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Genetically Disparate Fayoumi Chicken Lines Show Different Response to Avian Necrotic Enteritis

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Necrotic enteritis (NE) has reemerged as a significant problem as a result of growing restrictions of antibiotics in agricultural animal production and increasing concerns over antibiotic resistance in human pathogens. To enhance our understanding of host-pathogen immunobiology in NE, transcriptional analysis was conducted to compare changes in NE-induced intestinal transcripts and to identify immune-related genes whose expression are associated with NE disease resistance using two genetically disparate Fayoumi chicken lines, M5.1 and M15.2. NE was induced by co-infection of *Eimeria maxima* and *Clostridium perfringens* using an established disease model and two major NE-induced clinical signs, body weight loss and intestinal lesions, were measured in two inbred Fayoumi chicken lines, M5.1 and M15.2. In the clinical criteria, line M5.1 chickens were more resistant to NE compared to line 15.2 birds. Although they have the same genetic background, these two chicken lines are genetically disparate at their major histocompatibility complex (MHC) and this difference was reflected in the differential expression patterns of several inflammatory genes such as suppressor of cytokine signaling 3 (SOCS3), interleukin 8 (IL8), nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta (NFKBIZ), serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (SERPINF1), and gap junction protein, alpha 1, 43kDa (GJA1) between NE-afflicted and uninfected chickens. These results will lead to increased insights on the NE disease resistance mechanisms and the role of host genes controlling host immune response to *C. perfringens*.

Key words: disease resistance, Fayoumi, gene expression, NE, necrotic enteritis

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Introduction

As a result of growing concerns over antibiotic resistance in human pathogens and corresponding increased restrictions on the use of antibiotics in animal agriculture, necrotic enteritis (NE) has reemerged as one of the most important disease in poultry industry in the world, especially in the regions where antibiotics growth promoters (AGP) have been banned (Lee *et al*., 2011a; Van Immerseel *et al*., 2004). NE is an acute clostridial disease associated with depression, loss of appetite, and sudden death and has been controlled by the feed containing AGPs (Hermans and Morgan, 2007). The NE is commonly caused by *Clostridium perfringens*, a Gram-positive, rod-shaped, spore-forming, oxygen-tolerant anaerobe (Lee *et al*., 2011b). This bacterium is infected from soil, dust, contaminated feed and litter and also subsist in a component of the intestinal microbiota of humans and other animals (Songer, 1996). *C. perfringens* infection causes not only an economic loss but also food safety hazards because of its risk for transmission to humans during the process of poultry product in the food chain (Olkowski *et al*., 2008; Van Immerseel *et al*., 2004). *C. perfringens* is anaerobic and grows at temperatures between 15 and 50℃ and forms endospores that can tolerate 100℃ for 2 h. These properties make

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it difficult to get rid of this bacterium from the environment and feed for poultry production using standard procedures (Brynestad and Granum, 2002; Williams, 2005). In countries where the AGPs are banned, alternative methods to control NE outbreak need to be developed. One of the promising ways to reduce NE-related economic loss is the genetic selection for NE-resistant commercial chicken breeds. However, there is limited information on genetic markers associated with NE.

The Fayoumi breed, which originated in Egypt, was imported to the United States in 1954 primarily because of its reported resistance to the avian leukosis. Subsequently, it was shown to develop a robust protective response against *E. tenella* (Pinard-Van Der Laan *et al*., 1998). Derived from the original Fayoumi breeds, the M5.1 and M15.2 congenic pair of lines are highly inbred with an inbreeding coefficient of 0.99, and are genetically distant from the broiler and Leghorn lines. Lines M5.1 and M15.2 share an identical genetic background and differ only in the microchromosome bearing the Major histocompatibility complex (MHC) (Zhou and Lamont, 1999). The M5.1 Fayoumi chicken line was reported to be more resistant to *Eimeria maxima* infection compared with M15.2 line and both B-complex congenic lines showed differential gene expression profiles following *E. maxima* infection (Kim *et al*., 2008, 2009). MHC genotype was reported to show the differences in the percentage mortality and hen-day egg production in NE-afflicted laying hen flocks (Siegel *et al*., 1993). However, there has not been any study evaluate the susceptibility to NE between M5.1 and M15.2 chicken lines.

In our previous study (Kim *et al*., 2014), transcriptome profiling was applied to elucidate molecular mechanisms underlying the pathogenesis of NE and obtain further insights on host immune responses to NE infection. Therefore, based on the report, this study was conducted to compare the differences in the resistance to NE and the expression levels of immune related genes between two Fayoumi chickens, M5.1 and M15.2 lines following NE induction by functional genomics to identify host genes associated with NE disease resistance.

Materials and Methods

Experimental Animals

One-day-old two Fayoumi inbred lines (M5.1 and M15.2) were obtained from Iowa State University (Ames, IA), housed in the Petersime starter brooder units, and provided with feed and water *ad libitum*. Chickens were kept in brooder pens in an *Eimeria*-free facility for 14 days post-hatch and transferred to larger hanging cages with 2 birds/ cage at a separate location where they were co-infected with *C. perfringens* and *E. maxima* and kept until the end of the experimental period.

Experimental NE Disease Model

Chickens were infected with *E. maxima* strain 41A (1.0×10^4 oocysts/bird) by oral gavage on day 14 post-hatch followed by infection with *C. perfringens* strain Del-1 (1.0×10^9 colony forming units/bird) by oral gavage on day 18 (Jang *et al*., 2012). To facilitate development of NE, birds were fed an antibiotic-free, certified organic starter diet containing 17% crude protein between days 1–18 followed by a standard grower diet containing 24% crude protein between days 18–20. Uninfected control animals were housed in neighboring cages and given the same diet. Body weight gains (18 birds/ group) were measured and fecal samples were collected between days 14–20 post-hatch (days 0–7 post-infection with *E. maxima*). Oocyst numbers were determined using a McMaster chamber (HK Inc., Tokyo, Japan) (Yoo *et al*., 2009). Gut lesion scores were determined at day 20 (day 2 post-infection with *C. perfringens*) on a scale of 0 (none) to 4 (high) by three independent observers in a blinded fashion as described (Johnson and Reid, 1970; Lee *et al*., 2009). All protocols were approved by the Beltsville Area Institutional Animal Care and Use Committee.

RNA Extraction from Intestinal Intraepithelial Lymphocytes (IELs)

RNAs from chicken IELs were prepared as described Kim *et al*. (2011). The 10-cm segments of mid-jejunum was cut longitudinally, and washed three times with ice-cold Hank's balanced salt solution (HBSS). Harvested tissue samples were incubated in HBSS containing 0.5 mM EDTA and 5% FBS for 20 min at 37℃ with constant swirling. The supernatant were passed through nylon wool (Robbins Scientific, Sunnyvale, CA, USA), and washed twice with HBSS containing 5% FBS. IELs were purified by Percoll density gradient centrifugation and washed three times with HBSS containing 5% FBS. Total RNA was isolated using Trizol (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instruction.

Quantitative (q)RT-PCR

Differential genes expression between lines M5.1 and M15.2 were confirmed by qRT-PCR as described Kim *et al*. (2008). Equivalent amounts of intestinal IEL RNA samples from uninfected or *C. perfringens*/*E. maxima* co-infected Fayoumi M5.1 and M15.2 chickens were pooled into 3 samples from 18 birds each treatment group (6 birds/sample) and reverse-transcribed using the AffinityScript Multiple Temperature cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA, USA). PCR amplification was performed using the oligonucleotide primers listed in Table 1 with the Mx3000P system and Brilliant SYBR Green qRT-PCR master mix (Agilent Technologies). Standard curves were generated using $\log_{10}$ diluted standard RNAs and the levels of individual transcripts were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the Q-gene program (Muller *et al*., 2002). The cycle threshold (C_t) value of the target gene was normalized to GAPDH and calibrated to the relevant control value. Each analysis was performed in triplicate.

Statistical Analyses

Data from body weight gains, lesion scores and mRNA expression levels were expressed as the mean±S.D. values. Comparisons of the mean values were performed by ANOVA and the Student's *t*-test using SPSS software (SPSS

Table 1. **Oligonucleotide primers used for quantitative RT-PCR**

Symbol		Primer Sequence (5′-3′)	Entrez Gene Name	GenBank Accession No.
MTTP	Forward	TTCCTATATGCCTGTGGATT	microsomal triglyceride transfer protein	NM_001109784.1
	Reverse	AGGTACATCCTCACGTTGTC		
ALCAM	Forward	CTATTCGAGTGCTGTCACAA	activated leukocyte cell adhesion molecule	NM_205179
	Reverse	CAGATAGTGCACAGTGATGG		
CALB1	Forward	GCTTGGACTTAACACCTGAA	calbindin 1, 28kDa	NM_205513.1
	Reverse	TCCTCAGAATCAATGAAACC		
ANXA1	Forward	CAATGATGCAAGGGCCTTAT	annexin A1	NM_206906.1
	Reverse	CTTCATTGCCAGGTGGAGTT		
IL8	Forward	GGCTTGCTAGGGGAAATGA	interleukin 8	AJ009800
	Reverse	AGCTGACTCTGACTAGGAAACTGT		
APP	Forward	GGAAGCGATGATAAGGTGGTAGAAGAACAA	amyloid beta (A4) precursor protein	NM_204308
	Reverse	CATCACCATCATCATCGTCATCATCATCAG		
ARHGEF6	Forward	ACTGCTGGGAAATGTGGAGGAAATC	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	NM_001006432
	Reverse	ACGTCAGGTACAGGGAGCGGAACT		
BCL2	Forward	GATGTGCGTCGAGAGCGTCAA	B-cell CLL/lymphoma 2	NM_205339
	Reverse	GTGCAGGTGCCGGTTCAGGT		
COL1A2	Forward	CTCAGCTTTGTGGATACGCGGATTTTG	collagen, type I, alpha 2	NM_001079714
	Reverse	GCCCTGCAGATGCCTCACTCACA		
GJA1	Forward	GTCTTCATGCTGGTAGTGTCTTTGGTGTCT	gap junction protein, alpha 1, 43kDa	NM_204586
	Reverse	CTGTGGGAGTAGGGGTCGGTTTTTC		
HSP90B1	Forward	CTGGCTCTGGCATGCACGCTTCT	heat shock protein 90kDa beta (Grp94), member 1	NM_204289
	Reverse	CTTCATCATCAGTTCGGGACCCTTCTCTAC		
IRF2	Forward	GATCTCCCCTGTCTCGTCCTATGC	interferon regulatory factor 2	NM_205196
	Reverse	TGCCTTCAATGTTTTTCTTCTGCCAATG		
NFKBIZ	Forward	CCAGGTCCTCCAGGCAATCCAAAAG	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	NM_001006254
	Reverse	AGTGCAGGGCTGTCAAACCATCGTAG		
SERPINF1	Forward	CGGCAGCAGACAAGGGGAAGGATT	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	NM_001257289
	Reverse	TGAAGTAAGCAGCCCCAGCAAGGAG		
SOCS3	Forward	GACACCAGCCTGCGCCTCAAGA	suppressor of cytokine signaling 3	NM_204600
	Reverse	GCCCGTCACCGTGCTCCAGTAGA		
SOCS6	Forward	CAGATATCTTTGTGGACCAGGCAGTGAA	suppressor of cytokine signaling 6	NM_001127312
	Reverse	GGTAGCAAAGGTGAAAGTGGAGGGACATC		
TAB3	Forward	CACCGCAAAGACCTGGGACTG	TGF-beta activated kinase 1/MAP3K7 binding protein 3	XM_416787
	Reverse	GTGGGTGCTGGTTTCGTTGAGATGGT		
TCF12	Forward	CTCGGGGAAACCTGGAACAACCTACTA	transcription factor 12	NM_205375
	Reverse	GGGGGCACCTTTCTTACTTTCTTTGTCT		
TNFRSF11B	Forward	CATCTCGGCCAACCAAGTCTCACCT	tumor necrosis factor receptor superfamily, member 11b	NM_001033641
	Reverse	CGCTCGATATCTTCTTTTCCCACTTTCTTG		
TRAF3	Forward	CGTCTCGGCGCCACTTAGGA	TNF receptor-associated factor 3	XM_421378
	Reverse	GGGCAGCCAGACGCAATGTTCA		
VEGFA	Forward	GGCCTAGAATGTGTCCCTGTGG	vascular endothelial growth factor A	NM_001110355
	Reverse	ATGTGCGCTATGTGCTGACTCTGA		
LITAF	Forward	TGTGTATGTGCAGCAACCCGTAGT	lipopolysaccharide-induced TNF factor	AY765397
	Reverse	GGCATTGCAATTTGGACAGAAGT		
GAPDH	Forward	TGCTGCCCAGAACATCATCC	glyceraldehyde-3-phosphate dehydrogenase	NM_204305
	Reverse	ACGGCAGGTCAGGTCAACAA		

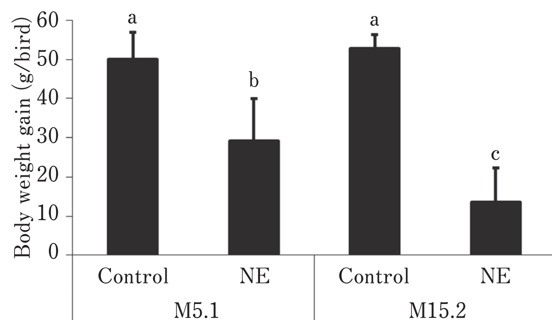

Fig. 1. **Effect of NE on body weight gain in Fayoumi chicken inbred lines M5.1 and M15.2.** Body weight gains were measured at 0 and 9 days post-infection. Each bar represents the mean±S. D. value (n=16). The different letters show the significant differences within the treatments (P<0.05).

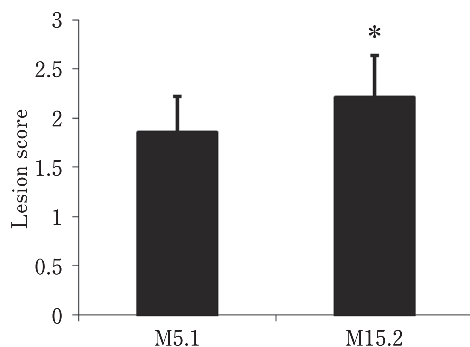

Fig. 2. **Effect of NE on gut lesion scores in Fayoumi chicken inbred lines M5.1 and M15.2.** Gut lesion scores were determined at day 20 (2 days post-infection with *C. perfringens*) on a scale of 0 (none) to 4 (high) in a blinded fashion by three independent observers. Each bar represents the mean±S.D. value (n=16). *, P<0.05.

15.0 for Windows, Chicago, IL) and were considered significant at P<0.05.

Results

Effects of NE on Body Weight Gains and Lesion Scores

Two Fayoumi inbred lines, M5.1 and M15.2, were induced for NE, and body weight gain and lesion score were compared. In both Fayoumi chicken lines, NE significantly reduced body weight gain, however, M5.1 showed less reduction in NE-induced body weight loss (42%) compared to M15.2 (74%) (Fig. 1). In the intestine, more severe lesions associated with NE were observed on the inner wall of intestine in M15.2 chickens compared with M5.1 chickens (Fig. 2).

Effects of NE on Intestinal Immune-related Gene Expression

In lines M5.1 and M15.2, the levels of 22 significantly altered immune-related genes were determined with or without NE induction. The genes were selected from the transcripts significantly altered by NE induction (>2.0-fold differential expression) in the IELs of broilers in our previous report (Kim *et al*., 2014), and also identified as the biological functions related with immune response by functional genomics. Interestingly, most genes analyzed in this study showed the decreased expression in both NE-infected Fayoumi chicken lines compared with non-infected control chickens (Table 2). However, the expression levels of suppressor of cytokine signaling 3 (SOCS3), interleukin 8 (IL8), and nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta (NFKBIZ) were increased in NE-afflicted M5.1 chickens compared with non-infected control group (Fig. 3). Meanwhile, serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (SERPINF1) and gap junction protein, alpha 1, 43 kDa (GJA1) were increased by NE infection in Fayoumi M15.2 chickens. The expression of lipopolysaccharide-induced TNF factor (LITAF) was increased after NE induction in both Fayoumi lines, M5.1 and M15.2 (Fig. 4).

Discussion

In this study, the differential effect of NE on two genetically disparate Fayoumi chicken lines with well-established disease susceptibility to coccidiosis and salmonellosis (Cotter *et al*., 1998; Kim *et al*., 2008, 2009) is reported. The results clearly showed that M5.1 chickens are more resistant to NE than M15.2 based on higher body weight gain and lower gut lesions.

The expression levels of 22 transcripts associated with immune-related functions were analyzed in IELs of both chicken lines to correlate the different NE resistance profile of M5.1 and M15.2 chickens which have differential expression patterns. While most transcripts decreased expression levels following NE induction in both Fayoumi chicken lines, M5.1 and M15.2, three genes, IL8, SOCS3, and NFKIZ were increased in NE-afflicted M5.1 chickens, and other two genes, SERPINF1 and GJA1 were induced in NE-afflicted M15.2 compared with non-infected control group. NE increased the expression of LITAF in both M5.1 and M15.2 lines.

We previously reported (Park *et al*., 2008) that IL8 was significantly upregulated in early stage of *C. perfringens* infection, but decreased at 2 days post *C. perfringens* exposure in the experimental model of NE produced by *E. maxima* and *C. perfringens* coinfection. In this study, IL8 was upregulated in the NE-resistant M5.1 line while decreased in M15.2 at 2 days post *C. perfringens*. IL8 is one of CXC chemokine family which is a chemoattractant to recruit immune cells to the site of infection, and plays an important role in inducing neutrophil infiltration and activation in various types of acute inflammation in innate immunity (Harada *et al*., 1994). IL8 thus could enhance the innate immune response to NE. SOCS3 is a negative regulator of inflammatory diseases, and induced by various cytokines,

Table 2. **The gene expression levels of immune related genes in two inbreed chicken lines, Fayoumi M5.1 and M15.2 with or without NE infection**

Gene	Treatment	Fayoumi M5.1	Fayoumi M15.2
ALCAM	CTRL	2.7E-18±1.6E-18[b]	9.1E-17±7.7E-19[a]
	NE	4.7E-19±6.6E-20[b]	1.3E-17±4.7E-18[b]
ANXA1	CTRL	8.2E-03±4.9E-04[a]	7.9E-03±1.8E-04[a]
	NE	2.8E-03±2.0E-04[b]	4.1E-03±1.7E-04[b]
MTTP	CTRL	1.8E-06±5.0E-08[b]	7.7E-06±9.8E-08[a]
	NE	1.1E-06±7.3E-08[b]	1.4E-06±2.8E-08[b]
HPS60B1	CTRL	7.1E-03±1.7E-04[b]	1.5E-02±3.6E-04[a]
	NE	4.3E-03±3.3E-04[c]	5.5E-03±9.9E-05[bc]
ARHGEF6	CTRL	5.8E-04±1.4E-05[b]	1.7E-03±2.8E-05[a]
	NE	3.8E-04±5.0E-05[b]	5.1E-04±2.1E-05[b]
VEGFA	CTRL	7.9E-02±1.5E-03[a]	4.1E-02±1.6E-03[b]
	NE	1.5E-02±1.0E-03[c]	9.5E-03±6.0E-05[d]
CALB1	CTRL	2.4E+00±1.2E-01[a]	1.2E+00±7.0E-03[b]
	NE	7.2E-01±1.1E-01[c]	1.6E-01±4.7E-03[d]
IRF2	CTRL	7.8E-03±3.4E-04[b]	1.5E-02±2.8E-04[a]
	NE	5.4E-03±1.5E-04[d]	9.5E-03±1.2E-04[c]
TNFRSF11B	CTRL	4.8E-07±6.6E-08	4.8E-07±6.6E-08
	NE	3.3E-07±6.3E-08	1.8E-07±2.2E-08
COL1A2	CTRL	2.6E-04±2.5E-05[ab]	4.4E-04±2.6E-05[a]
	NE	8.8E-05±5.5E-06[b]	2.7E-04±1.6E-05[ab]
TCF12	CTRL	8.3E-05±3.9E-06[b]	1.5E-04±1.9E-06[a]
	NE	2.0E-05±3.3E-06[d]	4.9E-05±2.0E-06[c]
APP	CTRL	1.5E-02±4.4E-04[b]	2.1E-02±1.5E-04[a]
	NE	4.6E-03±2.8E-05[d]	8.2E-03±1.7E-04[c]
BCL2	CTRL	8.7E-05±3.9E-06[b]	2.1E-04±6.8E-06[a]
	NE	3.5E-05±2.6E-06[c]	1.1E-04±2.2E-06[b]
TAB3	CTRL	1.2E-03±1.7E-05[a]	1.3E-03±1.0E-04[a]
	NE	3.4E-04±2.2E-05[b]	5.8E-04±1.6E-05[b]
TRAF3	CTRL	7.2E-04±4.4E-05[b]	9.8E-04±3.4E-05[a]
	NE	2.8E-04±2.6E-05[c]	4.1E-04±1.6E-05[c]
SOCS6	CTRL	2.0E-04±1.6E-05[b]	5.2E-04±2.3E-05[a]
	NE	8.0E-05±1.3E-06[c]	1.2E-04±4.2E-06[c]

Within each gene the different letters are significantly different according to the ANOVA test ($P<0.05$).

including IL6, IL10, IL8, and interferon-gamma (IFN-γ) (Schmitz *et al*., 2000; Stevenson *et al*., 2004; Hokenson *et al*., 2013; Spence *et al*., 2013).

In the present study, SOCS3 expression was enhanced in NE-afflicted M5.1 line which showed higher resistance to NE whereas decreased in M15.2. This may indicate that SOCS3 could regulate excessive inflammatory response initiated by NE in M5.1 lines.

Another inflammation related gene, NFKBIZ induced by diverse PAMPs such as peptidoglycan (PGN), bacterial lipoprotein, flagellin, MALP-2, R-848, and CpG DNA, regulates NF-κB activity, possibly to prevent excessive inflammation caused by bacterial components (Eto *et al*., 2003; Yamamoto *et al*., 2004; Yamazaki *et al*., 2001).

SERPINF1, also known as pigment epithelium derived factor (PEDF), has been reported to interfere with neovascu-

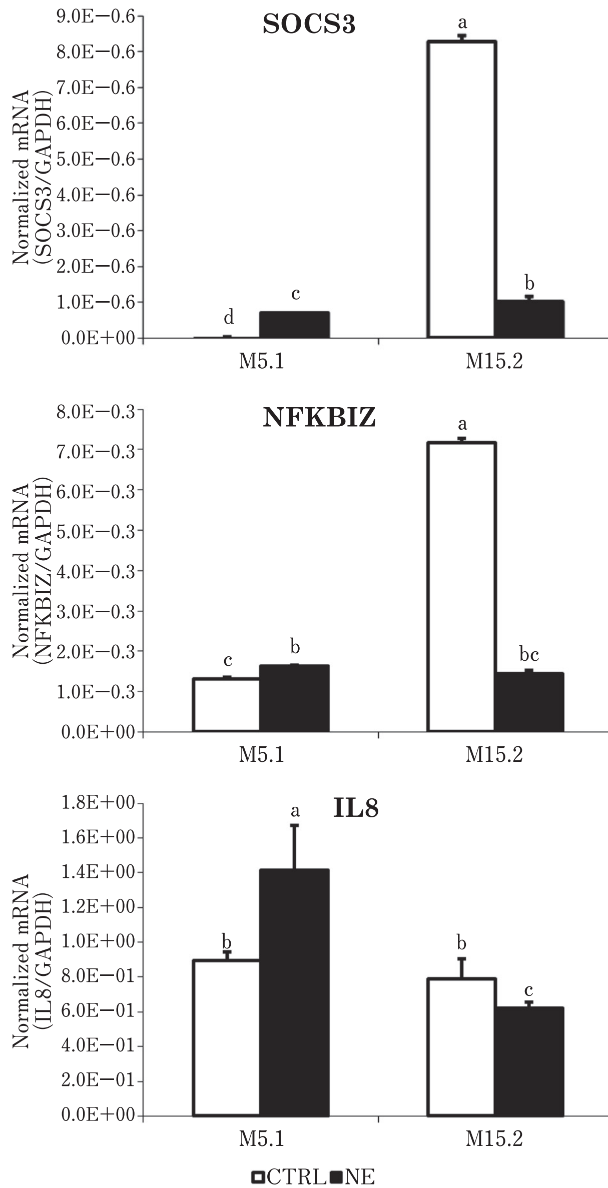

Fig. 3. **Effects of NE on the changes of expression levels of immune related genes in the intestine of Fayoumi chicken inbred lines M5.1 and M15.2.** Intestinal intraepithelial lymphocytes (IELs) transcripts for suppressor of cytokine signaling 3 (SOCS3), interleukin 8 (IL8), and nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta (NFKBIZ) were measured by quantitative RT-PCR and normalized to GAPDH transcript levels. Each bar represents the mean±S.D. value ($n=3$). The different letters show the significant differences within the treatments ($P<0.05$).

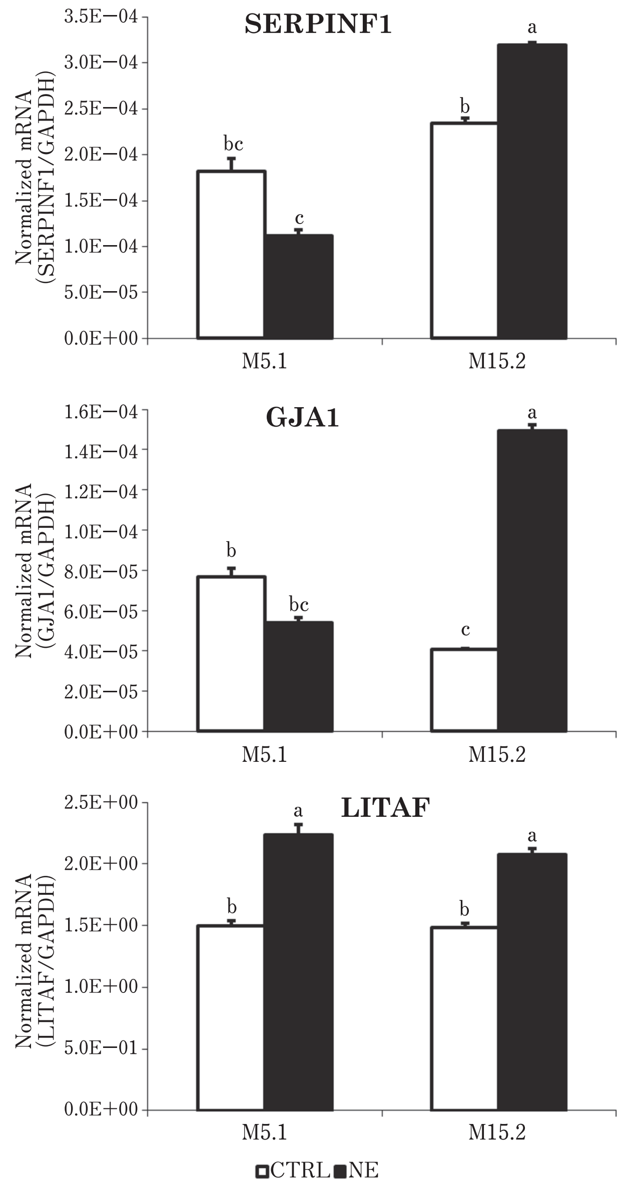

Fig. 4. **Effects of NE on the changes of expression levels of selected immune related genes in Fayoumi chicken inbred lines M5.1 and M15.2.** Intestinal intraepithelial lymphocytes (IELs) transcripts for serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (SERPINF1) and gap junction protein, alpha 1, 43kDa (GJA1), and lipopolysaccharide-induced TNF factor (LITAF) were measured by quantitative RT-PCR and normalized to GAPDH transcript levels. Each bar represents the mean±S.D. value ($n=3$). The different letters show the significant differences within the treatments ($P<0.05$).

larization and antagonize angiogenic factors such as VEGF, basic-fibroblast growth factor, platelet derived growth factor (PDGF), and IL-8 reinforcing a protective role at physiological levels for serpins (Richardson *et al*., 2006).

GJA1 has been found to play an important role in the regulation of gap junctional communication and be modulated by circulating proinflammatory cytokines such as tumor necrosis factor (TNF) α and IL-1 (Fortin *et al*., 2006).

The expression of LITAF is associated with inflammatory cytokine secretion and it functions as a tumor suppressor (Hong *et al*., 2006; Ji *et al*., 2011; Zhou and Lamont, 1999). LITAF is one of the factors responsible for the maintenance of SOCS3 and the deficiency of TNF-α (LITAF in chicken) in macrophages results in accelerated degradation of the LPS-induced SOCS3 protein (Dagvadorj *et al*., 2010; Ji *et al*., 2011). In commercial broiler chickens, LITAF was significantly upregulated at day 1 post *C. perfringens* infection. However, at 2 days post NE infection, the expression level was decreased by the same level with non-infected control group in the *E. maxima* and *C. perfringens* coinfection model of NE (Park *et al*., 2008). In this study, LITAF expression was increased in both Fayoumi inbred lines M5.1 and M15.2. From the above studies and the present results, the LITAF gene expression may be altered depend on the disease progress and inflammatory status.

In conclusion, co-infection model of NE using *E. maxima* and *C. perfringens* in two genetically disparate Fayoumi chickens, M5.1 and M15.2, showed differential effects on the clinical signs for NE in body weight loss and intestinal lesions. On the basis of clinical signs, line M5.1 showed higher resistance to NE compared to line 15.2. The expression levels of several inflammatory genes showed differential patterns between NE-afflicted and uninfected chickens suggesting a role of MHC in NE response. These results increase the understating of the mechanisms for the disease resistance to NE, and will facilitate the development of more effective control strategies against these enteric pathogens, thereby, promoting the profitability of the poultry industry and addressing the food safety concerns of consumers.

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