Selection for pro-inflammatory mediators yields chickens with increased resistance against *Salmonella enterica* serovar Enteritidis

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ABSTRACT *Salmonella* is a leading cause of foodborne illness and can be transmitted through consumption of contaminated poultry; therefore, increasing a flock's natural resistance to *Salmonella* could improve food safety. Previously, we characterized the heterophil-mediated innate immune response of 2 parental broiler lines and F_1 reciprocal crosses and showed that increased heterophil function and expression of pro-inflammatory mediators corresponds with increased resistance against diverse pathogens. A preliminary selection trial showed that individual sires had varying inherent levels of pro-inflammatory mediators and selection based on a high or low phenotype was passed onto progeny. Based on these results, we hypothesized selection of broilers for higher levels of the pro-inflammatory mediators IL-6, CXCLi2, and CCLi2 would produce progeny with increased resistance against *Salmonella* Enteritidis. Peripheral blood leukocytes were isolated from 75 commercial broiler sires, screened, and 10 naturally high and low expressing sires were selected and mated to randomly selected dams to produce the first generation of "high" and "low" progeny. The mRNA expression of CXCLi2 and CCLi2 were significantly ($P \leq 0.02$) higher in the high progeny and were more resistant to liver and spleen organ invasion by *Salmonella* Enteritidis compared with low progeny. Production of the second generation yielded progeny that had differences ($P \leq 0.03$) in all 3 mediators and further improved resistance against *Salmonella* Enteritidis. Feed conversion ratio and percent breast meat yield were calculated and were equal, whereas the high birds weighed slightly, but significantly, less than the low birds. These data clearly demonstrate that selection based on a higher phenotype of key pro-inflammatory mediators is a novel means to produce broilers that are naturally more resistant to *Salmonella*, one of the most important foodborne pathogens affecting the poultry industry.

Key words: broiler, pro-inflammatory, resistance, *Salmonella*, selection

2014 Poultry Science 93:535–544
http://dx.doi.org/10.3382/ps.2013-03559

INTRODUCTION

As the poultry industry changes to meet global requirements, breeding based on live weight alone can no longer be the sole parameter that drives selection. At least 10 parameters are taken into consideration by current breeder companies: eggs, hatchability, weight, breast meat, meat quality, immune response, growth profile, feed conversion, heart and lung fitness, and skeletal integrity (Laughlin, 2007).

Our laboratory has been evaluating the innate immune system of poultry to determine the possibility of implementing a breeding strategy based on an efficient innate immune response that would produce chickens with improved resistance against diverse poultry and foodborne pathogens. Heterophils are the primary polymorphonuclear cells in poultry, and as such are the "first responders" to infectious insult and injury. Heterophils exhibit biochemical and cytoskeletal activities including adhesion, chemotaxis, phagocytosis, microbicidal activities of degranulation and oxidative burst, and produce cytokines and chemokines (Genovese et al., 2013). We have characterized the heterophil-mediated innate immune response of 2 parental broiler lines and their F_1 reciprocal crosses and show increased in vitro heterophil function (Swaggerty et al., 2003a,b) corresponds with increased in vivo resistance against *Salmonella* Enteritidis (Ferro et al., 2004; Swaggerty et al., 2005a), *Enterococcus gallinarum* (Swaggerty et al., 2005b), *Campylobacter jejuni* (Li et al., 2008), and *Eimeria tenella* (Swaggerty et al., 2011). Additionally, there is an increase in mRNA expression of pro-inflammatory cytokines and chemokines in the resistant line compared with the susceptible line (Ferro et al., 2004; Swaggerty et al., 2004) that is maintained (Swaggerty et al., 2006).

Received August 15, 2013.
Accepted November 24, 2013.
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Strong pro-inflammatory cytokine and chemokine responses are associated with increased resistance against disease (Heinrich et al., 2001; Sebastiani et al., 2002; Coussens et al., 2004; Ferro et al., 2004; Withanage et al., 2004). Heterophils migrate to the liver and intestinal villi of newly hatched chickens infected with *Salmonella* Typhimurium, accompanied by elevated levels of pro-inflammatory cytokines in tissues (Withanage et al., 2004) and the ileum of *Salmonella* Enteritidis-infected chickens (Chappell et al., 2009). The pro-inflammatory cytokine, IL-1β, and chemokine, IL-8 (now called CXCLi2), are found in the gut of newly hatched chickens and mRNA expression continues to increase the first week posthatch (Bar-Shira and Friedman, 2006). The CXCLi2 mRNA is upregulated in *Salmonella*-resistant chickens (Sadeyen et al., 2004), and IL-1β and IL-6 mRNA expression increases following infection with *Salmonella* Enteritidis (Cheeseman et al., 2007).

The results of earlier studies by our laboratory indicated the sire is more influential than the dam. Therefore, we conducted a preliminary selection trial that shows individual broiler breeder sires have varying inherent levels of pro-inflammatory cytokine and chemokine mRNA and selection based on a high or low phenotype is passed onto the first generation of progeny (Swaggerty et al., 2008). The objectives of the present study were to confirm and advance the preliminary selection trial by 1) screening a new population of broiler sires and identifying a small number of individuals with naturally high and low levels of key pro-inflammatory mediators (IL-6, CXCLi2, and CCLi2), 2) using the identified high and low sires to produce 2 lines of progeny with a high and low phenotype, 3) conducting challenge trials using the high and low lines to determine if there are differences in resistance against *Salmonella* Enteritidis, and 4) monitoring industry performance standards including live weight, feed conversion ratio (**FCR**), and percent breast meat yield in the selected flocks.

MATERIALS AND METHODS

Selection Procedures

A commercial broiler line served as the parental generation (**G**) for the selection study, and the selection procedures are summarized in Table 1. From the G population, 75 males were randomly selected and tested for expression of pro-inflammatory mediators. For a sire to be used in matings, it must have the highest or lowest mRNA expression of at least 2 out of 3 of the pro-inflammatory mediators. The 10 males with the highest cytokine and chemokine expression were assigned to the high line and each was mated to 15 randomly selected females for a total of 150 hens. The 10 males with the lowest cytokine and chemokine expression were assigned to the low line and similarly mated to 150 females. These 2 mating groups produced the high and low lines of the F_1 generation, and the 2 lines were handled according to standard procedures of commercial lines in a breeding program. At 16 wk of age, we selected 119 and 121 males in the high and low line, respectively, on the basis of performance and then they were evaluated for expression of pro-inflammatory mediators. Five sires with the highest pro-inflammatory mediator expression from the high line and 5 sires with the lowest expression of pro-inflammatory mediators from the low line were selected and mated to 15 randomly selected hens from the high and low lines, respectively, and produced the F_2 generation. The F_2 generation was similarly handled except that the breeding group from this population was composed of 10 sires and 150 hens for each of the selected lines.

Blood Collection and Cell Isolation

The chickens used in the present study were derived from a broiler line reared on a commercial farm following industry standards and supervision of the company's Animal Welfare Committee. Blood collection was conducted according to federal regulations and overseen by veterinarians. Sire blood samples were collected at 40 wk of age and progeny were bled at 16 wk of age. In both instances, blood (3–5 mL) was collected from the large vein in the wing as previously described (Swaggerty et al., 2008). Briefly, the samples were shipped overnight and upon receipt, the blood and EDTA were transferred to conical tubes and 1% methylcellulose added, mixed thoroughly, and centrifuged at $35 \times g$ for 20 min at 4°C. The supernatant containing all peripheral blood leukocytes [**PBL** (including heterophils, monocytes, and thrombocytes)], was transferred to a new tube and clear RPMI added, and the cells pelleted by centrifugation ($485 \times g$ for 15 min at 4°C). The cells were resuspended and washed in fresh RPMI and pelleted, the supernatant removed, and the cells

Table 1. Selection procedures

Population	Description	Line	No. produced	No. evaluated	Matings (male × female)
G	Parental			75	
		High			10 × 150
		Low			10 × 150
F_1	1st selected generation	High	2,854	119	5 × 75
		Low	2,680	121	5 × 75
F_2	2nd selected generation	High	1,063	95	10 × 150
		Low	1,064	94	10 × 150

Table 2. Quantitative real-time reverse-transcription PCR probes and primers

RNA target	Probe/primer sequence	Exon boundary	Accession number[1]
28S			
Probe	5′-(FAM[2])-AGGACCGCTACGGACCTCCACCA-(TAMRA)-3′		X59733
F[3]	5′-GGCGAAGCCAGAGGAAACT-3′		
R[4]	5′-GACGACCGATTTGCACGTC-3′		
IL-6			
Probe	5′-(FAM)-AGGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)-3′	3/4	AJ250838
F	5′-GCTCGCCGGCTTCGA-3′		
R	5′-GGTAGGTCTGAAAGGCGAACAG-3′		
CXCLi2			
Probe	5′-(FAM)-TCTTTACCAGCGTCCTACCTTGCGACA-(TAMRA)-3′	1/2	AJ009800
F	5′-GCCCTCCTCCTGGTTTCAG-3′		
R	5′-TGGCACCGCAGCTCATT-3′		
CCLi2			
Probe	5′-(FAM)-ACACAACACCAGCATGAGGGCACTG-(TAMRA)-3′	2/3	L34553, AJ243034
F	5′-GGCAGACTACTACGAGACCAACAG-3′		
R	5′ ACGGCCCTTCCTGGTGAT-3′		

[1]Genomic DNA sequence.

[2]5-carboxyfluorescein.

[3]Forward.

[4]Reverse.

resuspended in lysis buffer (Qiagen RNeasy mini RNA extraction kit, Qiagen Inc., Valencia, CA), and frozen at −20°C until the RNA was isolated.

Isolation of Total RNA

The lysed PBL were transferred to QIAshredder homogenizer columns (Qiagen Inc.) and centrifuged for 2 min at ≥8,000 × *g* at room temperature. Total RNA was extracted from the homogenized lysates according to the manufacturer's instructions, eluted with 50 μL of RNase-free water, and stored at −80°C until quantitative real-time reverse-transcription (**qRT**)-PCR analyses were performed.

qRT-PCR

Cytokine and chemokine mRNA expression was quantified using a well-described method. Primers and probes for cytokines, chemokines, and 28S RNA-specific amplification have been described (Kaiser et al., 2000; Kogut et al., 2003; Swaggerty et al., 2008) but are provided for clarity (Table 2). The qRT-PCR was performed using the TaqMan one-step RT-PCR master mix reagents (Applied Biosystems, Branchburg, NJ). Amplification and detection of specific products were performed using the Applied Biosystems 7500 Fast Real-Time PCR System with the following cycle profile: 1 cycle of 48°C for 30 min, 95°C for 20 s, and 40 cycles of 95°C for 3 s and 60°C for 30 s. Quantification was based on the increased fluorescence detected by the 7500 Fast Sequence Detection System due to hydrolysis of the target-specific probes by the 5′ nuclease activity of the *rTth* DNA polymerase during PCR amplification. To correct for differences in RNA levels between samples within the experiment, the correction factor for each sample was calculated by dividing the mean C_t value for 28S rRNA-specific product for each sample by the overall mean C_t value for the 28S rRNA-specific product from all samples. The corrected cytokine mean is calculated: (average of each replicate × cytokine slope)/28s slope × 28s correction factor. The data shown are corrected 40-C_t values.

Bacteria Preparation

A poultry isolate of *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) was obtained from the National Veterinary Services Laboratory (Ames, IA) and selected for resistance to nalidixic acid and novobiocin and maintained in tryptic soy broth (Difco Laboratories, Sparks, MD) containing antibiotics (20 μg/mL nalidixic acid and 25 μg/mL novobiocin; Sigma Chemical Co., St. Louis, MO). A stock culture was prepared in sterile PBS and adjusted to a concentration of 1×10^9 cfu/mL as previously described (Swaggerty et al., 2005a). The viable cell concentration of the challenge dose for each experiment was determined by colony counts on XLT4 agar base plates with XLT4 supplement (Difco) and nalidixic acid and novobiocin (XLT-NN).

Experimental Chickens

Fertilized eggs from the high and low lines were obtained from the commercial company and were incubated and hatched under standard conditions (Stromberg, 1975). Each line was coded to conceal its identity. On day of hatch, straight-run chickens were placed in floor pens (3 m × 3 m) containing wood shavings, supplemental heat, water, and a balanced, unmedicated corn and soybean meal-based chick starter diet ad libitum. The feed contained 23% protein and 3,200 kcal of ME/kg of diet, and all other nutrient levels met or exceeded

Table 3. Cytokine and chemokine mRNA expression (40-C_t) for the parental population (G) of sires (n = 75)

Item	Range	Median	Average ± SEM
IL-6	8.79–19.66	16.47	16.04 ± 0.23
CXCLi2	2.70–12.78	5.86	6.86 ± 0.32
CCLi2	8.23–14.14	14.03	13.87 ± 0.22

established requirements (NRC, 1994). At the conclusion of each experiment, all birds were euthanized via CO_2 asphyxiation. The birds were not vaccinated at any time during the experiment nor did they receive any medications. Experiments were conducted according to the guidelines established by the USDA animal care and use committee.

Organ Invasion

Day-old chicks from the high and low lines were randomly placed into either control or challenged groups and maintained in floor pens housed in separate isolation rooms under the same conditions described above. Day-old chickens were challenged orally with *Salmonella* Enteritidis (0.5 mL); doses ranged from 10^1 to 10^7 cfu per chick. The specific numbers of chicks used in each challenge trial are provided in the respective results section. At 24 h postchallenge, chickens were euthanized and necropsied. At necropsy, the entire liver and spleen were aseptically removed, placed in a single tube containing tetrathionate enrichment broth (25 mL, Difco) and incubated overnight at 41°C. Following enrichment, 10 μL were streaked onto XLT-NN plates, incubated 24 h at 41°C, then the plates examined for nonlactose fermenting NN-resistant *Salmonella* colonies. Representative colonies were confirmed positive by plate agglutination using specific Group D_1 antisera (Difco). Both generations of progeny were subjected to 2 separate challenge trials. Noninjected and nonchallenged chickens (n = 5) were included for each experiment to confirm the flocks were free of *Salmonella* Enteritidis.

Performance

Basic performance data were collected on the high and low flocks. The total number of birds, FCR, live weight, and percent breast meat yield were determined. The FCR is feed consumed/weight gain. Live weight was measured at 6 wk of age. The percent breast meat is the amount of breast meat/live weight.

Statistical Analyses

There were no statistical analyses performed on the G sires. The data obtained on the initial sires showed a range of values for each cytokine/chemokine and allowed for the identification of sires with naturally high and low baseline values. The median, mean, and SEM for each cytokine and chemokine were calculated for the high and low F_1 and F_2 populations and statistical analyses performed (Student's *t*-test). A Student's *t*-test analysis was also conducted on challenge trials showing *Salmonella* Enteritidis organ invasion; comparisons were made for comparable doses. For all analyses, significance was considered if $P \leq 0.05$. A Student's *t*-test analysis was conducted on flock performance data. Each generation was analyzed separately.

RESULTS

G Population

Seventy-five sires were randomly selected from a commercial line of broilers to serve as the G population for production of the high and low lines. The PBL were

Table 4. Cytokine and chemokine mRNA expression (40-C_t) of sires used to produce high and low lines

Population	Cytokine/ chemokine	High			Low		
		Range	Median	Average ± SEM	Range	Median	Average ± SEM
G[1]	IL-6	16.69–17.13	16.86	16.88 ± 0.08	10.65–14.02	12.38	12.23 ± 0.59
	CXCLi2	3.11–10.83	5.02	5.78 ± 1.31	3.24–9.54	7.86	7.33 ± 1.1
	CCLi2	13.91–15.54	15.03	14.73 ± 0.32	9.14–13.08	10.52	10.84 ± 0.64
F_1[2]	IL-6	10.96–11.85	11.42	11.4 ± 0.15	6.05–8.13	7.42	7.2 ± 0.38
	CXCLi2	13.93–15.5	15.02	14.72 ± 0.3	5.98–6.89	6.50	6.51 ± 0.16
	CCLi2	12.22–13.49	13.19	13.04 ± 0.24	6.36–7.76	6.90	6.98 ± 0.26
F_2[1]	IL-6	14.06–17.32	15.83	15.7 ± 0.34	ND[3]–13.26	11.20	10.44 ± 1.2
	CXCLi2	9.92–16.88	13.15	13.14 ± 0.6	5.3–11.18	8.83	8.74 ± 0.52
	CCLi2	15.2–19.59	16.47	16.58 ± 0.37	9.06–12.03	10.70	10.61 ± 0.29

[1]n = 10 sires.
[2]n = 5 sires.
[3]Not detectable.

Table 5. Cytokine and chemokine mRNA expression (40-C_t) of progeny from selected high and low sires

Population	Cytokine/ chemokine	High Range	High Median	High Average ± SEM	Low Range	Low Median	Low Average ± SEM	*P*-value
F_1[1]	IL-6	5.0–11.85	9.23	9.03 ± 0.17	2.87–10.78	9.03	8.89 ± 0.1	0.47
	CXCLi2	4.69–16.01	11.71	10.86 ± 0.26	5.34–16.77	9.63	9.78 ± 0.17	0.001
	CCLi2	5.01–13.78	10.34	10.03 ± 0.19	1.59–13.26	9.73	9.49 ± 0.13	0.02
F_2[2]	IL-6	10.33–17.32	13.76	13.65 ± 0.03	ND[3]–16.75	13.11	13.17 ± 0.24	0.03
	CXCLi2	7.88–16.88	11.25	11.22 ± 0.17	ND–13.62	10.21	10.03 ± 0.21	4×10^{-6}
	CCLi2	10.18–19.59	13.96	14.04 ± 0.16	ND–17.68	12.61	12.68 ± 0.21	1.3×10^{-8}

[1]Total number of progeny for high and low lines was 119 and 121, respectively.

[2]Total number of progeny for high and low lines was 95 and 94, respectively.

[3]Not detectable.

isolated from a blood sample from each sire and the mRNA expression (40-C_t) of IL-6, CXCLi2, and CCLi2 quantified using qRT-PCR (Table 3). The mRNA expression of IL-6 varied from 8.79 to 19.66, and the average was 16.04 ± 0.23 for the 75 sires. Expression of CXCLi2 was from 2.70 to 12.78 with an average of 6.86 ± 0.32, and the range of CCLi2 expression was 8.23 to 14.14 with an average of 13.87 ± 0.22. The dams were not evaluated in the present study.

Of the original 75 males evaluated, 10 males with the highest and 10 males with the lowest mRNA expression of IL-6, CXCLi2, and CCLi2 were selected to produce the high and low lines, respectively (Table 4). The average expression levels of IL-6 for the high and low line sires were 16.88 ± 0.08 and 12.23 ± 0.59, respectively. The average expression of CXCLi2 was 5.78 ± 1.31 and 7.33 ± 1.1 for high and low, respectively, and 14.73 ± 0.32 and 10.84 ± 0.64 for CCLi2.

F_1 Population: Cytokine/Chemokine Expression

The G sires produced the first generation (F_1) of high and low line progeny. The mRNA expression of IL-6, CXCLi2, and CCLi2 were determined for every individual from both lines (Table 5). There were no differences in IL-6 between the high and low lines (9.03 ± 0.17 and 8.89 ± 0.1, respectively). The mRNA expression of CXCLi2 was significantly ($P = 0.001$) greater for the high line progeny compared with the low line (10.86 ± 0.26 and 9.78 ± 0.17, respectively). There were also significant ($P = 0.02$) differences observed for CCLi2 expression between the high and low line progeny (10.03 ± 0.19 and 9.49 ± 0.13, respectively).

F_1 Population: Salmonella Enteritidis Challenge

Two separate challenge trials were conducted using 2 separate hatches. In trial 1, high (n = 109) and low (n = 91) chicks were challenged with 8×10^4 cfu *Salmonella* Enteritidis. Significantly ($P = 0.006$) fewer high line chicks (88%) were positive for *Salmonella* Enteritidis compared with the low line (97.8%; Table 6). Because most chicks were positive in the initial challenge trial, subsequent challenges used varying doses of *Salmonella* Enteritidis.

In trial 2, the *Salmonella* Enteritidis challenge doses were 10^2 to 10^4 cfu/chick (high line = 71 chicks per dose; low line = 67–68 chicks per dose). Regardless of the dose administered, chicks from the high line were significantly ($P \leq 0.05$) more resistant to organ invasion by *Salmonella* Enteritidis compared with the low line chicks (Table 7). The high line chicks were 45.1 to 60.5% positive, whereas the low line ranged from 76.1 to 86.7% positive over the 3 doses of *Salmonella* Enteritidis evaluated. In each challenge trial, mock-infected control chicks were administered sterile PBS and were negative for *Salmonella* Enteritidis (data not shown).

F_1 Population Sires

Five roosters from high and 5 from the low F_1 lines were selected to produce the F_2 generation (Table 4). The average high line sire mRNA expression for IL-6, CXCLi2, and CCLi2 were 11.4 ± 0.15, 14.72 ± 0.3, and 13.04 ± 0.24, respectively. The low line sire values for IL-6, CXCLi2, and CCLi2 were 7.2 ± 0.38, 6.51 ± 0.16, and 6.98 ± 0.16, respectively. For each pro-inflamma-

Table 6. F_1 progeny *Salmonella* Enteritidis challenge trial 1

Line (cfu *Salmonella* Enteritidis administered)	No. of *Salmonella* Enteritidis positive chicks/ total no. challenged	Percent positive	*P*-value
High (10^4)	96/109	88	0.006
Low (10^4)	89/91	97.8	

Table 7. F_1 progeny *Salmonella* Enteritidis challenge trial 2

Line (cfu *Salmonella* Enteritidis administered)	No. of *Salmonella* Enteritidis positive chicks/ total no. challenged	Percent positive	*P*-value
High (10^2)	32/71	45.1	0.0001
Low (10^2)	51/67	76.1	
High (10^3)	43/71	60.5	0.05
Low (10^3)	51/67	76.1	
High (10^4)	41/71	57.7	0.0001
Low (10^4)	59/68	86.7	

tory mediator, the average value of the high sires was significantly greater than the low line sires ($P \leq 0.05$).

F_1 Population: Performance

Basic performance data were collected on the high (n = 2,854) and low (n = 2,680) individuals from F_1 (Table 10). At 6 wk of age, the live weight of the high line birds was significantly lighter compared with the low line (2.10 kg compared with 2.13 kg). The FCR for both flocks were comparable (high flock = 1.69; low flock = 1.68). Percent breast meat yield was also the same for the high and low flocks (22.4 and 22.3%, respectively).

F_2 Population: Cytokine/Chemokine Expression

The F_1 sires produced the second generation of progeny (F_2), and there were 95 high line progeny and 94 low line progeny that were evaluated for IL-6, CXCLi2, and CCLi2 mRNA expression (Table 5). For each proinflammatory mediator that was assessed, the F_2 high line progeny had significantly ($P \leq 0.03$) higher mRNA expression compared with the low line progeny. The IL-6 expression for the high line progeny ranged from 10.33 to 17.32 with an average of 13.65 ± 0.03, whereas the low line progeny ranged from nondetectable (**ND**) to 16.75 with an average of 13.17 ± 0.24 ($P = 0.03$). Expression of CXCLi2 for P2 high line progeny was 7.88 to 16.88 with an average of 11.22 ± 0.17 and the low line values were from ND to 13.62 with an average of 10.03 ± 0.21 ($P = 4 \times 10^{-6}$). The mRNA expression of CCLi2 in the high line F_2 population was also highly significant ($P = 1.3 \times 10^{-8}$) compared with the low line. The range observed for the high line was 10.18 to 19.59 with an average of 14.04 ± 0.16, whereas the low line progeny ranged from ND to 17.68 with an average of 12.68 ± 0.21.

F_2 Population: Salmonella Enteritidis Challenge

As with the F_1 generation of progeny, 2 separate *Salmonella* Enteritidis challenge trials were conducted to determine if the F_2 high line chicks were still more resistant than low line chicks. In the first trial, high and low chicks were dosed with 10^5 to 10^7 cfu/chick (high line = 22–23 chicks per dose; low line = 19–21 chicks per dose; Table 8). The percentage of high line chicks that were *Salmonella* Enteritidis positive over the 3 doses ranged from 0 to 8.7%, whereas the percentage of low line positive chicks ranged from 14.3 to 26.3%, but was not significant ($P = 0.06$–0.15).

A second challenge was conducted and chicks were dosed with 10^2 to 10^4 cfu *Salmonella* Enteritidis (high line = 36 chicks per dose; low line = 33–34 chicks per dose). The data are summarized in Table 9 and are consistent with the first 3 challenges where the high line chicks were more resistant against organ invasion by *Salmonella* Enteritidis compared with the low line chicks. The high line chicks were highly ($P \leq 1.1 \times 10^{-8}$) resistant against all doses of *Salmonella* Enteritidis (27–38% positive) compared with the low line chicks (88–100% positive; Table 9). In each challenge trial, mock-infected control chicks were administered sterile PBS and were negative for *Salmonella* Enteritidis (data not shown).

Table 8. F_2 progeny *Salmonella* Enteritidis challenge trial 1

Line (cfu *Salmonella* Enteritidis administered)	No. of *Salmonella* Enteritidis positive chicks/ total no. challenged	Percent positive	*P*-value
High (10^5)	1/22	4.5	0.06
Low (10^5)	5/19	26.3	
High (10^6)	0/23	0	0.08
Low (10^6)	3/21	14.3	
High (10^7)	2/23	8.7	0.15
Low (10^7)	5/19	26.3	

Table 9. F_2 progeny *Salmonella* Enteritidis challenge trial 2

Line (cfu *Salmonella* Enteritidis administered)	No. of *Salmonella* Enteritidis positive chicks/ total no. challenged	Percent positive	*P*-value
High (10^2)	10/36	27.8	2.4×10^{-9}
Low (10^2)	31/34	91.2	
High (10^3)	14/36	38.9	1.1×10^{-8}
Low (10^3)	33/33	100	
High (10^4)	12/36	33.3	2.0×10^{-8}
Low (10^4)	30/34	88.2	

F_2 Population Sires

The cytokine/chemokine expression of the sires used to produce the F_2 population is summarized in Table 4. The average IL-6 mRNA expression for the high sires was 15.7 ± 0.34 and the low sires was 10.44 ± 1.2. Expression of CXCLi2 was also determined for the high and low sires (13.14 ± 0.6 and 8.74 ± 0.52, respectively) as well as CCLi2 mRNA expression (16.58 ± 0.37 and 10.61 ± 0.29, respectively). For each pro-inflammatory mediator, the average value of the high sires was significantly greater than the low line sires ($P \leq 0.05$).

F_2 Population: Performance

Basic performance data were collected on the F_2 population of high (n = 1,063) and low (n = 1,064) flocks (Table 10). At 6 wk of age, the live weight of the high line birds was significantly lighter compared with the low line (2.23 kg compared with 2.13 kg). The FCR for both flocks were comparable (high flock = 1.79; low flock = 1.77). Percent breast meat yield was also the same for the high and low flocks (22.8 and 23.1%, respectively).

DISCUSSION

The poultry industry has made significant progress improving the overall health of flocks by implementing improved genetic selection and husbandry/biosecurity practices. All meat-producing industries are being limited in how and when antibiotics can be administered to animals. In 2006, the European Union implemented a ban on the use of antibiotics as growth promoters (Castanon, 2007; Cogliani et al., 2011). We believe one method to reduce the need for antibiotics is by utilizing birds that are naturally more resistant, which puts control of infectious agents in the host animal and therefore makes it less likely for producers to rely on antibiotics and other medications. High-yielding poultry raised in the modern commercial setting are thought to be more susceptible to diseases (Jie and Liu, 2011). As poultry breeding companies keep up with global consumption, consumer demands, and increasing regulations while looking for ways to improve overall robustness and livability, addressing animal welfare issues, and reducing production costs, novel methods for identification and selection of poultry that are naturally resistant to poultry and foodborne pathogens will become increasingly important.

There are numerous experimental populations of chickens that have undergone selection pressures including antibody responses (Zhao et al., 2012), resistance or susceptibility to salmonellosis (Bumstead and Barrow, 1988), *Salmonella* carriage (Beaumont et al., 2009), group productivity (Cheng et al., 2001), and other parameters (Cheng and Lamont, 1988). Transgenic chickens have also been generated that exhibit improved resistance or decreased ability to transmit viral diseases (Salter and Crittenden, 1989; Lyall et al., 2011). However, the use of transgenic animals is not likely to be accepted by the consumer and is therefore not likely to be used by the poultry industry. All of these research lines are invaluable for scientific advancement, but are not directly useful for the poultry industry with regard to implementing new breeding and selection strategies. One of the key facets that distinguishes our selection study from basic research lines is that the birds used in the current study originated from a commercial broiler line, were selected and housed by the commercial breeder, and were not merely an experimental line of birds maintained within a research facility. From a his-

Table 10. Performance of high and low flocks

Population	Parameter	High	Low	*P*-value
F_1	n	2,854	2,680	
	Live weight (kg)	2.10 ± 0.005	2.13 ± 0.005	0.01
	FCR[1]	1.69 ± 0.004	1.68 ± 0.004	0.18
	Breast (%)	22.4 ± 0.034	22.3 ± 0.035	0.63
F_2	n	1,063	1,064	
	Live weight (kg)	2.23 ± 0.009	2.29 ± 0.009	0.01
	FCR	1.79 ± 0.007	1.77 ± 0.006	0.18
	Breast (%)	22.8 ± 0.049	23.1 ± 0.048	0.44

[1]FCR = feed conversion ratio.

torical perspective, a study by Scholes and Hutt (1942) showed it was possible to identify familial differences in susceptibility to *Salmonella pullorum* based on differences in body temperature where the birds with naturally higher body temperature were more resistant to *S. pullorum* induced mortality compared with the line with inherently lower body temperatures. The selection procedures were similar between that study and the one described herein in that individuals from a common population were screened, selected, and then used to produce distinct high and low lines that were subjected to challenge trials where the high line was more resistant compared with the low line. However, to our knowledge, there are no comparable modern selection studies of commercial poultry that are similar to the one presented herein.

In the present study, an innovative selection strategy based on a phenotype of inherently higher pro-inflammatory mediators including IL-6, CXCLi2, and CCLi2 showed the profile of the sire was passed onto progeny. Additionally, the progeny generated from sires selected for high pro-inflammatory mediators were more resistant to *Salmonella* Enteritidis than progeny from sires selected for the lower phenotype. These data provide evidence that these inflammatory mediators are potential biomarkers that can be used for selection purposes. This approach is very different from other selection strategies that are seeking to improve resistance against single pathogens including avian influenza (Lyall et al., 2011), avian leukosis virus (Salter and Crittenden, 1989), and Marek's disease virus (Heifetz et al., 2007). The chicken MHC is associated with resistance or susceptibility to a broad range of diseases and the B^{21} allele is correlated with increased resistance against Marek's and *Escherichia coli* (Zekarias et al., 2002; Cavero et al., 2009) but has not been associated with systemic salmonellosis (Barrow et al., 2004). Even though MHC was not examined in our study, it could be a valuable parameter to characterize in birds selected for a high and low pro-inflammatory mediator phenotype. Another aspect to consider for future studies is to determine the *Salmonella* carrier state in the lines selected for high and low pro-inflammatory mediators as there are differences in carrier state in egg-type chickens depending on the bird's age (Beaumont et al., 2009). This could be an important consideration for pedigree pure lines, great-grand parent, grand parent, and parent lines, but maybe less important for end product meat birds meant strictly for a short-term grow out and consumption.

The cytokine (IL-6) and chemokines (CXCLi2 and CCLi2) that were used as markers for selection in the present study are pro-inflammatory mediators, and were selected as markers based on literature indicating a strong pro-inflammatory response is associated with increased resistance to *Salmonella* Enteritidis. A few examples include elevated mRNA expression of IL-6, CXCLi1, and CXCLi2 in heterophils, monocyte-derived macrophages, the ceca, and cecal tonsil (Ferro et al., 2004; Wigley et al., 2006; Cheeseman et al., 2008; Setta et al., 2012). Upregulation of IL-6 has also been reported in birds more resistant to Marek's disease (Xu et al., 2012) and necrotic enteritis (Hong et al., 2012).

The high and low line birds were also challenged with *C. jejuni* (n = 1) and differences were observed; the percentage of *C. jejuni* positive birds in the high line was 65% compared with 100% of the low line birds (C. L. Swaggerty and M. H. Kogut, unpublished data). These preliminary data are in agreement with a study showing resistance against *C. jejuni* is characterized by increased expression of genes associated with an innate immune response and cytokine signaling (Connell et al., 2012). Lameness and other related leg issues including tibial head necrosis are often caused by secondary bacterial infections by *Staphylococcus* or *Enterococcus* spp., or both. The high and low line birds were evaluated for lameness in an established model (Wideman et al., 2012) and the low line had an increased incidence of tibial head necrosis at 2 and 3 wk of age compared with the high line (Robert F. Wideman Jr., University of Arkansas, Fayetteville, personal communication). These studies must be repeated, but certainly provide provisional data supporting our hypothesis that selection based on pro-inflammatory mediators protects birds from a broad range of poultry and foodborne pathogens.

Innate immunity, which includes pro-inflammatory mediators, is very effective at defending against pathogens. In our study, the FCR and percent breast meat yield was comparable between the lines under nonchallenged conditions. Even though FCR was not determined in the challenge studies, poultry grown under commercial conditions are exposed to a myriad of naturally occurring pathogenic and commensal bacteria, vaccinations, feed changes, and so on, suggesting our selection strategy did not adversely affect nutrient utilization. A recent meta-analysis showed selection for growth alone does compromise immune function, but selection for immune function does not necessarily affect growth (van der Most et al., 2011). Our findings suggest there could be a slight decrease in weight of the birds selected for higher levels of pro-inflammatory mediators. Additional rounds of selection and performance data collection will have to be carried out to determine if the initial differences in weight make this selection method too costly for the poultry industry to implement. Antibiotic use, antibody production, and body temperature should also be monitored in subsequent flocks. It is possible that the high line birds require less therapeutic interventions than the low line. Selection based on pro-inflammatory mediators could be valuable in light of stricter regulations with respect to antibiotic use, and may provide the poultry industry with a viable option to enhance selection for improved robustness, livability, and resistance against a broad range of poultry and foodborne pathogens.

ACKNOWLEDGMENTS

Mention of commercial products is for the sole purpose of providing specific information, not recommendation or endorsement by the USDA.

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