Effects of heat stress on cyto(chemo)kine and inflammasome gene expression and mechanical properties in isolated red and white blood cells from 4 commercial broiler lines and their ancestor jungle fowl

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ABSTRACT Commercial broilers have been selected for high growth rate and productivity; however, this has negatively impacted their susceptibility to heat stress (**HS**). Insight into the molecular mechanisms underlying this vulnerability can help design targeted strategies for improvement of HS tolerance. Red blood cells (**RBC**) and white blood cells (**WBC**) were isolated from red jungle fowl and 4 lines of commercial modern broilers. Lines A and B are considered standard-yielding lines, whereas Lines C and D are high-yielding. Cells were cultured at either 37°C or 45°C for 2 h to induce heat stress (HS). Gene expression of cytokines, chemokines, and inflammasome components were measured. Heat shock proteins 27 and 70 (**HSPs**) in RBC were significantly affected by line ($P < 0.05$), whereas HSP27 and 60 were affected by temperature ($P < 0.05$). In WBC, there was a significant line effect on HSP gene expression ($P < 0.05$), and a significant increase ($P < 0.05$) in HSP90 in Line D in HS compared to TN conditions. In RBC, there was a main effect of HS on TNFα, CCL4, and CCLL4 ($P < 0.05$). HS significantly increased IL-8L1 (>30-fold, $P < 0.0001$) in Line C. Inflammasome genes (NLRP3, NLRC5 and NLRC3) were significantly affected by the line studied ($P < 0.05$). In WBC, the effect of line was significant for all cytokines, chemokines, and inflammasome components studied ($P < 0.05$). To examine the mechanical properties of isolated RBC from the 4 commercial lines and jungle fowl, RBC were placed into nematic liquid crystals, where Lines B and D were the most strained, and Line A and the jungle fowl were the least strained. Together, these findings indicate not only the dynamic nature of circulating cells, but the differences in the stress and inflammatory response among commercially available lines and their common ancestor. These profiles have the potential to serve as a future marker for stress responses in broilers, though further study is warranted.

Key words: broiler, red blood cell, white blood cell, heat stress, nematic liquid crystal

INTRODUCTION

Sustainable agricultural production is necessary to meet the growing demand for high quality protein due to the projected increases in world population (United Nations, 2017). Broiler chickens play an important role in worldwide meat production, as they are one of the most efficient food sources, and support the livelihoods and food security of billions of people (Smith et al., 2013). Genetic selection for high growth rate and enhanced muscle development has made remarkable progress in term of breast yield, feed efficiency, and reduction of market age (Siegel, 2014);

however, this increase has not come without negative consequences, including hypersensitivity to high environmental temperatures. Heat stress (**HS**) adversely affects feed intake, growth, meat yield, and mortality in the modern broilers and results in an estimated annual economic loss to the U.S. poultry industry of more than $128 million (St-Pierre et al., 2003). As well as economic concerns, HS is a wellbeing issue, as it greatly increases the animals' discomfort and distress, an issue that has become a serious concern to the consumer.

HS is detrimental to all agricultural systems, but particularly poultry, as their selection for fast growth and high meat yield has decreased their thermotolerance and negatively affected immune competence of the modern broilers (Qureshi and Havenstein, 1994). In order to develop effective nutritional or managerial interventions to alleviate the negative consequences of HS, mechanisms underlying the HS response need to be

understood. As HS induces a plethora of undesirable health and production consequences, monitoring circulating cells presents a unique target for assessing the whole-bird status. To that end, we examine here the effects of HS on the circulating cells of ancestral Jungle Fowl and 4 commercial lines of modern broiler chickens, 2 of which are considered standard-yielding and 2 considered high-yielding. Though white blood cells (**WBC**) are considered primary components of the inflammatory response, red blood cells (**RBC**) are an under-studied cell type, typically considered only in terms of respiratory gas exchange. In chicken, however, RBC are nucleated, and represent a dynamic cell type, as they are transcriptionally active and have protein synthetic capabilities, and may contribute significantly to the immune response, through both receiving and sending of cellular signals (Morera et al., 2011). Based on the functions and characteristics of circulating cells, changes in cytokine and chemokine expression profile can be important indicators of physiological changes in both WBC and RBC function in chickens. In addition, Nayani and co-workers recently developed a unique, rapid, liquid-crystal based tool for quantifying the mechanical properties of mammalian RBC to apply to research and diagnostic procedures (Nayani et al., 2020). Here, we additionally sought to optimize and apply this technique to avian RBC, and explore its potential as an indicator of bird health.

MATERIALS AND METHODS
### Animals, Experimental Design, and Sampling

This study was conducted in accordance with the recommendations in the guide for the care and use of laboratory animals (National Research Council, 2011) from the National Institutes of Health and all protocols were approved by the University of Arkansas Animal Care and Use Committee under protocol # 21050. Chicks from the South East Asian jungle fowl (**JF**), the wild-type ancestor of the modern broiler and 4 commercial lines (lines A-D, n = 4/line) were provided by Dr. Sara

Orlowski and reared at the University of Arkansas Poultry Research Farm. Birds were sexed and wing tagged for identification, and reared in a floor pen with fresh shavings and ad libitum access to feed and water. Lines A and B are standard yielding commercial strains that normally go to market at approximately 2 kg body weight, whereas Lines C and D are considered high-yielding, with a market weight around 4 kg. Approximately 1 mL of whole blood was collected via heart puncture from euthanized birds using a 3 mL syringe and a 1-inch 20-G needle from 7-day-old males and placed into K_2 EDTA blood collection tubes (Becton Dickinson, Franklin Lakes, NJ) to prevent coagulation. Blood samples were individually identified, and circulating cells were isolated and analyzed from individual birds.

RBC and WBC Isolation and HS Treatment

RBC were collected as previously described (Jin et al., 2020). Briefly, blood collection tubes were centrifuged at 1,500 g for 3 min at 4°C. The supernatant was removed, and RBC were washed with phosphate-buffered saline, pH 7.8, three times. Isolated cells were diluted in RPMI1640 (Life Technologies, Carlsbad, CA) supplemented with 10% FBS and 1% penicillin/streptomycin and seeded at a density of 2×10^6 cells per well in 6-well tissue culture dishes (Greiner Bio-One, Monroe NC). WBC were isolated as described by Swaggerty et al. (2008). Briefly, 5mL of whole blood was mixed with 10mL of 1% methyl cellulose in RPMI1640. The tube was vortexed briefly, then centrifuged at $100 \times g$ for 20 min at 4°C. The supernatant was then mixed with 10 mL of RPMI1640 and centrifuged at $500 \times g$ for 15 min at 4°C. The cell pellet was washed 3 times with RPMI1640, then diluted in RPMI1640 supplemented with 10% FBS and 1% penicillin/streptomycin and seeded at a density of 2×10^6 cells per well in 6-well culture dishes (Greiner Bio-One). All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Representative images of isolated RBC and WBC are shown in Figure 1. Heat stress was induced by
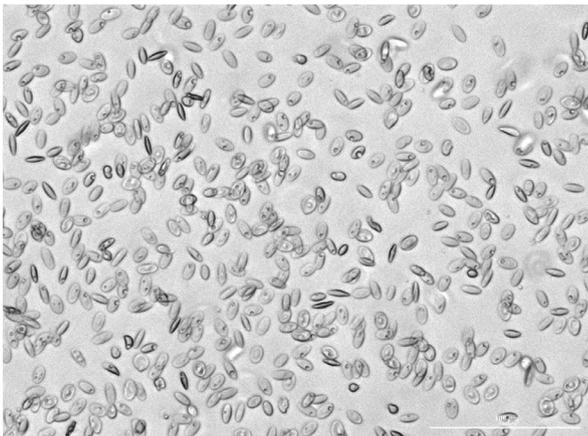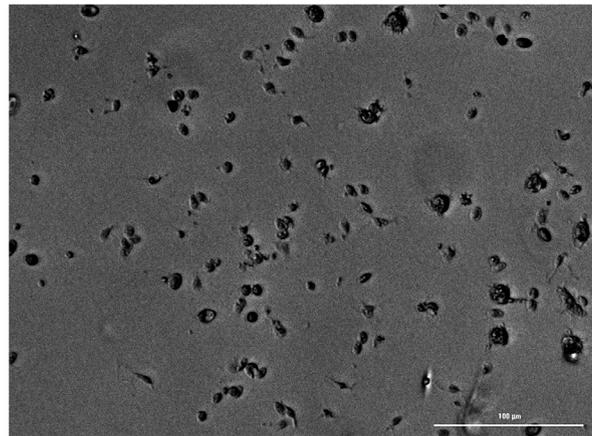

Figure 1. Representative images of isolated (A) RBC and (B) WBC.

incubating cells at 45°C in a humidified atmosphere of 5% CO_2 and 95% air for 2 h. The 2 h time for HS was selected based on our lab's previous experience across multiple cell types (Nguyen et al., 2017; Dhamad et al., 2020). Cells were then removed from the culture plate and pelleted via centrifuge ($300 \times g$ for 5 min). Excess media was removed, and 1 mL of TriZol added to the cell pellet. Samples were stored at -80°C until mRNA extraction and analysis.

RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from RBC and WBC using Trizol reagent (Life Technologies) according to the manufacturer's instructions, and concentration and quality were determined by using the Take3 micro-volume plate and the Synergy HT multimode microplate reader (Bio-Tek, Winooski, VT). RNA was reverse transcribed using qScript cDNA Sythesis Supermix (Quanta Biosciences, Gaithersburg, MD), and amplified by qPCR (Applied Biosystems 7500 Real Time System) with PowerUp SYBR green master mix (Life Technologies) as previously described (Dhamad, Greene, Sales, Nguyen, Beer, Liyanage and Dridi, 2020). Relative expression of the target genes was determined using the $2^{-\Delta\Delta CT}$ method, with normalization to r18s expression (Schmittgen and Livak, 2008). The cells isolated from JF under TN conditions were used as the control group for all analyses. Oligonucleotide primer sequences specific for chicken have be previously reported (Nguyen, Greene, Kong, Bottje, Anthony and Dridi, 2017; Mullenix et al., 2021).

Dispersing RBCs in Lyotropic Liquid Crystals

Isolated chicken RBCs were re-dispersed in an isotonic solution of NaCl (154 mM) following centrifugation at room temperature for 5 min at 7,500 rpm. Lyotropic liquid crystals containing disodium cromoglycate (DSGC; Sigma-Aldrich, St. Louis, MO) were prepared by mixing 17.3 wt % of DSCG with 82.7 wt % deionized-distilled water (18.2 MΩcm). The mixture was placed on a shaker for 4 to 8 h to ensure homogeneity. Then, 0.5 vol% (0.5 μL) of RBCs dispersed in isotonic aqueous NaCl solutions were added to the (100 μL) DSCG solution and gently stirred to disperse the RBCs in the DSCG solution. The pH of the 17.3 wt % DSCG solution as prepared was 9.2, and was lowered to 7.4 with 1 M HCl. The experimental results presented were found to be independent of pH for values between 9.2 and 7.4.

Microscopy

Images were obtained with an Olympus BX41 fitted with 40X objectives (Olympus, Center Valley, PA). Polscope imaging was performed with an Olympus CX60 microscope equipped with a Cambridge Research Incorporated Abrio LC-PolScope package. The LC PolScope used monochromatic illumination at 640 nm. A Carl-Zeiss LSM 700 laser scanning confocal microscope (Carl Zeiss Microscopy, White Plain, NY) was used to obtain the confocal micrographs using a 632-nm laser.

Statistics

Gene expression data were analyzed by 2-way ANOVA, with line (JF, lines A-D) and temperature (TN, HS) as factors using GraphPad Prism v. 7.03 (San Diego, CA). When significant main effects were detected, means were compared using Tukey's multiple range test. Significance was set at $\alpha = 0.05$. All data are represented as means $\pm$ SEM.
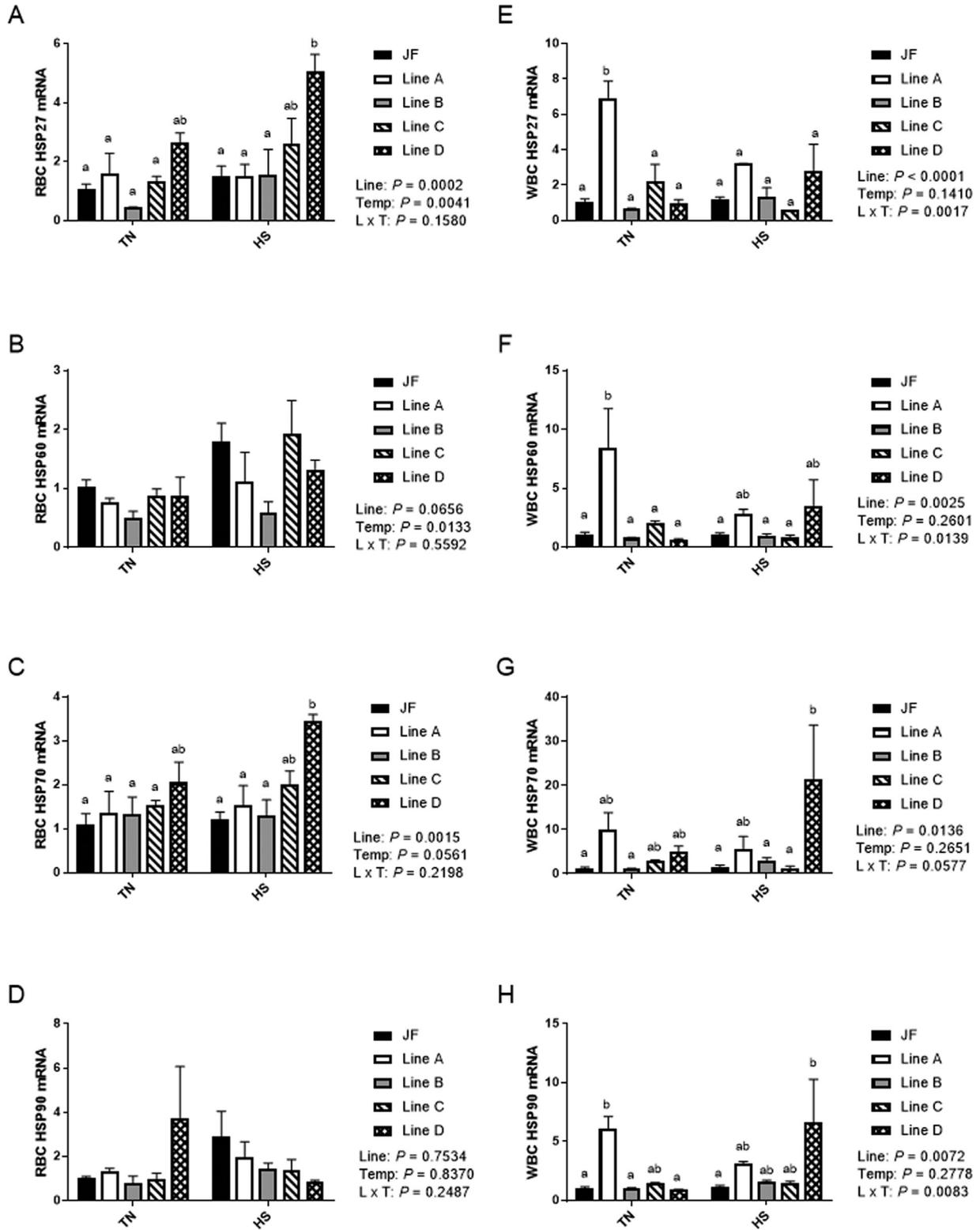
RESULTS

Heat Shock Protein Response in Isolated RBC and WBC From JF and 4 Commercial Lines of Broilers

There was a main effect of HS on heat shock proteins 27 and 60 (HSP27 $P < 0.01$, HSP60, $P < 0.05$) in RBC. Heat shock proteins 70 and 27 (HSP70, HSP27) showed a similar pattern of gene expression in RBC, where high-yielding Line D showed the greatest response to 2 h of HS exposure (Figures 2A−2D). In isolated WBC, there was a significant line effect ($P < 0.05$) on gene expression of HSP27, 60, 70, and 90 (Figures 2E−2H).

Cytokine and Chemokine Gene Expression in Isolated RBC and WBC From JF and 4 Commercial Lines of Broilers

Though all of the interleukins measured (IL-4, IL-6, IL-8L1, IL-10, IL-18) were expressed in RBC, there were no main effects of HS on their gene expression ($P > 0.05$, Figures 3 and 4), except for IL-8L1 showed main effects of HS ($P < 0.001$) and line ($P < 0.0001$), where there was a significant increase (>30-fold, $P < 0.0001$) in Line C during HS as compared to TN conditions (Figure 3B). In contrast, there was a main effect of line ($P < 0.001$) on all cytokines measured in WBC, as well as an effect of HS ($P < 0.05$) on IL-4 (Figure 4C), where gene expression was decreased with acute HS. In RBC, there was a main effect of HS ($P < 0.05$) on tumor necrosis factor α (**TNFα**), CCL4 and CCLL4, where gene expression was increased by HS, an effect primarily driven by the high-yielding lines (Line C and D, Figures 3E, 5A and 5C). Additionally, there was a main effect of line on CCL4 mRNA abundance ($P < 0.05$). In WBC, line was significant ($P < 0.05$) for all chemokines studied (Figures 4E−4H).
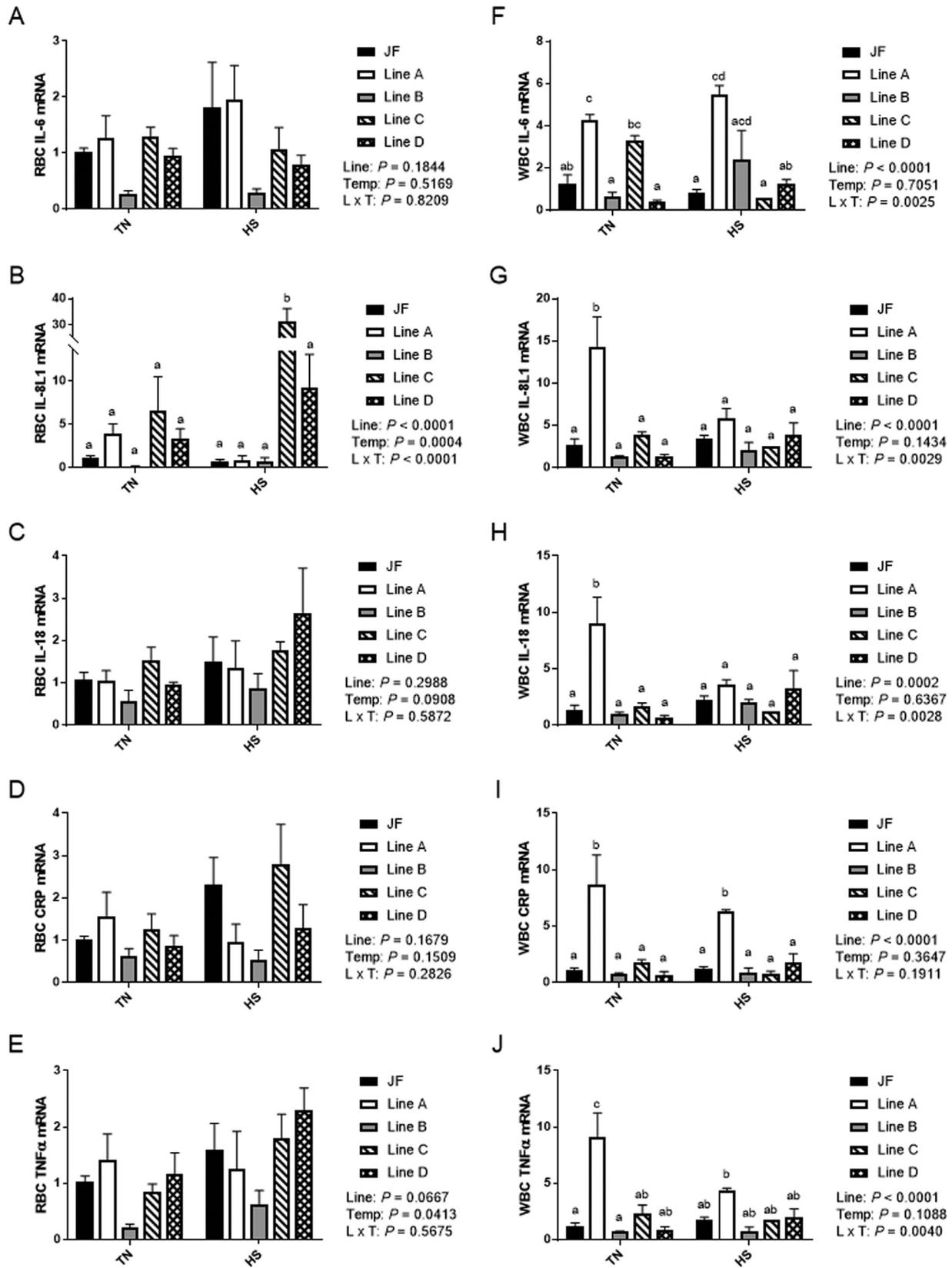
Figure 2. Effect of HS on heat shock protein gene expression in RBC and WBC isolated from jungle fowl and 4 commercial lines of broilers. The relative gene expression of HSP27 (A, E), HSP60 (B, F), HSP70 (C, G), and HSP90 (D, H) in RBC (A–D) and WBC (E–H) was determined by qPCR and analyzed by $2^{-\Delta\Delta Ct}$ method using JF-TN as a calibrator. Data are presented as mean ± SEM. Main effects of line, temperature, and the interaction are presented next to each graph. Different letters indicate significant difference at $P < 0.05$.

Expression Profile of Inflammasome Genes in Isolated RBC and WBC From JF and 4 Commercial Lines of Broilers

In RBC, gene expression of NLRP3, NLRC5, and NLRC3 showed a main effect of line of the chicken ($P < 0.05$, Figures 6A–6C). In the WBC, NLRP3, NLRC5, and NLRC3 gene expression were also significantly affected by line ($P < 0.01$), but NLRC3 and NLRC5 also showed a main effect of temperature ($P < 0.05$). The interaction of line and HS was also significant for the 3 inflammasome markers $(P < 0.05)$, whereby gene

Figure 3. Effect of HS on gene expression of proinflammatory cytokines in RBC and WBC isolated from jungle fowl and 4 commercial lines of broilers. The relative gene expression of IL-6 (A, F), IL-8L1 (B, G), IL-18 (C, H), CRP (D, I), and TNFα (E, J) in RBC (A−E) and WBC (F−J) was determined by qPCR and analyzed by $2^{-\Delta\Delta Ct}$ method using JF-TN as a calibrator. Data are presented as mean ± SEM. Main effects of Line, Temperature, and the interaction are presented next to each graph. Different letters indicate significant difference at $P < 0.05$.
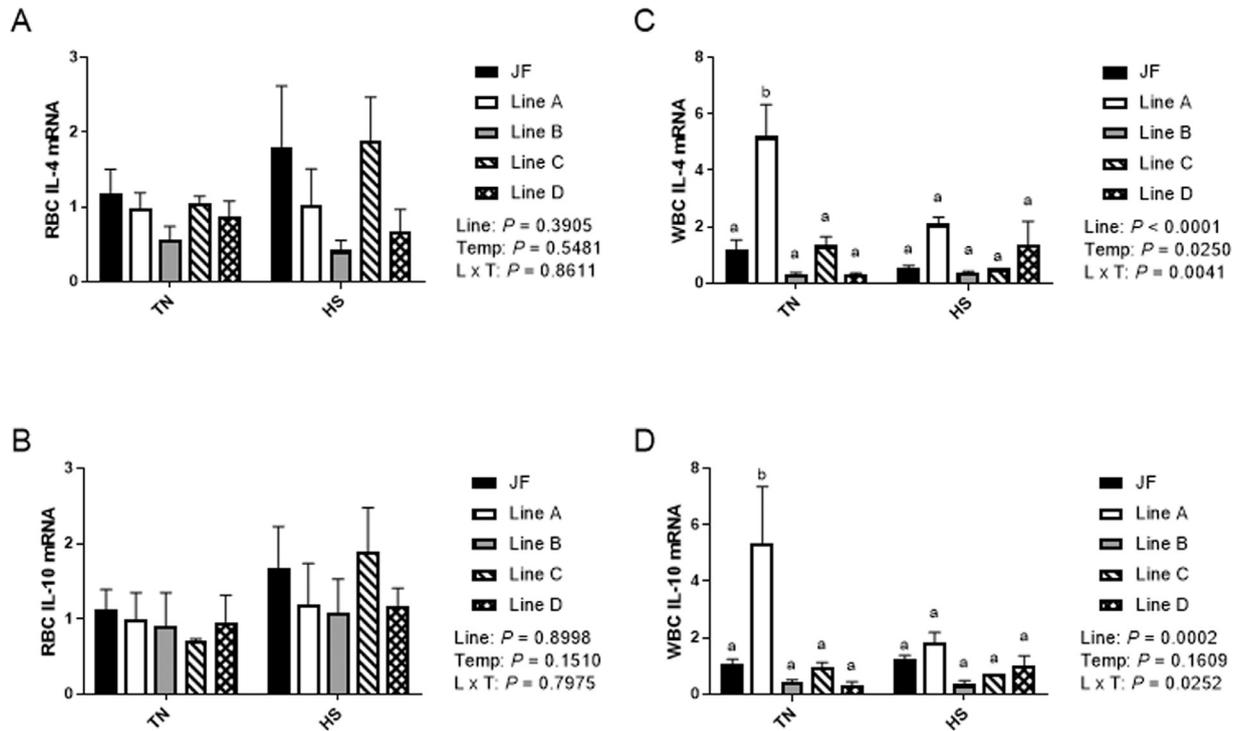
Figure 4. Effect of HS on gene expression of anti-inflammatory cytokines in RBC and WBC isolated from jungle fowl and 4 commercial lines of broilers. The relative gene expression of IL-4 (A, C), and IL-10 (B, D) in RBC (A and B) and WBC (C and D) was determined by qPCR and analyzed by $2^{-\Delta\Delta Ct}$ method using JF-TN as a calibrator. Data are presented as mean ± SEM. Main effects of line, temperature, and the interaction are presented next to each graph. Different letters indicate significant difference at $P < 0.05$.

expression decreased during HS in Line A ($P < 0.05$) but remained unchanged in others (Figures 6D–6F).

Mechanical Properties of RBC in Liquid Crystals Differ Among JF and Commercial Lines of Broilers

The RBCs were suspended in 154 mM NaCl to ensure the RBCs maintained their elliptical shapes; this also confirmed that the osmotic pressure of the interior of the RBC is 280 mosm/kg (Ohno et al., 2009). Previous work has shown that 17.3 wt% aqueous DSCG is isotonic with the interiors of RBCs (Nayani et al., 2020), which ensures that the RBC shape changes were limited to the mechanical interaction between the liquid crystals and RBCs. The changes in the shapes of the RBCs vary for different fowl types (Figure 7). From the optical microscopy images, Lines B and D are the most strained by liquid crystals, and Line A and the jungle fowl are the least strained. To further characterize the shapes of the RBCs in lyotropic DSCG, the aspect ratio for multiple cells within the microscopy images was calculated (Nayani et al., 2020). The aspect ratios, r_x/r_y, for ten strained Line D RBCs, are polydisperse, ranging from 1.0 to 3.0, while the aspect ratios r_x/r_y of strained jungle fowl RBCs ranges from 1.12 to 2.04 (Figure 7). The equivalent aspect ratios of RBCs before straining are almost monodisperse, with r_x/r_y ∼1.04. The broad distribution of values of r_x/r_y for strained RBC indicates a variation of mechanical properties with the cell population.

DISCUSSION

As global temperatures rise, it is important to understand the underlying mechanisms of HS on avian species, in order to best develop strategies to maintain sustainable production of this important protein source. To that end, we have characterized the gene expression of cytokines, chemokines, and inflammasomes of circulating cells (RBC and WBC) isolated from 4 commercial lines of modern broilers, as well as their ancestor, the red jungle fowl, in response to HS. As standard and high yielding broiler lines are 2 main types of broilers in the commercial market, we categorized the lines as such, with Lines A and B considered standard, and Lines C and D considered high-yielding. High yielding lines have been selected to have increased breast meat yield, while standard yielding lines have improved feed conversion (Mehaffey et al., 2006), production characteristics which may affect multiple metabolic systems, including thermotolerance. Additionally, unlike mammalian RBC, chicken RBC are nucleated, and therefore actively produce proteins that can influence not only their own cellular function, but interactions with other cells within the circulation, making them a key player in the stress response and a prime target for study.

Many of the observed effects of HS can be explained by the aggregation of proteins and an overall imbalance of protein homeostasis within the cell. Heat shock proteins are critical mediators of the stress response, where members have various roles in protein quality control systems to ensure cellular survival or in activation of
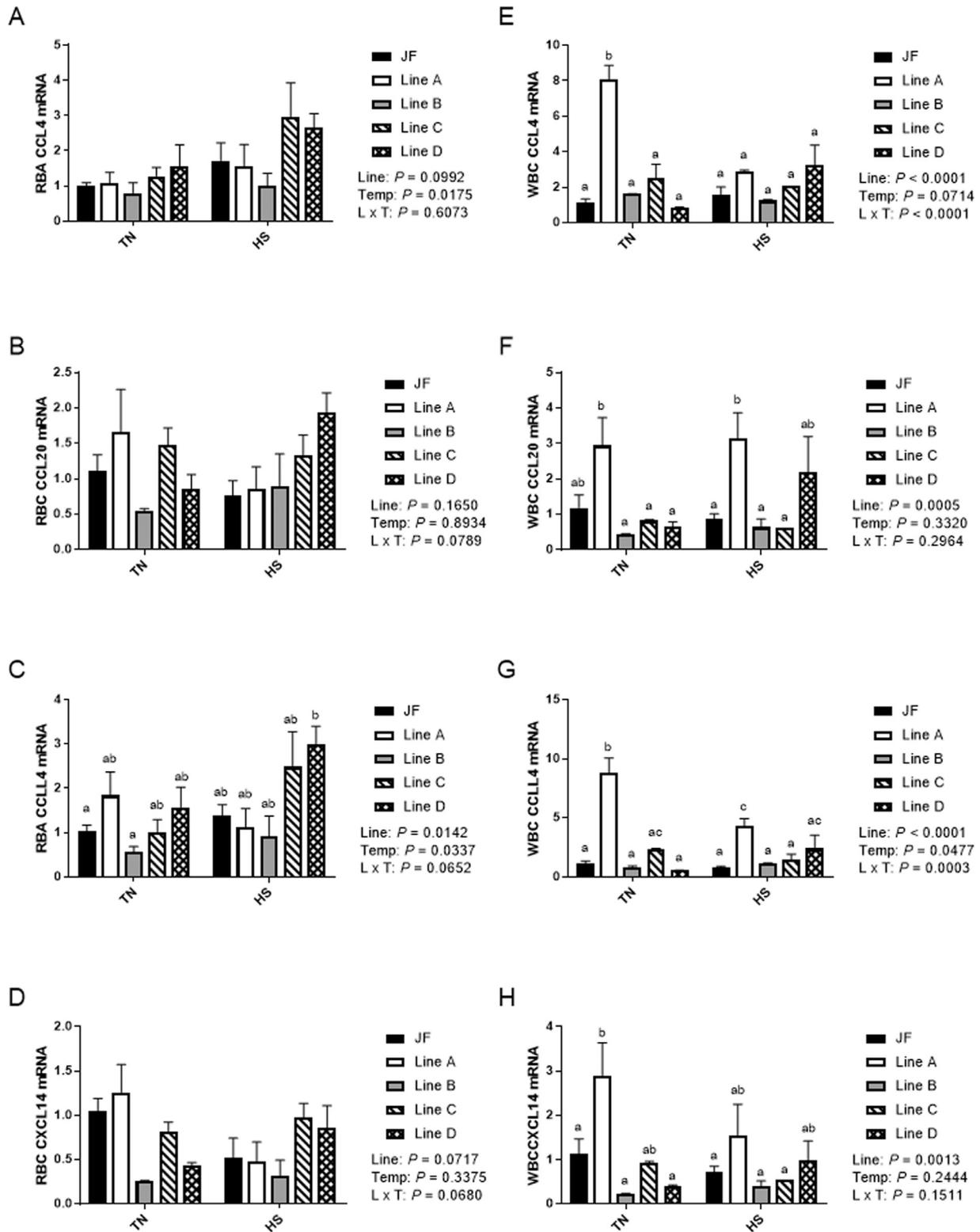
Figure 5. Effect of HS on chemokine gene expression in RBC and WBC isolated from jungle fowl and 4 commercial lines of broilers. The relative gene expression of CCL4 (A, E), CCL20 (B, F), CCLL4 (C, G), and CXCL14 (D, H) in RBC (A−D) and WBC (E−H) was determined by qPCR and analyzed by $2^{-\Delta\Delta Ct}$ method using JF-TN as a calibrator. Data are presented as mean ± SEM. Main effects of line, temperature, and the interaction are presented next to each graph. Different letters indicate significant difference at $P < 0.05$.

stress signaling cascades that result in cell-death pathways (Santoro, 2000). RBC from the high-yielding Line D showed the greatest response in HSP27 and HSP70 when exposed to acute (2 h) HS. The lack of a response in the other lines may be indicative of a greater tolerance to short term HS, which has been shown in slow-growing as compared to fast-growing stains (Yunis and Cahaner, 1999), as well as in breeds indigenous to tropical environments (Soleimani and Zulkifli, 2010; Soleimani et al., 2011). Previous studies examining the HS response of slow-growing as compared to fast-growing chicken implicated the increased growth negatively
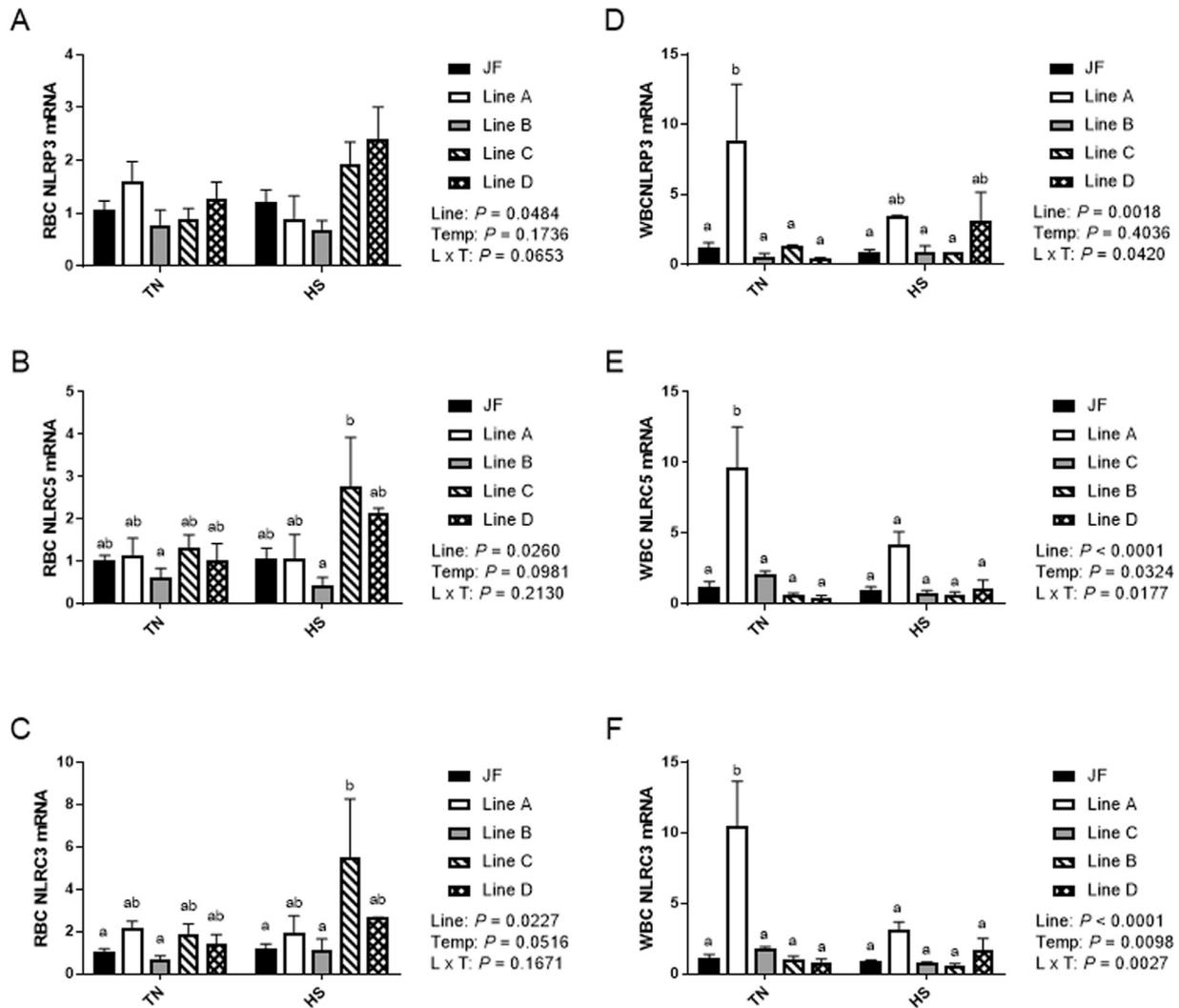
Figure 6. Effect of HS on inflammasome gene expression in RBC and WBC isolated from jungle fowl and 4 commercial lines of broilers. The relative gene expression of NLRP3 (A, D), NLRC5 (B, E), and NLRC3 (C, F) in RBC (A−C) and WBC (D−F) was determined by qPCR and analyzed by $2^{-\Delta\Delta Ct}$ method using JF-TN as a calibrator. Data are presented as mean ± SEM. Main effects of line, temperature, and the interaction are presented next to each graph. Different letters indicate significant difference at $P < 0.05$.

in the birds' susceptibility to HS (Berrong and Washburn, 1998; Soleimani, Zulkifli, Omar and Raha, 2011), which may also be reflected in the greater response seen in the high yielding lines in this study. HSP70 was upregulated in both RBC and WBC to the greatest degrees in high-yielding Line D. HSP70 and 90 have also been shown to be upregulated by acute HS in the nucleated RBC of trout (Lewis et al., 2010), and may play a role in maintenance of RBC membrane integrity during HS conditions (Lund and Tufts, 2003; Zhang et al., 2018; Balogi et al., 2019), factors which may be critically important for the maintenance of cellular structure and flexibility during stress conditions.

Recently, chicken erythrocytes have been identified as transcriptionally active players in the immune response, where in response to Marek's Disease (Jahejo et al., 2020), *Candida albicans* infection (Passantino et al., 2007), or poly I:C treatment (Morera et al., 2011), these cells upregulate numerous immune regulatory factors, including toll-like receptors, interleukins, chemokines, and interferon-α. Additionally, HS has been shown to significantly impact cytokine production (Bouchama

and Knochel, 2002; Shini and Kaiser, 2009; Carroll et al., 2012). Here, we show that, in response to HS, TNFα, IL-8L1, CCL4, and CCLL4 gene expression is increased in chicken RBC. This HS effect seems to be primarily driven by increase in the high-yielding birds (Lines C and D). TNFα has only recently been characterized in chicken, though it is well-studied in inflammation and in response to HS in mammals (Lu et al., 2004; Yun et al., 2012). Recently, we showed that TNFα gene expression in whole blood also increased by chronic HS in broilers (Greene et al., 2021), an effect similar to that seen in the isolated RBC in this study. TNFα stimulates chemokines, which can then activate recruitment of dendritic cells and monocytes, leading to inflammation. In particular, IL-8 is activated by TNFα, and was first identified as a neutrophil-activating cytokine in mammals (Baggiolini et al., 1989). It is produced in a wide variety of cells, including monocytes, lymphocytes, neutrophils, vascular endothelial cells, dermal fibroblasts, keratinocytes, and hepatocytes (Harada et al., 1994), and its gene expression has been shown to be induced by HS in human respiratory epithelial cells (Singh et al.,
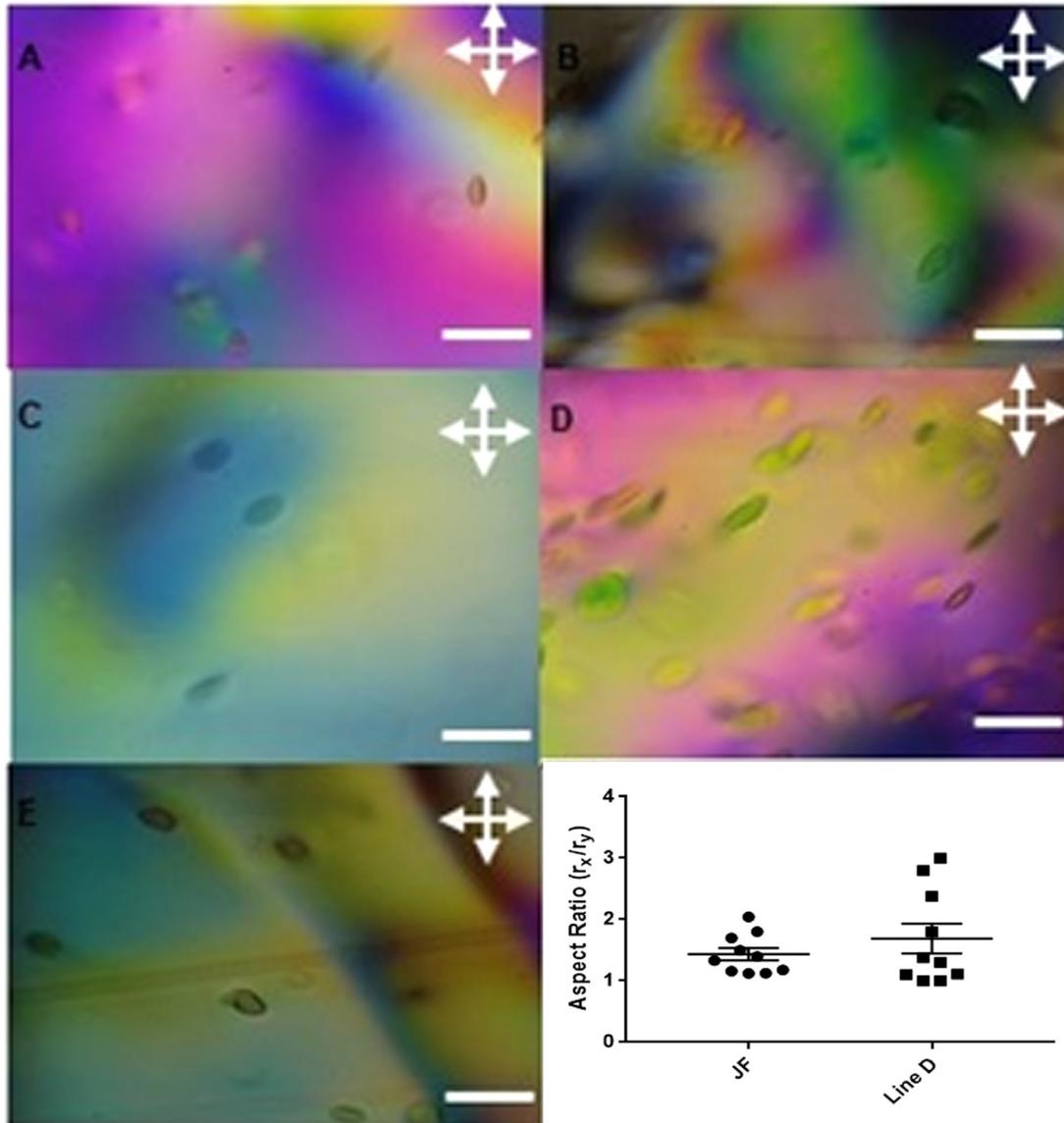
Figure 7. RBC from jungle fowl and 4 lines of commercial broilers are differentially strained by nenatic liquid crystals. (A) Optical micrograph (40X objective) of strained Line A RBCs suspended in nematic liquid crystal phase (17.3% DSCG aqueous solution) at 25°C. (B) Optical micrograph (40X objective) of strained Line B RBCs suspended in nematic liquid crystal phase (17.3% DSCG aqueous solution) at 25°C. (C) Optical micrograph (40X objective) of strained Line C RBCs suspended in nematic liquid crystal phase (17.3% DSCG aqueous solution) at 25°C. (D) Optical micrograph (40X objective) of strained Line D RBCs suspended in nematic liquid crystal phase (17.3% DSCG aqueous solution) at 25°C. (E) Optical micrograph (40X objective) of strained jungle fowl RBCs suspended in nematic liquid crystal phase (17.3% DSCG aqueous solution) at 25°C. (F) Histogram showing the variation in values of rx/ry for jungle fowl and Line D RBCs strained by a nematic liquid crystal phase.

2008) and dental pulp cells (Chang,et al., 2009). Here, we show a dramatic upregulation of IL-8L1 gene expression in high-yielding Line C. In a cell similar to chicken RBC, transcriptome profiling of the RBC of heat stressed rainbow trout recently showed upregulation of gene transcripts related to immune response, as well as apoptosis, hematopoiesis, and stress proteins (Lewis, Hori, Rise, Walsh and Currie, 2010).

The inflammasome is an intracellular complex that is the mediator between a cellular stimulus and the inflammatory response. Here, the only inflammasome affected by temperature in RBC was NLRC3, primarily due to the increases in the high-yielding lines. This inflammasome has been shown to have an anti-inflammatory role through inhibition of the NF-κB pathway (Schneider et al., 2012), and it may potentially be

upregulated to counter the greater inflammatory state caused by HS in these lines. Interestingly, in human studies, moderate HS activates the innate immune system and improves the efficiency of its response. However, severe HS further induces inflammation and decreases the performance of the innate immune system (Presbitero et al., 2021). As the responses in both RBC and WBC differ among lines, the level and/or duration of HS required to elicit inflammatory responses may differ. Taken together, the cytokine, chemokine, and inflammasome gene expression profile in RBC may serve as a future marker for stress responses in broilers, though further study is warranted.

Interestingly, the isolated WBC showed a high amount of variation in expression of measured genes. This may be due to the fact that this isolation method

generates a non-homogeneous population, in which does not differentiate between neutrophils, lymphocytes, basophils, eosinophils, and monocytes. We did not characterize the different populations obtained from each line, and it is possible that the contribution of each WBC type was different. Indeed, it has been previously shown that different lines of commercial birds can have significantly different WBC profiles (Talebi et al., 2005; Bílková et al., 2017; Ifelayo et al., 2020).

Red blood cells dynamically adapt to mechanical forces such as the shear force generated by passing through narrow capillaries by regulating their cytoskeleton (Bao and Suresh, 2003; Kumar and Graham, 2015). These properties have been studied in mammalian RBC in the context of hematological diseases such as sickle cell anemia, where disease cells differ in shape and mechanical properties from healthy cells (Maciaszek and Lykotrafitis, 2011). A larger in-plane aspect ratio is indicative of a greater strain placed on the plasma membrane of the cell, and a greater deformation from the normal elliptical shape of the chicken RBC towards a more spindle shape. This may be considered either positive, whereby the membrane is more elastic and able to withstand pressure and fit through small capillaries, or negative, where the deformation is greater, but less reversible, and the cell is more susceptible to changes due to stressors. Interestingly, the variation seen in deformation of the cells of Line D, from almost none (1.0) to extreme (3.0), was greater than that of the other lines studied. This line also had the largest upregulation of HSPs gene expression after HS, which may play a role in the elasticity of their plasma membranes (Horváth et al., 2008). Additionally, it is possible that the generation of cytokines and chemokines may be driven by a subset of the population that is more- or less-elastic, leading to a differential susceptibility to HS (or other stressors) among different lines of chickens. It has been shown in rainbow trout that RBC change in properties including cytoplasmic shape and loss of organelles with aging (Lund et al., 2000). As fish and birds possess similarly nucleated RBC, the age of RBC in the birds may be a potential factor in their response, where Line D may have a longer half-life and therefore cells in a wider age range. Interestingly, we also observed that the nucleus of the cell also becomes strained when placed in the liquid crystal media (data not shown). This observation, along with RBC turnover, would be important to explore further, particularly in relation to differences under stress conditions, as a more- or less-resilient nucleus may have implications for the cellular ability to respond to the stressor.

Together, these findings highlight not only the dynamic nature of circulating cells, but the differences in the stress and inflammatory response among commercially available broiler lines and their common ancestor. Additionally, we are the first to use nematic liquid crystal technology to characterize and study avian RBC. These profiles have the potential to serve as a minimally invasive future marker for stress responses in broilers, though further study is warranted.

DISCLOSURES

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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