) and Gram-negative bacterial infections (Ferro *et al.*, 2004; Swaggerty *et al.*, 2005a). In addition, we have shown increased mRNA expression levels of pro-inflammatory cytokines in heterophils isolated from the more resistant lines compared with the susceptible lines (Swaggerty *et al.*, 2004). Straight-run, not separated by sex, F1 reciprocal crosses (C = B sire $\times$ A dam; D = A sire $\times$ B dam) have a differential heterophil response (D [fast feathering] > C [slow feathering]) (Swaggerty *et al.*,

2003b) and cytokine profile (Ferro *et al.*, 2004). Collectively, our data indicate there are clear and measurable differences in heterophil function and innate response that are under specific genetic control.

The feathering gene is located on the short arm of the Z sex chromosome and codes for feather growth. The fast feather allele, *k*, is the wild-type recessive trait responsible for rapid feather growth, whereas the slow feather allele, *K*, is dominant and responsible for slow feather growth. Our previous results suggest the presence of the fast feathering gene (*k*) may enhance heterophil functional efficiency while the slow feathering gene (*K*) may reduce the functional efficiency of isolated heterophils.

Based on *in vitro* heterophil functional differences, we decided to evaluate chickens from the F1 reciprocal crosses separated by sex to determine whether *in vitro* heterophil function is linked to or merely associated with the feathering gene. The objective of this study was to determine whether there was linkage between the feathering gene and the *in vitro* heterophil functions of degranulation and oxidative burst, cytokine/chemokine

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mRNA expression levels in heterophils, or *in vivo* organ invasion by *S. enteritidis*. With respect to the feathering gene, the F1 reciprocal cross males are the same in both lines (slow males [SM] and fast males [FM] = K/k) whereas the females are different (slow females [SF] = $K/-$; fast females [FF] = $k/-$). Therefore, if *in vitro* heterophil functional efficiency was associated with the fast feathering gene, we would expect heterophils from FF to be functionally more efficient than heterophils from SF, while the functional efficiency of heterophils from SM and FM would be the same.

Materials and Methods

Chickens. F1 reciprocal cross chickens (F1 cross C = B sire $\times$ A dam; F1 cross D = A sire $\times$ B dam) were hatched at a commercial hatchery and separated into males and females. F1 cross D chickens were feather sexed, while F1 cross C chickens were vent sexed. The male and female progeny from slow feathering cross C were referred to as slow male (SM) and slow female (SF), while the fast feathering cross D males and females were FM and FF, respectively. With respect to the feathering gene, the F1 reciprocal cross males are the same in both lines (SM and FM = K/k) whereas the females are different (SF = $K/-$; FF = $k/-$). Chickens were transported to our facility on the day of hatch in standard chick boxes containing hydration gel packs in an enclosed, temperature-controlled vehicle. Upon arrival chickens were placed in 2m $\times$ 2m floor pens containing wood shavings, provided supplemental heat, water, and un-medicated corn and soybean meal-based chick starter diet *ad libitum*. The feed met or exceeded standards established by the National Research Council (1994).

Bacteria. A poultry isolate of *S. enteritidis* (#97-11771) was obtained from the National Veterinary Services Laboratory (Ames, Iowa, USA) and approved by the United States Department of Agriculture (USDA). *S. enteritidis* was cultured in tryptic soy broth (Difco Laboratories, Becton Dickinson Co., Sparks, Maryland, USA) overnight at 41°C. Stock *S. enteritidis* (1×10^9 colony forming units [CFU]/ml) were prepared as previously described (Swaggerty *et al.*, 2003b). *S. enteritidis* was prepared in RPMI or phosphate-buffered saline for *in vitro* and *in vivo* assays, respectively. *S. enteritidis* were opsonized with normal chicken serum (NCS-OpSE) as previously described (Kogut *et al.*, 2001).

Heterophil isolation. Heterophils were isolated from peripheral blood of 150 chickens per group, 2 days post-hatch. Blood was collected in vacutainer tubes containing disodium ethylenediamine tetraacetic acid (BD vacutainer, Franklin Lakes, New Jersey, USA) and mixed thoroughly. Following blood collection, heterophils were isolated as previously described (Swaggerty *et al.*, 2003b). Heterophil preparations were consistently 95% pure and >95% viable. All tissue culture reagents and chemicals were obtained from Sigma Chemical Company (St Louis, Missouri, USA), unless otherwise noted.

Phagocytosis. Heterophils were assayed for the ability to phagocytize live *S. enteritidis* as previously described (Kogut *et al.*, 2001). Briefly, heterophils (2 ml of 5×10^6 cells/ml) and *S. enteritidis* (2 ml of 5×10^7 CFU/ml) were added to diluted heterophils (10 *S. enteritidis*:1 heterophil) and centrifuged at 2000 $\times g$ for 15 min. The pelleted heterophils and *S. enteritidis* were incubated 1 h at 39°C and then submerged in ice for 15 min. The samples were washed with equal volume of ice-cold RPMI and centrifuged at 2000 $\times g$ for 15 min. The pellets were resuspended in ice-cold gentamicin (100 μg /ml in RPMI) and incubated for 1 h at 37°C on a rotary shaker. Heterophils were washed three times with ice-cold RPMI (3 to 5 ml per wash and centrifuged at 2000 $\times g$ for 15 min), and the pellet was re-suspended in 2 ml RPMI. Cytospin smears were prepared from each sample in a Shandon Cytospin 3 (Shandon Inc., Pittsburgh, Pennsylvania, USA), stained with Hematology 3-step stain (Biochemical Sciences, Inc., Swedesboro, New Jersey, USA), and examined by light microscopy with an oil immersion objective (100 $\times$).

Degradation. The release of intracellular granules was monitored by quantifying the amount (μM) of β -d-glucuronidase in the supernatant of heterophils as previously described (Kogut *et al.*, 2001). Heterophils (8×10^6 cells/ml) were treated with OpSE or RPMI for 1 h at 39°C on a rotary shaker. The samples were immersed in ice for 10 min. Cells were removed by centrifugation (250 $\times g$ for 15 min at 4°C), the supernatants collected then assayed for β -d-glucuronidase activity. Briefly, samples and standards (25 μl) were added to a black flat-bottom enzyme-linked immunosorbent assay plate and incubated with 50 μl freshly prepared substrate (10 mM 4-methylumbelliferyl- β -d-glucuronide dissolved in 0.1 M sodium acetate buffer containing 0.1% Triton X-100; pH 4.0) for 4 h at 41°C. The reaction was stopped by the addition of 200 μl stop solution (0.05 M glycine, 5 mM ethylenediamine tetraacetic acid; pH 10.4). Released 4-methylumbelliferone was measured on a GENios Plus Fluorescence Microplate Reader (TECAN US Inc, Research Triangle Park, North Carolina, USA) (excitation = 355 nm, emission = 460 nm) and converted using a standard curve of known concentrations.

Oxidative burst. Production of reactive oxygen species by heterophils during oxidative burst was measured by oxidation of DCFH-DA to fluorescent DCF as previously described (He *et al.*, 2003). Briefly, heterophils (1 ml, 8×10^6 cells/ml) were incubated with 5% chicken serum, DCFH-DA (10 μg /ml), and phorbol A-myristate 13-acetate (1 μg /ml) for 1 h at 37°C and 5% CO₂. Heterophils (150 μl) were then dispensed to a black 96-well plate and the fluorescence intensity measured using a GENios Plus Fluorescence Microplate Reader at 485 nm excitation and 530 nm emission wavelengths (manually set gain at 50). The mean relative fluorescent units (RFU) were recorded after 1 h.

RNA isolation. Heterophils (1×10^7) were treated with 300 μl RPMI or *S. enteritidis*, for 30 min at 39°C on a rotary shaker. Treated heterophils were pelleted, washed with RPMI (485 $\times g$ for 15 min at 4°C), the supernatant discarded, the cells re-suspended in lysis buffer (Qiagen RNeasy mini RNA extraction kit; Qiagen Inc., Valencia, California, USA), and frozen. Total RNA was extracted from homogenized (QIAshredder columns) lysates according to the manufacturer's instructions.

Quantitative real-time reverse transcriptase-polymerase chain reaction. Cytokine/chemokine mRNA expression levels in control and agonist-treated heterophils were quantitated using a method described by Kaiser and colleagues (Kaiser *et al.*, 2000). Primers and probes have been described previously (Kaiser *et al.*, 2000; Kogut *et al.*, 2003; Swaggerty *et al.*, 2004). Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the Reverse Transcriptase qPCR Master Mix RT-PCR kit (Eurogentec, San Diego, California, USA). Amplification and detection of products were performed using the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Warrington, UK). All samples were standardized using 28S RNA. Results were shown as control 40-cycle threshold (Ct), *S. enteritidis*-treated 40-Ct values, and the fold-change from control. Fold change is calculated using the following formula $2^{(S. enteritidis \text{ corrected mean} - \text{control corrected mean})}$.

Organ invasion. Thirty 2-day-old chickens from each group were challenged orally with 5×10^6 CFU *S. enteritidis* (0.5 ml, 1×10^7 CFU/ml stock). Twenty-four hours post-challenge, chickens were euthanized and necropsied. At necropsy, livers and spleens were aseptically removed and incubated overnight at 41°C in tetrathionate enrichment broth. Following enrichment, 10 μl were streaked onto brilliant green agar plates with carbenicillin and novobiocin, incubated 24 h at 41°C, then the plates examined for non-lactose fermenting carbenicillin and novobiocin-resistant *Salmonella* colonies. Representative colonies were confirmed *S. enteritidis*-positive by plate agglutination using specific Group D₁ antisera (Difco Laboratories). Non-challenged chickens from each group were included as negative controls. Three experimental trials were conducted and the data pooled for presentation and statistical analysis.

Statistical analyses. Anti-coagulated blood from 150 chickens per group was pooled and heterophils isolated for the *in vitro* assays. Each blood

collection and heterophil isolation was conducted on three separate days; therefore, heterophils were pooled from a total of 450 chickens. Data from 90 chickens per line (30 for three separate challenges) were used for *S. enteritidis* organ invasion. For both *in vitro* and *in vivo* studies, the mean and standard error of the mean were calculated from pooled data. Comparisons were made only between the females (SF and FF), and then a separate analysis for males (SM and FM). Statistical analyses (Student's *t* test) were performed using Microsoft® Excel 2000 ($P < 0.05$).

Results

Phagocytosis. Heterophils isolated from FF, FM, SF, and SM chickens were evaluated for the ability to phagocytize *S. enteritidis*. Specifically the percentage of heterophils with *S. enteritidis* and the average number of *S. enteritidis* per heterophil was determined. There were no differences between any of the groups with regard to percentage of heterophils that engulfed *S. enteritidis*, nor in the number of *S. enteritidis* per heterophil. The percentage of heterophils from males and females that engulfed *S. enteritidis* was all between 78.7 and 80.7%, and all groups phagocytized seven to nine *S. enteritidis* per heterophil (data not shown).

Degranulation. The ability of heterophils to release bactericidal granules (degranulation) following stimulation was also measured in heterophils isolated from FF, FM, SF, and SM chickens (Figure 1). Heterophils isolated from FF chickens released a significantly ($P < 0.05$) higher concentration (μM) of β -d-glucuronidase, the enzyme associated with heterophil-mediated degranulation, than heterophils from SF chickens (183 ± 8.9 and 149 ± 3.7 μM , respectively). Comparisons between heterophils from FM and SM showed no differences in degranulation (156 ± 5.1 and 146 ± 2.2 μM , respectively).

Oxidative burst. Oxidative burst was measured in heterophils from FF, SF, FM, and SM chickens following stimulation with phorbol A-myristate 13-acetate (Figure 2). As seen with degranulation, heterophils from FF chickens underwent a significantly greater ($P < 0.05$) oxidative burst response compared with

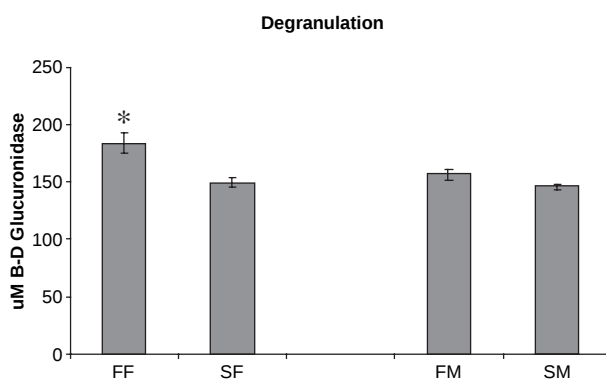


Figure 1. Degranulation of heterophils isolated from 2-day-old chickens was compared to determine their ability to release toxic granules when stimulated with opsonized *S. enteritidis*. Data are pooled and presented as the mean $\pm$ standard error of the mean of the μM β -d glucuronidase released from triplicate assays from three separate experiments ($n=450$ chickens). All statistical differences are for comparisons between females (FF vs SF) or between males (FM vs SM) ($*P < 0.05$).

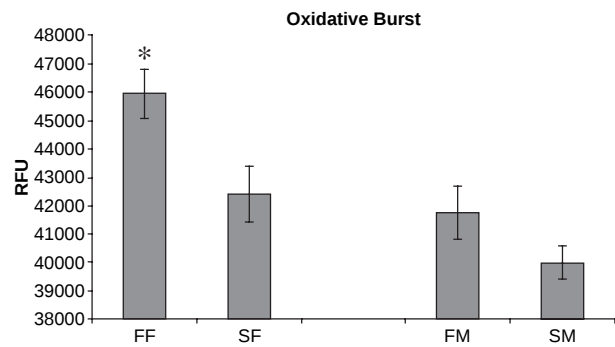


Figure 2. Heterophils isolated from FF, SF, FM, and SM 2-day-old chickens were compared to determine their ability to generate an oxidative burst response following stimulation with the inflammatory agonist phorbol A-myristate 13-acetate. Data are pooled and presented as the mean $\pm$ standard error of the mean of the RFU of triplicate assays from three separate experiments ($n=450$ chickens). All statistical differences are for comparisons between females (FF vs SF) or between males (FM vs SM) ($*P < 0.05$).

heterophils from SF chickens (4.6×10^4 and 4.2×10^4 RFU, respectively). Additionally, there were no differences in the oxidative burst response between heterophils isolated from the males (FM = 4.1×10^4 RFU; SM = 4.0×10^4 RFU).

Cytokine mRNA expression. We also evaluated heterophils for mRNA expression levels of the pro-inflammatory cytokine interleukin (IL)-6, the pro-inflammatory chemokine CXCLi2 (formerly IL-8), and interferon (IFN)- α (Table 1). There were no differences in the control or *S. enteritidis*-stimulated mRNA expression levels of IL-6, CXCLi2, or IFN- α between heterophils from the FF and SF chickens, therefore indicating there was no linkage with the feathering gene. Likewise, no differences were observed for IL-6 and CXCLi2 for FM and SM heterophils (control or *S. enteritidis*-stimulated). There was a statistical ($P < 0.05$) difference in the IFN- α mRNA expression levels observed between the control males (FM > SM) and a change in mRNA expression levels following *S. enteritidis*-stimulation (SM > FM).

Organ invasion. In addition to the *in vitro* heterophil functional studies, an *in vivo* *S. enteritidis* challenge was conducted to determine whether there were differences in extraintestinal organ invasion between the FF and SF chickens and the FM and SM chickens (Figure 3). The FF chickens had numerically ($P = 0.09$) fewer positive chickens compared with the *S. enteritidis*-infected SF (36.7% and 49.4%, respectively). However, there was a statistical ($P < 0.05$) difference between the males. There were 15.6% *S. enteritidis*-positive FM chickens, while 34.4% from the SM were positive for *S. enteritidis* organ invasion. All control chickens were negative for *S. enteritidis*. There was no linkage between the feathering gene and protection against *S. enteritidis* organ invasion (liver and spleen).

Discussion

Previous studies by our laboratory examined *in vitro* heterophil function and resistance/susceptibility to bac-

Table 1. Control, *S. enteritidis*-treated, and fold change of IL-6, CXCLi2, and IFN- α mRNA expression levels in heterophils isolated from F1 cross C and D chickens separated by sex^a

Line	IL-6			CXCLi2			IFN- α		
	Control (40-Ct)	<i>S. enteritidis</i> -treated (40-Ct)	Fold change ^b	Control (40-Ct)	<i>S. enteritidis</i> -treated (40-Ct)	Fold change	Control (40-Ct)	<i>S. enteritidis</i> -treated (40-Ct)	Fold change
FF	11.6 $\pm$ 0.6	11.9 $\pm$ 1.0	1.8 $\pm$ 0.8	16.7 $\pm$ 0.6	16.8 $\pm$ 0.7	1.2 $\pm$ 0.5	18.2 $\pm$ 0.03	13.5 $\pm$ 0.4	0.04 $\pm$ 0.01
SF	12.3 $\pm$ 1.4	12.5 $\pm$ 1.5	3.2 $\pm$ 2.7	18.7 $\pm$ 0.2	18.4 $\pm$ 0.2	0.8 $\pm$ 0.1	17.2 $\pm$ 1.8	14.7 $\pm$ 1.1	0.27 $\pm$ 0.1
FM	10.7 $\pm$ 0.5	10.5 $\pm$ 0.6	1.0 $\pm$ 0.4	16.3 $\pm$ 0.2	16.6 $\pm$ 0.4	1.3 $\pm$ 0.3	20.7 $\pm$ 0.1*	19.9 $\pm$ 1.7	1.75 $\pm$ 0.9
SM	11.3 $\pm$ 0.5	11.3 $\pm$ 1.1	1.8 $\pm$ 0.8	18.4 $\pm$ 0.2	18.0 $\pm$ 0.4	0.8 $\pm$ 0.2	16.0 $\pm$ 1.7	19.0 $\pm$ 0.7	9.83 $\pm$ 3.7*

^aComparisons were made only between the females (SF and FF), and then a separate analysis for males (SM and FM). Statistical analyses (Student's *t* test) were performed using Microsoft® Excel 2000 (**P* < 0.05).

^bFold change is calculated using the formula $2^{(S. \text{ enteritidis corrected mean} - \text{control corrected mean})}$.

terial challenge with *S. enteritidis* and *Enterococcus gallinarum* in parental broilers (Line A [fast feathering] and Line B [slow feathering]) and their F1 reciprocal crosses (C and D) (Swaggerty *et al.*, 2003b, 2005b; Ferro *et al.*, 2004). With respect to the feathering gene, the F1 reciprocal cross males are the same in both lines (SM and FM = *K/k*+) whereas the females are different (SF = *K/-*; FF = *k +/-*). The initial studies by our group, as well as all subsequent evaluations, have shown a dichotomy of innate immune responsiveness between fast and slow feathering lines at the parent level and F1 generations. In all immunologic evaluations of the lines of these chickens, fast-feathering chickens have a more effective heterophil-mediated innate immune response compared with their slow-feathering counterparts (Swaggerty *et al.*, 2003a,b, 2004, 2005a,b, 2006; Ferro *et al.*, 2004). The objective of the present study was to determine whether the feathering gene was actually linked to, not just associated with, increased *in vitro* heterophil function, the cytokine/chemokine mRNA expression levels produced by heterophils, or increased resistance to *in vivo* liver/spleen organ invasion by *S. enteritidis*.

To our knowledge and with the exception of our initial report (Swaggerty *et al.*, 2003a), there are no studies evaluating the role of the feathering gene in either innate immune responsiveness or heterophil function in chick-

ens. Therefore, our results showing that the bactericidal killing mechanisms of degranulation and oxidative burst were linked to the recessive *k* fast feathering gene were the first report to show direct linkage between the feathering gene and innate immunity in broilers. These data do not indicate that the feathering gene is the sole gene responsible for degranulation and oxidative burst as heterophils from both fast and slow feathering chickens have a response.

Slow feathering in poultry is a result of a qualitative Z-sex-linked dominant gene (*K*), while fast feathering is actually the wild-type trait associated with the recessive allele (*k*) (Moreng and Avens, 1985; Austic and Nesheim, 1990). Slow feathering is a mutation resulting from the insertion of endogenous retroviral DNA (ev21) into the genome, which then becomes inherited like any other host gene (Bacon *et al.*, 1988; Iraqi & Smith, 1995). In addition to the feathering alleles *K* and *k* mentioned in the present study, there are two other feathering alleles, *Kⁿ* and *K^s*, which are associated with varying degrees of feathering (Somes, Jr., 1969; Chambers *et al.*, 1994). Ultimately, *Kⁿ* is the dominant feathering allele and the most severe, but none of the chickens utilized in the present study fall into this genotype. Therefore, for the chickens we evaluated in this study, the slow-feathering *K* allele was dominant. There are numerous reports relating the feathering gene to nutrition and growth (Ajang *et al.*, 1993; Fotsa *et al.*, 2001; Hamoen *et al.*, 2001; Kalinowski *et al.*, 2003; Sikur *et al.*, 2004). However, most of the studies involving the immune function of fast-feathering and slow-feathering chickens are directed towards viral immunity or antibody production (Bacon *et al.*, 1986; Smith & Fadly, 1988; Fadly & Smith, 1997; Smith & Crittenden, 1998). Slow-feathering progeny from a slow-feathering dam displayed an "inadequate immune response" based on increased viral infections and decreased antibody production indicating a poor acquired immune response (Harris *et al.*, 1984). The equivalent slow-feathering progeny from slow-feathering dams in the present study were the SM and SF. Similarly, we observed a decreased immune function as demonstrated by a decreased bactericidal killing and increased susceptibility to *S. enteritidis* organ invasion. As we now know the acquired immune response is controlled by the innate immune response, therefore, a poor acquired response would be directly related to an initial poor innate response (Bendelac *et al.*, 1997; Janeway, Jr. & Medzhitov, 2002; Pasare & Medzhitov, 2005).

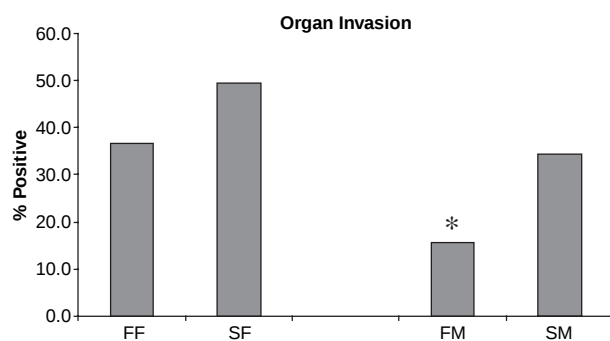


Figure 3. Two-day-old chickens were challenged orally with *S. enteritidis* (5×10^6 CFU/chicken) and liver/spleen organ invasion was determined using standard microbiological techniques. Data are pooled from 90 chickens and presented as the mean $\pm$ standard error of the mean of triplicate organ invasion experiments. Data shown are the percentages obtained from the number of *S. enteritidis*-positive chickens/90. All comparisons are between females (FF vs SF) or between males (FM vs SM) (**P* < 0.05).

The observation that phagocytosis and cytokine/chemokine mRNA expression levels were not under genetic control of the feathering gene was not surprising. These actions are very complex and are under the control of numerous genes and gene families and redundant pathways (Underhill and Ozinsky, 2002; Jutras and Desjardins, 2005; Stuart and Ezekowitz, 2005). In fact, IL-6 and CXCLi2 are encoded on chromosomes 2 and 4, respectively (Kaiser *et al.*, 2005); therefore, it is logical that there is no linkage between the feathering gene that is on the Z chromosome. Even though IFN- α is encoded on the Z sex chromosome, the data indicate its mRNA expression levels are not linked to the feathering gene, also present on the same chromosome. The results presented herein showed *S. enteritidis* liver/spleen organ invasion in young chickens was not under control of the feathering gene. Numerous studies indicate *S. enteritidis* organ invasion is controlled by multiple genes (Bumstead & Barrow, 1988; Mariani *et al.*, 2001; Lamont *et al.*, 2002; Wigley *et al.*, 2002; Wigley 2004; Beal *et al.*, 2005). Lamont and colleagues show there are at least four genes associated with *S. enteritidis* colonization of the spleen in 1-day-old chickens; however, the role of the feathering gene is not addressed in that study (Lamont *et al.*, 2002). Even though the feathering gene was not linked to *S. enteritidis* organ invasion in young broilers, our previous studies indicate there is an association between fast feathering and increased resistance to bacterial infections (Ferro *et al.*, 2004; Swaggerty *et al.*, 2005a,b). Additional studies are required to further characterize the innate immune responsiveness of the parent lines (A and B) and the F1 reciprocal crosses (C and D) including the role of the sire in conferring resistance/susceptibility to *S. enteritidis* organ invasion.

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Non-English Abstracts

The feathering gene is linked to degranulation and oxidative burst not cytokine/chemokine mRNA expression levels or *Salmonella enteritidis* organ invasion in broilers

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Dans le passé, nous avons démontré des différences dans la fonction hétérophile entre les parentales des poulets de chair (A [emplumement rapide] > B [emplumement lent]) et leurs croisements réciproques de leurs descendants à la génération F1 (D [emplumement rapide] > C [emplumement lent]). Dans la présente étude, nous avons évalué la relation entre le gène de l'emplumement et la fonction hétérophile, les niveaux d'expression de l'ARNm des cytokines/chémokines pro-inflammatoires et de la résistance à l'invasion des organes par *Salmonella enteritidis* (SE). Les hétérophiles ont été isolés chez les poussins C et D, mâles et femelles âgés de deux jours (mâles et femelles à emplumement lent [SM et SF] et mâles et femelles à emplumement rapide [FM et FF]). Les fonctions hétérophiles de dégranulation et d'explosion respiratoire ont été mesurées. Les hétérophiles des poussins FF ($183 \pm 8,9$) ont libéré plus de β -D-glucuronidase (μ M) que les hétérophiles des poulets SF ($149 \pm 3,7$), ($P < 0,05$); les hétérophiles des poulets FF ($4,6 \times 10^4$) ont généré significativement plus d'explosion respiratoire (moyenne RFU) comparés aux hétérophiles de poulets SF ($4,2 \times 10^4$), ($P < 0,05$). Les niveaux d'expression de l'ARNm de l'interleukine (IL)-6, du CXCLi2, et de l'interféron (IFN)- α ont été mesurés par RT PCR quantitative en temps réel. Aucune différence n'a été observée entre les hétérophiles des poulets SM et FM ou entre ceux des poulets SF et FF. Enfin, les poussins âgés de deux jours qui ont reçu SE, ont fait l'objet d'une quantification de l'invasion du foie et de la rate par la bactérie. Aucune différence n'a été observée entre le nombre de poulets FF et SF positifs vis-à-vis de SE mais les poussins FM ont été significativement plus résistants à l'invasion des organes par SE que les poulets SM ($P < 0,05$). Ces données indiquent que la dégranulation et l'explosion respiratoire sont liées au gène de l'emplumement; cependant les niveaux d'expression de l'ARNm de l'IL-6, du CXCLi2, et de l'IFN- α ne le sont pas. De plus, la sensibilité à l'invasion des organes *in vitro* n'est pas liée au gène de l'emplumement.

In vorherigen Untersuchungen konnten wir Unterschiede in der Heterophilenfunktion zwischen Mastel-terntieren (A [schnell befiedernd] > B [langsam befiedernd]) und ihren reziproken F1-Kreuzungsprodukten (D [schnell befiedernd] > C [langsam befiedernd]) darstellen. In der jetzigen Studie untersuchten wir die Verbindung des Befiederungsgens zur Heterophilenfunktion, zu den pro-inflammatorischen Zytokin/Chemokin-mRNS-Expressionsgehalten und zur Resistenz gegenüber der *Salmonella* Enteritidis (SE)-Organbesiedlung. Aus zwei Tage alten in männliche und weibliche (langsam befiedernde männliche und weibliche [SM und SF] und schnell befiedernde männliche und weibliche [FM und FF]) getrennte Hühnerküken (C und D) wurden Heterophile isoliert. Die Heterophilenfunktionen der Degranulierung und des oxidativen Ausbruchs wurden bei diesen Zellen bestimmt. Die Heterophilen der FF-Hühnerküken bildeten mehr ($p < 0,005$) β -D-Glucoronidase ($183 \pm 8,9 \mu$ M) als die Heterophilen der SF-Küken ($149 \pm 3,7$); die FF-Heterophilen ($4,6 \times 10^4$) erzeugten im Vergleich zu den SF-Heterophilen ($4,2 \times 10^4$) einen signifikant größeren ($p < 0,05$) oxidativen Ausbruch (mittlerer RFU). Die Interleukin (IL)-6-, CXCLi2- und Interferon (IFN)- α -mRNS-Expressionswerte wurden mittels quantitativer Real Time-RT-PCR ermittelt. Es wurden keine Differenzen zwischen den Heterophilen der SM- und FM- bzw. zwischen den SF- und den FF-Hühnerküken festgestellt. Schließlich wurden Eintagsküken mit SE inokuliert und die Leber/Milz-Organbesiedlung quantifiziert. Zwischen der Anzahl der SE-positiven FF- und SF-Küken bestand kein Unterschied, aber die FM-Küken waren gegenüber der SE-Organbesiedlung signifikant resistenter ($p < 0,05$) als die SM-Küken. Diese Daten weisen darauf hin, dass zwischen dem Befiederungsgen und der Degranulierung sowie dem oxidativen Ausbruch eine Verbindung besteht, während die IL-6, CXCLi2-

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und IFN- α -mRNS-Expression nicht mit diesem Gen verbunden sind. Außerdem konnte keine Verbindung zwischen dem Befiederungs-Gen und der Empfänglichkeit gegenüber einer SE-Organbesiedlung.

Anteriormente, mostramos las diferencias en las funciones heterofilicas entre pollos de engorde parentales (A [emplume rápido] > B [emplume lento]) y sus cruces recíprocos F1 (D [emplume rápido] > C [emplume lento]). En este estudio, evaluamos la relación del gen del emplume con la función heterofilica, los niveles de expresión de mRNA de citoquinas/quimioquinas proinflamatorias, y la resistencia a la invasión de órganos por *Salmonella enteritidis* (SE). Los heterófilos se aislaron de pollos de dos días de vida (C y D) separados en machos y hembras (machos y hembras lentos [SM y SF] y machos y hembras rápidos [FM y FF]). Se valoraron las funciones heterofilicas de degranulación y estallido oxidativo. Los heterófilos de pollos FF (183 ± 8.9) excretaron más ($P < 0.05$) β -D-glucuronidasa (μM) que los heterófilos de pollos SF (149 ± 3.7); los heterófilos FF (4.6×10^4) generaron un estallido oxidativo (media RFU) significativamente mayor ($P < 0.05$) que los heterófilos SF (4.2×10^4). Se cuantificaron los niveles de expresión de mRNA de interleuquina (IL)-6, CXCLi2, e interferón (IFN)- α mediante RT-PCR cuantitativa a tiempo real. No se observaron diferencias entre heterófilos SM y FM o entre heterófilos SF y FF. Finalmente, se administró SE a pollos de un día de vida y se cuantificó la invasión de hígado/bazo. No se observaron diferencias significativas entre el número de pollos FF y SF positivos a SE, pero los FM fueron significativamente más resistentes ($P < 0.05$) a la invasión de órganos por SE que los pollos SM. Estos resultados indican que la degranulación y el estallido oxidativo están ligados con el gen del emplume; sin embargo los niveles de expresión de mRNA de IL-6, CXCLi2, y IFN- α no lo están. Además, la susceptibilidad a la invasión de órganos *in vitro* de SE no estuvo ligada al gen del emplume.