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Intra- and inter-breed variation in immune response to acute and sub-chronic *Salmonella* infection and commercial immune-stimulant in two-layer breeds

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Abstract

Salmonella is one of the most hazardous diseases in poultry farms. Markedly, the application of active immunostimulants is illustrated as potential protective agents against infection in poultry farms. Thus, this work aimed to explore inter- and intra-breed variation in response to acute and subchronic *Salmonella enteritidis* infection in two-layer breeds (one commercial [Hy-line strain] and another native [Fayoumi breed]). Besides exploring the possible protective effect of a commercial immune modulator (STIMULAN) on the two breeds during the acute infection. The ELISA antibody titer in sub-chronic infections and the expression analysis of some selected genes (*IL-1β*, *LITAF*, *TGF-β*, *HSP90* and *HSP70*) are used as the clinical signs for acute infections to assess the possible protective role of a commercial immunomodulator (STIMULAN). Five groups were used during the acute experiment: G1—control; G2a—susceptible; G2b—resistant birds, G3—which received STIMULAN and G4—which received the infection + STIMULAN. The groups with sub-chronic infections include G1 (control), G2 (high antibody titer) and G3 (low antibody titer). The gene expressions among the susceptible birds during acute infection of both breeds are nearly similar. They only differ in the expression of *HSP90* in the Fayoumi breed. However, the resistant birds vary in their gene expression profile. The effect of STIMULAN as a feed additive in non-infected birds was an up-regulation of *LITAF*, *TGF-β*, *HSP90* in Fayoumi. Moreover, a powerful stimulatory role was observed when both breeds were infected. Both breeds were asymptomatic during the sub-chronic infection. Although, the increased expression of inflammatory-related genes in the Hy-line was considered as an indication of infection persistence. Fayoumi is capable of immune clearance for this infection. Thus, the Fayoumi breed is more resistant to acute *Salmonella* infection. *HSP90* plays a vital role in its resistance. We recommend the use of STIMULAN as an immunomodulator during *Salmonella* infection.

KEYWORDS
breed effect, gene expression, immune response, immunomodulator, *Salmonella enteritidis*

1 | INTRODUCTION

Salmonella is one of the most hazardous diseases on the poultry farm. *Salmonella enterica* can infect chickens at any time during their lives, and also it is one of the most common causes of food poisoning in humans through contaminated meat, eggs and egg products, causing severe economic and public health problems (Afshari et al., 2018).

Chickens' genetic variation in their resistance to *Salmonella* infection was reported among breeds and individuals within the same breeds; also, the genetic basis of the host determines the level of bacterial pathogenesis and the severity of the disease (Tohidi et al., 2018). *Salmonella enteritidis* caused acute systemic disease in susceptible chickens (Withanage et al., 2005). Some other birds do not show any clinical signs, and their response to the infection occurred in the form of mild cecum inflammation, with infiltration of heterophil and monocyte/macrophage in its mucosa. Furthermore, they have increased expression of pro-inflammatory cytokines, interferon-gamma (*IFN-γ*) and nitric oxide synthase (*iNOS*) in their intestine (Matulova et al., 2013), and other internal organs like the spleen, liver and cecal tonsils at 6–48-hr post-infection (Withanage et al., 2005).

The chronic, asymptomatic *Salmonella* carrier is thought to be a cause of maintenance of infection in the poultry house and also possible contamination of eggs by laying hens as the intestinal carriage may occur for several months after the infection depending on the bird adaptive immune response's ability for immune clearance of *Salmonella* infection (Withanage et al., 2005).

The characterizations of the immune response to acute and chronic *Salmonella* infection were reported in previous studies; including the expression of genes coding for chemokines, cytokines such as interleukin 1*β* (*IL-1β*), Lipopolysaccharide-induced *TNF-α* factor (*LITAF*), or antimicrobial peptides in the lymphatic organs and at the cecum (Crhanova et al., 2011). The role of the expression of heat shock proteins during infection was reported; they conferred powerful effects on the stimulation of innate immune responses, mediate phagocytosis and provide protection against inflammation by suppressing the production of inflammatory mediators (Malago et al., 2005). The transforming growth factor-*β*1 (*TGF-β*) gene is a well-known component of regulatory cytokines, which has pleiotropic effects in immune defense systems against bacterial infection; it also regulates the expression of chicken *HSP70* and *HSP90* (Takenaka & Hightower, 1992).

Preventing *Salmonella* in chicken flocks is one of the significant challenges, and many feed additives as essential oil, acetic acid, prebiotic and others were used for this purpose. They are used to reduce pathogenic effects of bacteria, improve bird's performance, decrease the cost of treatment of the infected flock, improve intestinal health and minimize the shedding of *Salmonella* in feces (Attia et al., 2012; Lee et al., 2018).

This work aimed to explore inter- and intra-breed variation in response to acute and subchronic *Salmonella* infection in two-layer breeds (one commercial [Hy-line strain] and another native [Fayoumi breed]). By using the clinical signs in acute infection, ELISA antibody titer in sub-chronic infection and the expression analysis of some selected genes as *IL-1β*, *LITAF*, *TGF-β*, heat shock protein 90 (*HSP90*) and heat shock protein 70 (*HSP70*). Also, explore the possible protective effect of a commercial immune modulator (STIMULAN) on the two breeds during the acute infection.

2 | MATERIALS AND METHODS

2.1 | Birds

Two-layer breeds were used, one commercial (Hy-line strain) and another native (Fayoumi breed). The Fayoumi chicks were obtained from an integrated poultry project in Fayoumi Governorate, Egypt, and Hy-line chicks were obtained from a private farm at Borg El Arab city in Egypt. One hundred and twenty birds of 1-day-old male chicks for each breed were used in this study. The birds were housed in concrete floor rooms covered with wood shavings, and wooden partitions were used to separate the different groups. The birds were fed a starter feed from 1–7 days old and grower feed from 8–45 days old. The chicks received a regular vaccination regime; optimum housing conditions include the availability of food, water, ventilation and 12 h light/dark system.

2.2 | Feed additives

The immunostimulant (STIMULAN) was added in a dose of 1 ml/L liter drinking water (Arabcomed-Egypt, Obour City, Cairo, Egypt). Each 1 L contains vitamin E (100 g), selenium (0.540 g), zinc (0.2835 g), iron (0.580 g), propylene glycol (100 ml) and water (carrier) up to 1 L.

2.3 | Experimental design and sample collection

2.3.1 | Bacteria

Salmonella enteritidis used for inducing experimental infection was obtained from the Animal Health Research Institute, Cairo, Egypt, in the form of slope and stored on glycerol at −20°C until use. They were dissolved in the nutrient broth for 48 h and were cultured on MacConkey agar media. The confirmatory test for *S. enteritidis* was done as a urease test, and a triple sugar test and serotyping to one slope was performed using PCR in a private diagnostic laboratory in Alexandria, Egypt. The colonies from nutrient agar media were used to make titration by Mackfirland reaction to estimate the LD50 (10^8 CFU) and 1/2 LD_{50} (5×10^7 CFU), which were used in this study (Ishola et al., 2008).

2.3.2 | Acute *Salmonella* infection

The birds of each breed were allocated into four groups: Group 1: Control group (G1) (20 birds). Group 2 (60 birds): Infected intra-esophageal at the age of 2 weeks with 10^8 CFU /bird of *S. enteritidis* according to Ishola et al. (2008), 48 h post-challenge, the birds in this group were divided into two subgroups depending on their clinical signs,

TABLE 1 Antibody titer of high and low immune response in Fayoumi and Hy-line using ELISA

Breed*	Immune status	ELISA- level
Fayoumi breed	High	2.38 ± 0.05^{a}
	Low	1.86 ± 0.04^{c}
Hy-line strain	High	2.19 ± 0.09^{b}
	Low	1.26 ± 0.06^{d}

*All values are expressed as Mean±SE. The number of birds in each group = 20.
Values with different letters within the same column are significant at ($p < 0.01$).

the susceptible birds (G2a) with diarrhoea, in-appetence and ruffled feathers and the resistant birds (G2b), which appeared normal. Group 3(G3): Receive immune modulator (STIMULAN), which was added to the drinking water at a dose of 1 ml/L from 1 day old until the end of the experiment (20 birds) and group 4(G4): infected with 10^8 CFU/bird at 2 weeks of age plus STIMULAN (1 ml/L) (20 birds). The birds were then euthanized by cervical dislocation at the age of 3 weeks, and spleen tissue was dissected, washed in saline and stored at −80°C until further use for RNA isolation and expression analysis of selected genes.

2.3.3 | Subchronic *Salmonella* infection

At 2 weeks of age, two groups of chicks were used, a control group (G1) without any treatment, and the other group was inoculated intra-esophageal with *S. enteritidis* with a dose of 5×10^7 CFU (1/2 LD_{50}) (20 birds/group). Three weeks following the infection, blood samples were collected and used for measuring the antibody titer by ELISA, and the antibody titer was a guide for subdivision of this group into a high immune response (high antibody titer) (G2) and low immune response (low antibody titer) (G3). At the age of 5 weeks, the spleen tissues were collected in the same manner as in acute infection groups for gene expression analysis.

2.4 | ELISA

ELISA was used to classify birds into high and low immune responses according to antibody titter in the subchronic infected group. High antibody titer ranged from 1.80 to 2.78, and low titer ranged from 0.97 to 1.79 (Table 1).

2.5 | Haematological parameters

The total leukocytic count was determined using the haemocytometer. Differential leukocyte count (basophils, eosinophils, monocytes, heterophils and lymphocytes) was performed.

2.6 | Isolation of RNA and cDNA synthesis

Total RNA was extracted using (Trisure reagent)® according to the manufacturer's instructions (Intron, Korea). Extracted RNA was stored at −80°C. Total RNA was reverse transcribed into cDNA by using a power first-strand cDNA synthesis kit (Intron, Korea) according to the manufacturer protocol. The obtained cDNA was stored at −20°C until further use. The cDNA was checked by the housekeeping gene (18s rRNA), then the product was then checked on 2% agarose gel electrophoresis.

2.7 | Quantitative Real-time polymerase chain reaction

Quantitative real-time PCR for the inflammatory-related genes (*IL-1β*, *LITAF*), *TGF-β*, *HSP90* and *HSP70* were done in the qPCR machine (3000 × Stratagene, USA). The qRT-PCR reaction was performed by using SensiFastTM SYPER Low-Rox kit (Enzynomic, USA). Primers annealing temperature and PCR products are listed in Table 2. In 0.2 ml PCR tubes, the following ingredients were included, 0.8 μl of each forward and reverse primer, 2 μl of cDNA, 6.4 μl RNAas free water and 10 μl

TABLE 2 Sequences of primers used in real-time PCR

Genes	Primer sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)	Acc. Number	Reference
IL-1β	F: CAGCCTCAGCGAAGAGACCTT R: CACTGTGGTGTGCTCAGAATCC	60°C	166	XM_015297469.1	Ghareeb et al. (2013)
HSP90	F: GAGTTTGACTGACCCGAGCA R: TCCCTATGCCGGTATCCACA	60°C	107	NM_001109785.1	Xie et al. (2014)
HSP70	F: CCAAGAACCAAGTGGCAATGAA R: CATACTTGCGGCCGATGAGA	60°C	72	NM_001006685.1	Rimoldi et al. (2015)
LITAF	F: CCCCTACCCTGTCCCACAA R: TGAGTACTGCGGAGGGTTCAT	63°C	67	XM_015294125.1	Ghareeb et al. (2013)
TGF-β	F: ACCAGGTCCTACTCCAGGAAGAC R: AAAGCAGACAGGTCCAGCAATAA	63°C	82	XM_015275817.1	Ghareeb et al. (2013)
18s	F: CGAAAGCATTTGCCAAGAAT R: GGCATCGTTTATGGTCGG	60°C	98	XM_015288030.1	Primer 3

TABLE 3 Differential leukocytic count in Fayoumi breed and Hy-line strain

Breed	Immune status	Lymphocytes Mean ± SE	Basophils Mean ± SE*	Eosinophils Mean ± SE	Monocytes Mean ± SE	Heterophils Mean ± SE
Fayoumi	High	60.27 ± 3.83^{b}	0.67 ± 0.13^{a}	2.93 ± 0.25^{c}	4.93 ± 0.28^{d}	60.27 ± 3.83^{b}
	Low	55.50 ± 4.86^{d}	0.38 ± 0.18^{c}	4.00 ± 0.38^{a}	6.63 ± 0.38^{a}	55.50 ± 4.86^{d}
Hy-line	High	56.82 ± 5.26^{c}	0.45 ± 0.16^{b}	3.27 ± 0.36^{b}	5.82 ± 0.52^{b}	56.82 ± 5.26^{c}
	Low	64.00 ± 4.18^{a}	0.22 ± 0.15^{d}	2.22 ± 0.22^{d}	5.56 ± 0.56^{c}	64.00 ± 4.18^{a}

*All values are expressed as Mean ± SE. The number of birds in each group = 20.
Values with different letters within the same column are significant at ($p < 0.01$).

SYPER green. The thermal cycler condition was one cycle of denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and annealing temperature of 60°C for 1 min. To ensure that a signal product was amplified, a melting curve analysis was performed on the PCR products at the end of each PCR run. The 18s rRNA was used as a housekeeping gene.

2.8 | Statistical analysis

Data analysis was done by one-way ANOVA. Statistical analysis was done using GraphPad Prism software version 6 (Graphprism Software, La Jolla, California, USA). The results were obtained as mean ± standard error (*SE*). The analysis of gene expression was performed by comparative threshold cycle method $2^{-\Delta\Delta ct}$ (Rao et al., 2013).

3 | RESULTS

3.1 | Differential leukocytic count of high and low immune response

Differential leukocytic count; in Fayoumi breed, acute *Salmonella* infection induces a significant increase in lymphocytes, basophils and heterophils in high immune response birds than low immune response, which have a significant increase in eosinophils and monocytes (Table 3).

Hy-line strain showed a significant increase in basophils, eosinophils and monocytes in high immune response birds compared with low immune response, which has a significant increase in lymphocytes and heterophil.

3.2 | Gene expression

3.2.1 | Acute infection of *Salmonella*

Acute *Salmonella* infection in susceptible Fayoumi birds (G2a) was associated with a significant up-regulation of inflammatory-related genes *IL-1β* to ninefolds, and mild up-regulation of *LITAF* gene to twofolds was recorded (Figure 1A and B), meanwhile; it is associated with a significant decrease of *TGF-β* and *HSP90* to −0.2 and −0.3 folds (Figure 2A and B). In resistant birds (G2b) (without clinical sign), they showed a significant up-regulation of inflammatory-related genes (*IL-1β* and *LITAF*) to 2- and 87-folds; no change was observed in the expression of *TGF-β*. Significant up-regulation of *HSP90* to eightfolds was observed.

Regarding the effect of STIMULAN (immune-modulator) alone (G3) in Fayoumi, no effect on *IL-1β* gene expression was observed; meanwhile, significant up-regulation of both *LITAF*, *TGF-β* and *HSP90* to 8-, 1.8- and 30-folds, respectively were observed. The combined

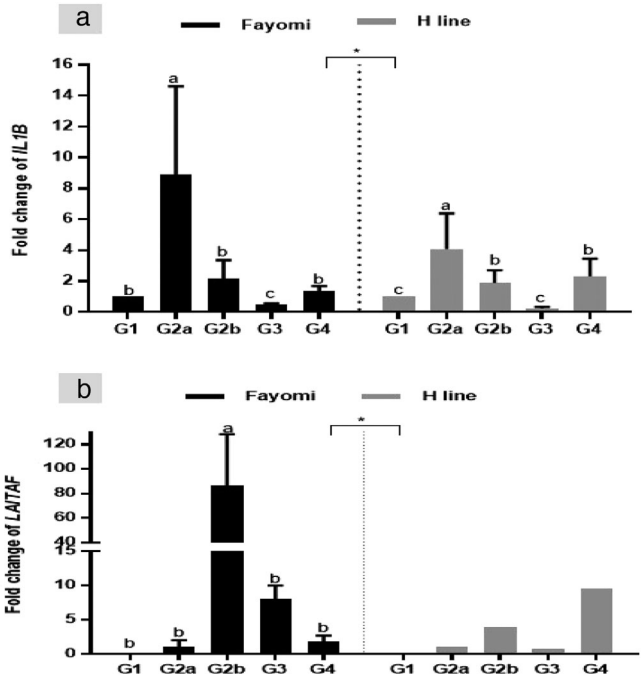

FIGURE 1 Relative gene expression of inflammatory-related genes (*IL1-1β*, *LITAF*) in Fayoumi breed and Hy-line strain in different treatment of acute infection. All values are expressed as Mean±SE. Values with different letters within the same breed are significant at ($p < 0.01$). Asterisks mean intra-breed significant. Groups; G1 (control), G2a (susceptible to infection with 10^8cfu /bird of *S. enteritidis* with clinical sign), G2b (resistant to infection with 10^8cfu /bird of *S. enteritidis* without clinical sign), G3 received STIMULAN only and G4 received STIMULAN and infected with 10^8cfu /bird of *S. enteritidis*

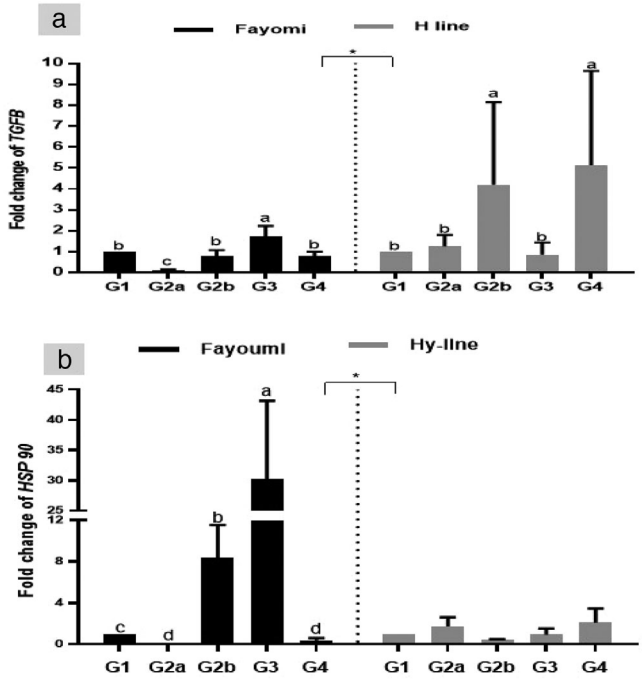

FIGURE 2 Relative gene expression of *TGF-β* and *HSP90* genes in Fayoumi breed and Hy-line strain in different treatment of acute infection. All values are expressed as Mean±SE. Values with different letters within the same breed are significant at ($p < 0.01$). Asterisks mean intra-breed significant. Groups; G1 (control), G2a (susceptible to infection with10^8 CFU/bird of *S. enteritidis*-with clinical sign), G2b (resistant to infection with10^8 CFU/bird of *S. enteritidis*- without clinical sign), G3 received STIMULAN only and G4 received STIMULAN and infected with 10^8 CFU/bird of *S. enteritidis*

administration of the STIMULAN with *Salmonella* infected birds in G4 significantly induced up-regulation of *IL-1β* to twofolds. Meanwhile, no effect was observed on the expression of the other genes; they were maintained within the control level.

Regarding the acute infection in the Hy-line strain, susceptible birds (G2a) showed a significant up-regulation of only *IL-1β* to fourfolds and had no marked change in the expression of the other genes. In the resistant birds (G2b), mild up-regulation of inflammatory-related genes (*IL-1β*, *LITAF*) was observed to 1.8 and fourfold, significant up-regulation of *TGF-β* to 4.5-folds and without change in the expression of *HSP90* was observed (Figure 2).

STIMULAN treatment in Hy-line strain alone (G3) showed no marked effect in all studied genes; meanwhile, the group that received the combination of STIMULAN and *Salmonella* infection showed different levels of gene expression; *IL-1β* gene significantly increased to 2.5-folds and *TGF-β* gene to fivefolds. Furthermore, up-regulation of *LITAF* and *HSP90* to 9- and 2.5-folds was observed (Figure 2).

Regarding the effect of the breed, the variable immune response was observed between Fayoumi and Hy-line, higher expression of inflammatory-related genes (*IL-1β* and *LITAF*) and *HSP90* in Fayoumi was observed; meanwhile, TGF-*β* gene expression increased in Hy-line strain (Figure 2).

3.2.2 | Sub-chronic infection of *Salmonella*

Sub-chronic *Salmonella* infection induced different gene expression profiles three-week post-challenge in both breeds (Figure 3 A, B, C, D and E). In Fayoumi, no change was observed in the expression of *IL-1β* and *HSP90* among the G2 (high immune response birds) and G3 (low immune response birds). An increase in *LITAF* and *HSP70* gene expression to 6.5- and 28-folds and significant down-regulation of *TGF-β* gene expression in high immune response birds (G2) was observed. In contrast, in the Hy-line strain, both *IL-1β* and *HSP90* had a significant up-regulation in their expression in G2 (high immune response birds) to 6.8- and 4.5-folds and additionally, both genes were increased at G3 (low immune response birds) to 5- and 2.8-fold (Figure 3). *LITAF* gene expression was increased to 4.8-folds in G2, and *TGF-β* gene expression increase to 6.2-folds in the G3 group, and no change was observed in *HSP70*.

4 | DISCUSSION

Acute inflammatory disease is usually associated with a change in white blood cell count. The inter- and intra-breed variation in the differential leukocytic count was observed in our study in Fayoumi and Hy-line breeds. Bílková et al. (2017) demonstrated varying leukocytic count among different chicken breeds, which reflect variable immunological adaptations based on their original need to control the infections. Hence the comparison of differential leukocytes between the two studied breeds may be of no value. However, within each breed, the distribution of WBCs between the resistance and susceptible birds differed significantly, and also, the types of cells present in the resistant bird in Fayoumi are varied from that present in the resistant Hy-line. Neto et al. (2007) demonstrated differences in blood parameters and the intensity of the organ lesions among three susceptible and three resistance lines of commercial layers infected with *Salmonella gallinarum*.

Inter- and intra-breed variation in response to acute *Salmonella* infection in the two studied layer breeds (Hy-line and Fayoumi) are definite. The performances of the susceptible birds of both breeds are nearly similar; the expression of the *IL-1β* gene is high, and the *TGF-β* and *LITAF* gene expressions are low compared with the resistant birds, and the only difference between the two susceptible breeds is the more expression of *HSP90* in Fayoumi breed. Although the resistant birds of both breeds showed mild up-regulation of *IL-1β*, the expressions of the other genes are variable. Fayoumi showed significant expression of *LITAF* and *HSP90*; meanwhile, *TGF-β* gene expression is unchanged, in Hy-line, significant up-regulation of *TGF-β*, mild up-regulation of *IL-1β*, *LITAF* and unchanged expression of *HSP90* were observed. Aricibasi et al. (2010) found that the genetic background between the most susceptible layer-types and the less susceptible broiler chickens influences the pathogenesis of the infectious bursal disease virus (IBDV). They demonstrated significant variation between circulating cytokine between the two breeds. Moreover, Swaggerty et al. (2004) demonstrated that chemokines and interleukins play a vital role during

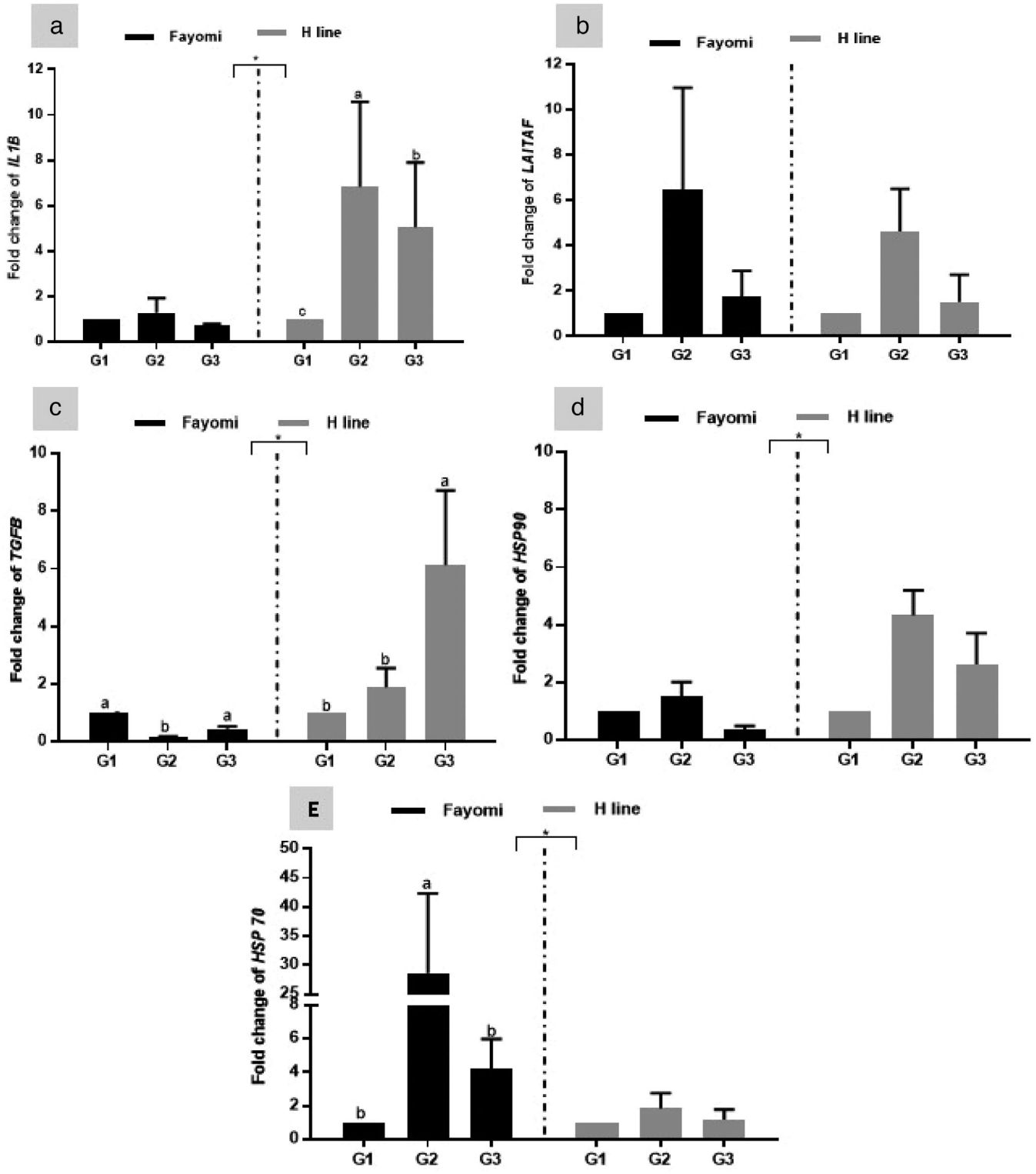

FIGURE 3 Relative expression of the studied genes in Fayoumi breed and Hy-line strain groups in different treatment of subchronic infection. All values are expressed as Mean±SE. Values with different letters within the same breed are significant at ($p < 0.01$). Asterisks mean intra-breed significant. Groups: G1 (control), G2 (high immune response birds-high antibody titer) and G3 (low immune response birds-low antibody titer)

Salmonella infections, they compared the mRNA level of different interleukins in heterophile between the resistant and susceptible chickens, and they demonstrated an increased expression of *IL-6*, *IL-8* and *IL-18* in resistant chicken as compared to the susceptible chicken. Additionally, they found a decrease in the transcripts' level of heterophile transforming growth factor (*TGF-β4*) of the resistant chicken, which is a coincidence within unchanged level of *TGF-β* in Fayoumi, which enables these chickens to quickly and efficiently initiate an acute pro-inflammatory response and the resistant state as observed by Withanage et al. (2005). The production of these cytokines such as *IL-1β*, *IL-6*, *IL-8*, *IL-12*, *IL-17*, *IFN-γ*, *LITAF* or *iNOS* in response to *Salmonella* infection of chickens have been reported in many studies (Berndt et al., 2007; Van Immerseel et al., 2002; Xie et al., 2018). The primary functions of these genes (*IL-1β*, *IL-8* and *IL-17*) are to attract leukocytes to the site of infection (Ivanov et al., 2008).

Variation in the expression of *HSP90* during the acute *Salmonella* infection between Hy-line and Fayoumi was observed. The induction of HSP by several stresses, including infection, was recorded by Malago et al. (2005) and Hassan et al. (2017). Furthermore, the breeds variation in HSP expression was recorded (Abdo et al., 2017; Xie et al., 2014).

When cells increase their HSP content, they protect themselves from lethal assaults, as the HSP interferes with the uncontrolled protein unfolding associated with stress (Bolhassani et al., 2019). We suggested that the more expression of *HSP90* during acute infection in Fayoumi breed in the resistant bird to ninefolds and also its elevation in response to STIMULAN (immune-modulator) to 30-folds are associated with increased resistance status to *Salmonella* infection and provide more protection to these birds compared with the Hy-line strain. Wallin et al. (2002) suggested that *HSP60*, *HSP70*, *HSP90* and *GP96*, have a role in stimulating innate immune-system cells directly and act as 'danger'-signaling molecules.

The effect of STIMULAN alone as a feed additive in non-infected birds is potent in Fayoumi breeds compared with Hy-line; it induced up-regulation of *LITAF*, *TGF-β* and *HSP90*. No effect was observed on the studied genes of the Hy-line strain. During infection, the expressions of these genes are changed in the groups received by the STIMULAN of both breeds. The more powerful role of STIMULAN in Hy-line strain in the form of significant up-regulation of *IL-1β*, *TGF-β* mild up-regulation of *LITAF* and significant downregulation of *HSP90*. Meanwhile, only significant up-regulation of *IL-1β* was observed in Fayoumi, while the other genes were maintained within the control level. The STIMULAN alone as a feed additive provided a significant immunomodulatory effect in Fayoumi, and during infection in both breeds, this may be due to its contents of the vitamins-mineral mix (vitamin E, selenium, zinc and iron) as each of them was recorded to have an immunomodulatory effect (Kaiser et al., 2012; Naz et al., 2016; Adedokun et al., 2019). Additionally, Sanda (2019) reported a significant increase in antibody titer to Newcastle Disease vaccine in chickens supplemented with vitamin-mineral mix compared with the control, and the group received the supplement only without a vaccine. Redmond et al. (2010) and El Nahas et al. (2019) suggested that different genetic backgrounds of chicken affect their response to immune enhancing.

The results of subchronic *Salmonella* infection three-week post-challenge revealed no change in *IL-1β* gene expression in both high and low immune response birds in the Fayoumi breed. Beal et al. (2005) reported a significant increase in the *IL-1β* mRNA level at 6-day post-infection in the spleen of the *Salmonella* infected birds followed by dropped in its expression to the control level at 13 and 20-day post-infection. Also, Chausses et al. (2011) observed the absence of the expression of *IL-1β* in infected birds with *Salmonella* at 3-week post-infection.

In our study, the sub chronically infected Hy-line, and Fayoumi breeds were asymptomatic, Withanage et al. (2005) suggested that the resistant bird may carry *Salmonella* infection for weeks without showing clinical signs. However, the gene expression profiles of both breeds under study are different. In Fayoumi, no change was observed in the expression of *IL-1β* and *HSP90* in both high and low immune response birds. An increase in the expression of *the LITAF* gene to sixfolds and significant down-regulation of *TGF-β* gene expression in high immune response birds were observed. Reverse in Hy-line strain both *IL-1β* and *HSP90* had significant up-regulation in their expression in high immune response birds to 7- and 4.5-folds. Additionally, both genes were increased at the low immune response birds. *LITAF* gene expression was also increased to fivefold in G2, and *TGF-β* gene expression increase to 6.2-folds in the resistant group. Ruby et al. (2012) revealed that chronically infected hosts who are asymptomatic could transmit disease through fecal shedding of bacteria. Additionally, Gast et al. (2019) suggested that the regulation of the inflammatory response could affect the persistent *Salmonella* infection. Furthermore, Chaussé et al. (2014) compared resistant and susceptible chicken lines to *Salmonella enteritidis* carrier-state, they identified 271 differentially expressed genes; no difference was observed in the expression of regulatory cytokines in both lines, including *IL-10* and *TGF-β* and enhanced expression of cytokines, including *IL-4* and *IL-13* and other genes which supports the immune clearance of avian salmonellosis in resistance birds, and a carrier states in susceptible birds.

We suggest that the immune clearance of *Salmonella* infection in Fayoumi breed (both high and low immune response) is better than Hy-line strain, which had a high inflammatory response of *IL-1β*, *LITAF*, *TGF-β* and also *HSP90* 3-week post-infection.

5 | CONCLUSION

In conclusion, intra and inter-breed variation in the immune response to acute and sub-chronic *Salmonella* infection in Hy-line and Fayoumi layers was observed. Fayoumi breed is more resistant to acute *Salmonella* infection; *HSP90* plays a vital role in this resistance. We recommend the use of STIMULAN as immunomodulatory during *Salmonella* infection.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION

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