MANAGEMENT AND PRODUCTION

Processing evaluation of random bred broiler populations and a common ancestor at 55 days under chronic heat stress conditions

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ABSTRACT As a result of genetic selection, the modern broiler is more efficient, higher yielding, and faster growing than the bird of the 1950s. Unfortunately, as a result of improvement in growth rate, the modern broiler has the potential to struggle under heat stress conditions. The present study evaluates 3 different random bred populations and a common ancestor under both a thermal neutral and heat stress conditions after a 54-D grow-out period. The lines used in this study included the Athens Canadian Random Bred (**ACRB**), a 1995 Random Bred (**95RAN**), a 2015 Random Bred (**MRB**), and a Junglefowl (**JF**). Male chicks (n = 150/line) were placed by line in environmentally controlled chambers. An 8-h daily cyclic heat stress (36°C) was applied to half of the chambers beginning on day 28 (**HS**) and lasting until processing at day 55, while the remaining chambers remained thermal neutral (**TN**) at 26°C. Dock weights and carcass weights were lower in the HS-95RAN and HS-MRB, compared to their TN counterparts, while the ACRB and JF had no difference in dock and carcass weights regardless of environmental condition. The MRB line had the highest breast yield (27.79%) while the JF (12.79%) and ACRB (12.42%) had the lowest. The 95RAN line had the highest abdominal fat percentage (2.83%) while the MRB line had the lowest moisture uptake during chill. The HS exposure lowered overall breast yield and breast pH at 15 min and 4 h postmortem but did not have an impact on color (L*) or 24 h breast drip loss. The MRB was scored for both woody breast and white striping. The TN-MRB group had a higher incidence of moderate and severe woody breast and white striping than the HS-MRB group. Based on the results of this study, it appears that HS has a greater negative impact on the higher yielding lines (MRB and 95RAN) than the ACRB and JF and that clear line differences exist between the random bred lines and their common ancestor.

Key words: heat stress, broiler, genetic selection, carcass traits, meat quality

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INTRODUCTION

Poultry production worldwide has seen a dramatic increase over the past 50 yr. In fact, United States poultry meat production in 2017 totaled over 42 billion pounds, surpassing both pork and beef production (NAMI, 2017). This spectacular increase in poultry production can be attributed to the successes made in genetic selection on growth rate, yield, and feed efficiency (Rauw et al., 1998). In a study comparing the commercial broiler from 1957 to a commercial broiler from 1991 when fed a similar diet, the 1991 broiler outperformed the 1957 broiler having far greater body weights and better feed conversion. The results of this study identified that 85–90% of the differences observed between these lines were a result of intense genetic selection (Havenstein et al., 1994a,b). Since this study, genetic companies have continued improving the growth rate, yield, feed conversion, and welfare of commercially produced broilers in the United States.

While genetics plays an important role in the efficiencies of broiler production, environment and changes to the environment of the broiler can have a significant impact on performance traits such as growth, yield, and feed intake. In recent years, the average surface temperature of the earth has changed, causing higher than normal average temperatures throughout much of the world including the United States (Nasa.gov, 2019). Broilers are already more susceptible to heat stress

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conditions than red meat species due to their high metabolic rate, extensive amount of feather coverage, and lack of sweat glands (Loyau et al., 2013). High ambient temperatures in broiler production environments can cause a decrease in feed intake ultimately resulting in decreased body weight, increased feed conversion ratios, higher than normal mortality, and weakened gut health (Cahaner and Leenstra, 1992; Ahmad and Sarwar, 2006; Song et al., 2017; He et al., 2018). It is also known that broilers with high or rapid growth rates have a higher sensitivity to increases in temperatures (Cahaner and Leenstra, 1992) making the modern broiler much more prone to issues that may be associated with heat stress (Song and King, 2015).

In addition, chronic heat stress can impact breast muscle morphology and overall meat quality of product produced. It has been shown that heat stress can change the muscle morphology of broiler breast meat by decreasing muscle hypertrophy, the main form of post-hatch growth, through decreased muscle protein synthesis and downregulation of IGFs-mTOR signaling pathway (Ma et al., 2018). Chronic heat stress can negatively impact breast meat quality through changes in aerobic metabolism and increased intramuscular fat deposition (Lu et al., 2017). Similarly, heat stress causes a rapid pH decline in the breast muscle postmortem (Young et al., 2004) which can be associated with decreased meat quality through pale color and high drip loss (Wang et al., 2009). Breast pH is a strong indicator of poultry meat quality as it is strongly correlated with traits such as muscle color, drip loss, water holding capacity, and tenderness (Barbut et al., 1997).

With the unprecedented large-scale impact of global warming, it is important to understand the mechanisms of heat stress response and determine its effect on meat quality. The purpose of this study was to evaluate the processing and meat quality characteristics of random bred broiler populations representing the commercial broiler from 1950, 1995, and 2015 as well as their common ancestor under both a thermal neutral and chronic cyclic heat stress condition at a common processing age. It was also conducted to elucidate potential line differences in processing yields relative to body weight for the random bred populations raised under similar environmental conditions.

MATERIALS AND METHODS

Research Lines

The present study used four research lines that are housed and maintained at the University of Arkansas research farm. The first line of birds was the Athens Canadian Random Bred (**ACRB**), which represents a commercial broiler from the 1950s (Collins, et al., 2016). The 1995 random bred (**95RAN**), consisted of 7 parent stock male and 6 parent stock female lines commercially available in the 1990s (Harford et al., 2014). The third line is a Modern Random bred population (**MRB**) that was initially established in 2015 at the University of Arkansas. This line originally consisted of 4 commercially available broiler packages from 3 different broiler genetics companies; Cobb MX x Cobb 500, Ross 544 x Ross 308, Ross Yield + x Ross 708, and the Hubbard HiY package. The 4 packages have been integrated through 5 generations of random mating to create a homogenous population representing a commercially available broiler from 2015. The final population, the Junglefowl (**JF**), serves as the common ancestor to the commercial broiler (Gyles et al., 1966; Wall and Anthony, 1995). All lines have been maintained as closed populations since their creation and are randomly mated each generation with the avoidance of full and half sibs to control and minimize the accumulation of inbreeding in each population. All animal experiments were approved by the University of Arkansas (Fayetteville, AR) Animal Care and Use Committee (protocol # 18083) and were in accordance with recommendations in NIH's *Guide for the Care and Use of Laboratory Animals*.

Rearing and Heat Stress Exposure

Twelve environmentally controlled chambers were used in this study. Each chamber was divided by wire paneling into 2 identical pens to allow for 2 lines per chamber and a total of 6 pens per line. The chambers allow for the proper setting and tight control of temperature and lighting. At hatch, chicks were vent sexed and males from all lines were placed at a density of 25 birds per pen. From placement to day 21, all chambers were set to the same environmental conditions. The ambient room temperature was gradually decreased from 32°C from days 1 to 3, 31°C from days 4 to 6, 29°C from days 7 to 20, 27°C from days 11 to 14. From day 15 through the remainder of the study, the ambient room temperature remained at 25°C. A relative humidity of 20–40% was maintained throughout the experiment. From day 0 to day 7, birds were on a 23 h light/1 h darkness photoperiod. At day 7, they were transitioned to 20 h light/4 h darkness for the remainder of the study. A commercially available starter diet was fed from 0 to 28 D that was formulated to meet or exceed NRC recommendations (NRC, 1994). A commercially available finisher was fed for the remainder of the study. Birds were allowed constant access to feed and water throughout the trial.

The environmental treatments began the morning of day 29. Half of the chambers were kept in a thermal neutral (**TN**) environment at a constant temperature of 25°C for the remainder of the study. The remaining 6 chambers were subjected to an 8 h daily cyclic heat stress (**HS**) until processing. The rise in temperature to 36°C began an 8 AM each day and ending at 4 PM returning to an ambient temperature of 25°C. This resulted in 3 pens (75 birds) per line being subjected to either a TN or HS environment.

Processing

Birds were processed on d 55 at the University of Arkansas Pilot Processing Plant (Fayetteville, AR) using a commercial inline system. Feed was removed from the birds 10 h before load out and processing while free access to water remained. Live dock weights were recorded before being placed on a shackle line. Birds were processed randomly by line due to constraints on the picker from varying bird size. Once placed on the line, birds were electrically water bath stunned (11V, 11 mA, 10) and manually cut through the left carotid artery and allowed to bleed out for 3 min before the scald tank (55°C). The carcasses were picked using an in-line commercial picker and then eviscerated by hand. Abdominal fat weight, with-out giblets (**WOG**) weight, and 15 min breast pH was recorded before being placed into a 4-h ice bath chill. A Testo 205 pH probe was used to measure pH on the left side of the pectoralis major. After chill, chilled-WOG (**CWOG**) were weighed, manually deboned, and parts were weighed. After debone, a 4 h breast pH was recorded. For the MRB line only, breast fillets were subjectively hand-scored for both woody breast and visually scored for white striping on a scale of 0 to 3 with 0 being no signs of WB or WS and 3 being severe WB or WS. A score of 1 was mild, while a score of 2 was considered moderate for both traits. Breast fillets were placed in plastic Ziploc bags and refrigerated overnight in a 3°C cooler. Twenty-4 h postmortem, breast fillets were removed from the bag and weighed to account for breast muscle drip loss. A final pH measurement of the breast fillet was taken and color (L*, a*, b*) was recorded using a Minolta CR-400 colorimeter of the posterior side of the right breast fillet.

RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

Total RNAs were extracted from chicken beast muscle tissues by Trizol reagent (Life Technologies, Grand Island, NY) according to manufacturer's recommendations. After DNAse treatment and purification, total RNA concentrations were determined for each sample by Take 3 Micro-Volume Plate using Synergy HT multimode microplate reader (BioTek, Winooski, VT), and RNA integrity and quality were assessed by both OD260/OD280 nm absorption ratio (>1.9) and by using 1% agarose gel electrophoresis. For cDNA synthesis, total RNA (1 μg) was reverse transcribed using qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD) in a 20 μL total reaction. The reverse transcription reaction was performed at 42°C for 30 min followed by an incubation at 85°C for 5 min. Real-time quantitative PCR (Applied Biosystems 7500 Real-Time PCR system) was performed using 5 μL of 10X diluted cDNA, 0.5 μmol of each forward and reverse specific primer, and SYBR Green Master Mix (Thermo-Fisher Scientific, Rockford, IL) in a total 20 μL reaction as previously described by Lassiter et al. (2015) and Flees et al. (2017). Oligonucleotide primers specific for chicken nuclear receptor factor 1 (NRF1): forward 5′-GGCCAACGTCCGAAGTGAT-3′ and reverse 5′-CCATGACACCCGCTGCTT-3′, superoxide dismutase 1 (**SOD1**): forward 5′- TGGCTTCCATGTGCATGAAT -3′ and reverse 5′- AGCACCTGCGCTGGTACAC -3′, SOD2: forward 5′- GCTGGAGCCCCACATCAGT -3′ and reverse 5′- GGTGGCGTGGTGTTTGCT -3′, NFKB1; forward 5′- CAGTCAACGCAGGACC-TAAAGA-3′ and reverse 5′-GTGACGTGAAGTATTC-CAAGGTT-3′, NFKB2: forward 5′- AGATCTCGCGG ATGGACAAG-3′ and reverse 5′- CTCAATGT-CATCTTTCTGCACCTT-3′, RelA: forward 5′- CGCTGCGTGCACAGTTTC-3′ and reverse 5′-CTTCCAGTTCCCGTTTCTTCAC-3′, RelB: forward 5′- CCACGGCGCTAATAATTTGC-3′ and reverse 5′- GAAGGGCATTGCATGCATT-3′, and r18S as housekeeping gene: forward 5′- TCCCCTCCCGT TACTTGGAT -3′ and reverse 5′- GCGCTCGTCGG CATGTA -3′ were used. Relative expressions of target genes were determined by the 2-ΔΔCt method (Schmittgen and Livak, 2008). Samples extracted from JF at TN conditions were used as a calibrator.

Statistical Analysis

Processing results were analyzed using a two-way ANOVA in the JMP Pro 14 fit-model platform (JMP, 2019). The main effects analyzed were line (ACRB, 95RAN, MRB, JF) and environmental treatment (HS, TN) as well as their interaction. Pen was used as the experimental unit. Means were considered statistically significant at a P value < 0.05 with means separated by Tukey's HSD or the student t-test where appropriate. For traits where the interaction was not significant, main effects of line and environmental treatment are presented and discussed. For woody breast and white striping, only the main effect of environmental treatment was analyzed as only one line was evaluated. For statistical comparison, scores of normal (0) and mild (1) were grouped as "unaffected" while scores of moderate (2) and severe (3) were grouped as "affected" for both WB and WS. Results were compared by Fisher's exact test. Gene expression data were analyzed by 2-way ANOVA and means were compared using Tukey's multiple comparison tests in GraphPad Prism (Version 7.0, Graph Pad Software, La Jolla, CA).

RESULTS

A line by treatment interaction was present for Dock, WOG, and CWOG weights and a* and b*. For the MRB and 95RAN lines, the HS treatment (HS-MRB, HS-95RAN) had lower Dock, WOG, and CWOG weights than their TN counterparts (TN-MRB, TN-95RAN). However, there were no differences observed for those traits between the HS-ACRB, TN-ACRB, HS-JF or TN-JF groups (Table 1). In addition, a line by treatment interaction was present for breast muscle a* and b*; however, those results might be associated with an effect of breast muscle thickness differences between line and

treatment groups. No other line by treatment interactions were present.

Line effects were present for all traits measured (Table 2). Dock, WOG, and CWOG weights were highest in the MRB, and lowest in the ACRB and JF with the 95RAN an intermediate as expected. All parts were expressed as a percentage relative to the CWOG weight to account for a large difference in weights as a result of selection. Fat percentage was expressed relative to WOG weight. Percent fat was highest in the 95RAN and lowest in the ACRB and JF. Percent breast yield was highest in the MRB line and lowest in the ACRB and JF while percent leg quarter yield was highest in the ACRB and JF and lowest in the MRB. Breast muscle drip loss was highest in the MRB and 95RAN. For all pH time measurements, the MRB has the highest pH, while the JF had the lowest pH (Figure 1). Breast muscle color (L*) was highest or lightest in the JF and darkest in the MRB.

Differences for percentage breast yield, 15 min pH, and 4 h pH were observed for the main effect of environmental treatment. Percentage breast yield, 15 min pH, and 4 h pH was highest in the TN group when compared to the HS group. Yellowness (b*) was higher in the HS group with no differences observed for the remaining traits measured (Table 3). For the MRB line, the TN group had a higher incidence of severe (score of 3) white striping with a higher average WS score (Figure 2) and a higher incidence of moderate (2) and severe (3) woody breast with a higher average WB score (Figure 3). Fisher's exact test indicated that the HS and TN groups were different for both WB and WS at a *P*-value of 0.01.

As shown in Figure 4, under TN conditions, 95RAN and MRB lines exhibited a significant lower muscle expression of NFKB2 gene compared to JF and ACRB chickens (Figure 4B). SOD1 mRNA abundances were significantly higher in 95RAN, and NRF1 gene expression was significantly upregulated in ACRB compared to the other lines (Figures 4E, G). Heat stress exposure, however, significantly downregulated NFKB2 expression in the muscle of JF and ACRB lines, but not 95RAN and MRB (Figure 4B). Similarly, heat stress significantly decreased mRNA levels of SOD1 in 95RAN and NRF1 in JF and ACRB compared to their counterparts maintained at TN conditions (Figures 4E, G).

DISCUSSION

Although it is well known that genetic selection of the commercial broiler has improved yield, feed conversion, and livability, very few studies have been conducted to evaluate genetic lines representing different periods of selection under the same environmental conditions or challenges. One such study evaluated lines from 1957, 1978, and 2005. The results of this study showed an increase in breast muscle percentage from 1957 to 2005 of 79% in males and 85% in females as well as an increase in growth rate and decrease in feed conversion (Zuidhof et al., 2014). The 4 lines evaluated in this study have never

Table 1. Means[1] ± SEM for traits with significant line by treatment[2] interactions at 55 D of age.

	TN				HS			
Trait	MRB	95RAN	ACRB	JF	MRB	95RAN	ACRB	JF
Dock Wt (g)	4,409.57 ± 42.63^{a}	2,836.68 ± 48.41^{c}	907.1 ± 65.12^{e}	855.87 ± 54.5^{e}	3,913.27 ± 41.38^{b}	2,493.48 ± 53.6^{d}	855.81 ± 58.53^{e}	835.87 ± 54.5^{e}
WOG[3] (g)	3,360.08 ± 34.39^{a}	2,013.45 ± 39.06^{c}	595 ± 52.54^{e}	558.37 ± 43.96^{e}	3,011.15 ± 33.39^{b}	1,769.97 ± 43.24^{d}	558.96 ± 47.22^{e}	550.73 ± 43.9^{e}
CWOG (g)	3,247.04 ± 34.92^{a}	2,062.05 ± 39.65^{c}	616.05 ± 53.34^{e}	580.73 ± 44.62^{e}	3,068.23 ± 33.9^{b}	1,817.81 ± 43.9^{d}	580.23 ± 47.94^{e}	573.37 ± 43.65^{e}
a*	1.45 ± 0.15^{c,d}	1.81 ± 0.17^{c}	3.01 ± 0.23^{b}	3.59 ± 0.19^{a,b}	1.02 ± 0.15^{d}	1.69 ± 0.19^{c,d}	3.34 ± 0.21^{a,b}	4.03 ± 0.19^{a}
b*	8.36 ± 0.21^{b}	6.69 ± 0.23^{d}	8.66 ± 0.31^{b}	10.14 ± 0.26^{a}	8.16 ± 0.2^{b,c}	7.28 ± 0.26^{c,d}	10.53 ± 0.28^{a}	10.83 ± 0.27^{a}

[1]Means with no common letter are significantly different between line and treatment combinations at $P < 0.05$.
[2]MRB = modern random bred line (2015); 95 = 1995 random bred; ACRB = Athens Canadian random bred; JF = jungle fowl; TN = thermal neutral; HS = heat stress.
[3]WOG = with-out-giblets; CWOG=chilled-with-out-giblets.

Table 2. Means[1] ± SEM for traits measured during processing from 4 different genetic lines.[2]

Trait[3]	MRB	95RAN	ACRB	JF
Dock Wt (g)	4,161 ± 30^{a}	2,665 ± 36^{b}	881 ± 44^{c}	846 ± 39^{c}
WOG Wt (g)	3,186 ± 24^{a}	1,892 ± 29^{b}	577 ± 35^{c}	555 ± 31^{c}
Fat (%)	1.4 ± 0.06^{b}	2.83 ± 0.08^{a}	0.71 ± 0.1^{c}	0.68 ± 0.08^{c}
CWOG Wt (g)	3,248 ± 24^{a}	1,940 ± 29^{b}	598 ± 36^{c}	577 ± 32^{d}
MU (%)	1.95 ± 0.1^{c}	2.57 ± 0.12^{b}	3.69 ± 0.13^{a}	4.04 ± 0.15^{a}
Breast (%)	27.79 ± 0.19^{a}	18.85 ± 0.23^{b}	12.42 ± 0.27^{c}	12.79 ± 0.24^{c}
Tenders (%)	5.5 ± 0.16^{a}	5.27 ± 0.07^{a}	4.14 ± 0.09^{b}	4.26 ± 0.08^{b}
Wings (%)	9.66 ± 0.07^{d}	11.2 ± 0.08^{c}	14.31 ± 0.1^{b}	15.07 ± 0.09^{a}
LQs (%)	30.06 ± 0.16^{c}	31.76 ± 0.19^{b}	33.36 ± 0.24^{a}	32.86 ± 0.21^{a}
DL (%)	−0.52 ± 0.17^{a}	−0.35 ± 0.2^{a}	−1.95 ± 0.24^{b}	−2.86 ± 0.21^{c}
15 min pH	6.65 ± 0.02^{a}	6.35 ± 0.02^{b}	6.11 ± 0.03^{c}	6 ± 0.03^{d}
4h pH	6.08 ± 0.01^{a}	6.04 ± 0.02^{a}	5.9 ± 0.02^{b}	5.67 ± 0.02^{c}
24h pH	5.87 ± 0.01^{a}	5.77 ± 0.02^{b}	5.69 ± 0.02^{c}	5.6 ± 0.02^{d}
L*	57.49 ± 0.23^{a}	54.55 ± 0.28^{b}	55.59 ± 0.34^{b}	57.59 ± 0.3^{a}
a*	1.23 ± 0.1^{d}	1.75 ± 0.13^{c}	3.18 ± 0.15^{b}	3.8 ± 0.14^{a}
b*	8.26 ± 0.14^{c}	6.98 ± 0.17^{d}	9.6 ± 0.21^{b}	10.48 ± 0.19^{a}

Percentage values are based off CWOG weights.

[1]Means with no common letter within a row are significantly different between line at $P < 0.05$.

[2]MRB = modern random bred line (2015); 95RAN = 1995 random bred; ACRB = Athens Canadian random bred; JF = jungle fowl.

[3]WOG = with-out giblets; CWOG = chilled-with-out giblets; MU = moisture uptake; LQ = leg quarters; DL = drip loss; L* = lightness, a* = redness, b* = yellowness.

been compared at a common chronological processing age.

Each broiler population used in this study represents a snapshot in time of the broiler industry and its current stage of genetic selection in addition to a population considered the common ancestor of the modern commercial broiler. The ACRB line was created to represent a genetic population that would be found in the 1950s (Collins et al., 2016). Genetic selection around this time was not as extensive as it is now with most selection practices focusing solely on improving the live weight of the bird (Thuruvenkadan and Prabakaran, 2017). As genetic selection continues to advance, other agricultural and economically important traits such as feed conversion, livability, and welfare traits were included in modern selection programs eventually creating the broiler we see today. The 95RAN, representing the commercial broiler from the 1990s, creates a snapshot in time of the broiler industry, before the use of molecular methods in selection programs (Harford et al., 2014). The MRB line is a line representing the current state of poultry genetic selection and the use of molecular and marker-assisted selection (Thuruvenkadan and Prabakaran, 2017).

For the live weight or dock wt of the bird at 55 D as well as the carcass weights, it appeared that the period of chronic heat stress had a greater impact on the 2 higher yielding lines than the ACRB or JF. The heat-stressed MRB and 95RAN had lower body weights and carcass weights while no impact was seen on the 2 groups (HS or TN) for the ACRB and JF. Fast growth rate and high metabolic rate have a direct effect on heat sensitivity in broilers (Cahaner and Leenstra, 1992). In fact, several studies have concluded that the effects of heat stress are more pronounced in commercial flocks or flocks with faster growth rates (Washburn et al., 1992; Yunis and Cahaner, 1999). At molecular levels, the differential basal expression of NFKB2 between the modern broilers (95RAN and MRB) and their ancestors (JF and ACRB) might explain, at least partly, the differential resistance to heat stress between these lines. Indeed, the NFKB family of transcription factors plays a key role in the regulation of stress-associated and inflammatory gene expression (Oeckinghaus et al., 2011). Liu and coworkers have shown that NFKB signaling is essential for heat stress resistance in human UVEC cells (Liu et al., 2015) and a direct link between HSPs and NFKB signaling pathway has been observed (Park et al., 2003.). Although not presented, the growth rate of the 95RAN and MRB line far exceeds that of the ACRB and JF which was expected. The growth rate of the lines as well as their respective feed intakes played a role in the differences in body weight and carcass weight observed. With varying growth rates and extreme differences in the dock wt at 55 D for the 4 lines used, further research to evaluate the lines at a common weight may be beneficial in understanding the effect of heat stress on these populations. Processing birds at a common physiological age, rather than a common chronological age, could help elucidate further differences that exist among these lines under heat stress conditions.

As expected, absolute weights for all carcass parts were different among the lines so relative values were calculated to account for drastic differences in dock weights and carcass weights. Percentage fat was highest in the 95RAN line at 55 D while lowest in the ACRB and JF. Selection pressure of a broiler is focused heavily on traits such as body weight, feed conversion, breast yield, and rapid growth (Emmerson, 1997). Unfortunately, as a result of selection for these traits, abdominal fat which has been shown to be highly heritable has increased (Gaya et al., 2006) which would explain the increase in body fat between the ACRB and 95RAN. Genetic

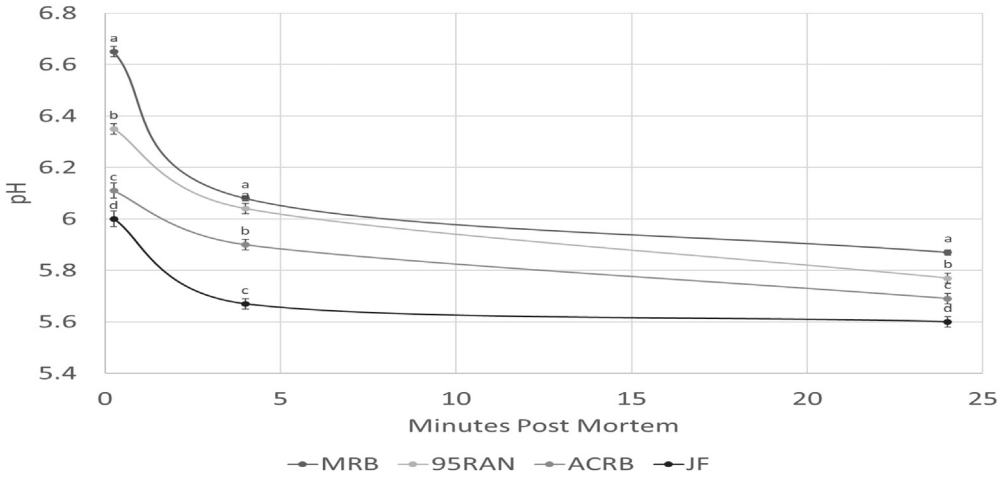

Figure 1. Twenty-four-hour pH decline by line for 4 chicken populations processed at 55 D. Means with no common letter within a time period are significantly different between line at $P < 0.05$. Abbreviations: MRB, modern random bred line (2015); 95RAN, 1995 random bred; ACRB, Athens Canadian random bred; JF, jungle fowl.

companies in more recent years have begun to select against the undesirable consequences that come with selection for body weight, growth, and efficiency such as abdominal fat (Konarzewski et al., 2000; Gaya et al., 2006). Selection for lower feed conversion ratio has also resulted in a reduction of overall carcass fat (Emmerson, 1997). As a result of these changes in selection, abdominal fat in the MRB line should be lower than the 95RAN line as indicated by the results of this study.

An emphasis on breast meat yield has taken hold over the last 20 yr as a result to changes in consumer preference and a push from a whole bird market to one of a cut-up and further processing (Tavarez and Solis de los Santos, 2016). With the MRB being a composite of relatively modern broiler lines, it was expected that this line would exhibit the highest breast yield as was shown by the data. The lowest breast yield was seen in both the ACRB and JF lines. A stepwise increase in the percentage breast yield from the ACRB to the 95RAN and finally the MRB line shows the improvements made through the genetic selection focus on breast meat yield. While percentage breast meat has increased throughout the years of selection of the modern broiler, it is interesting to note that with this focus on white meat, dark meat production has not seen the same relative improvement. The ACRB and JF had the highest percentage leg quarter yield, the MRB had the lowest with 95RAN an intermediate. Although not as drastic of a difference as was viewed with breast meat yield, leg quarter yield relative to body weight has decreased with changes in consumer preference.

Minimal differences were detected between the overall effects of environmental group treatment. Heat stress reduced overall Dock, WOG, and CWOG weights as heat treated birds were less active and therefore experienced decreased feed intake resulting in lower body weight gain (Cahaner and Leenstra, 1992). When considering the relative parts weights, only percentage breast yield was different between groups with the HS group having a lower breast yield than the TN group. As genetic selection has focused on both increased body weight and breast yield (Tavarez and Solis de los Santos, 2016), it would be expected that a treatment which reduced feed intake and body weight gain would also have the greatest impact on the relative part weight of the highest selected trait.

Meat quality results differed between line and environmental treatment. Both pH and color are strong indicators of meat quality as each trait can be associated with issues such as PSE-like and DFD-like meat (Barbut, 1997). Very little research has been done comparing the pH decline of genetic lines differing in growth rate. It is interesting to note that initial pH

Table 3. Means[1] ± SEM for traits measured during processing from 2 different environmental conditions during administered during rearing.[2]

Trait[3]	TN	HS
Dock Wt (g)	2,252 ± 27[a]	2,025 ± 26[b]
WOG Wt (g)	1,632 ± 22[a]	1,473 ± 21[b]
Fat (%)	1.39 ± 0.06[a]	1.42 ± 0.06[a]
CWOG Wt (g)	1,671 ± 22[a]	1,510 ± 21[b]
MU (%)	2.98 ± 0.09[a]	3.15 ± 0.09[a]
Breast (%)	18.26 ± 0.17[a]	17.67 ± 0.16[b]
Tenders (%)	4.85 ± 0.05[a]	4.73 ± 0.05[a]
Wings (%)	12.52 ± 0.06[a]	12.6 ± 0.06[a]
LQs (%)	31.88 ± 0.15[a]	32.14 ± 0.14[a]
DL (%)	−1.43 ± 0.15[a]	−1.42 ± 0.15[a]
15 min pH	6.32 ± 0.02[a]	6.24 ± 0.02[b]
4h pH	5.92 ± 0.01[a]	5.88 ± 0.01[b]
24h pH	5.74 ± 0.01[a]	5.72 ± 0.01[a]
L*	56.26 ± 0.21[a]	56.35 ± 0.21[a]
a*	2.46 ± 0.09[a]	2.52 ± 0.09[a]
b*	8.46 ± 0.13[b]	9.2 ± 0.13[a]

Percentage values are based off CWOG weights.

[1]Means with no common letter within a row are significantly different between treatment at $P < 0.05$.

[2]TN = thermal neutral; HS = heat stress.

[3]WOG = with-out giblets; CWOG = chilled-with-out giblets; MU = moisture uptake; LQ = leg quarters; DL = drip loss; L* = lightness; a* = redness; b* = yellowness.

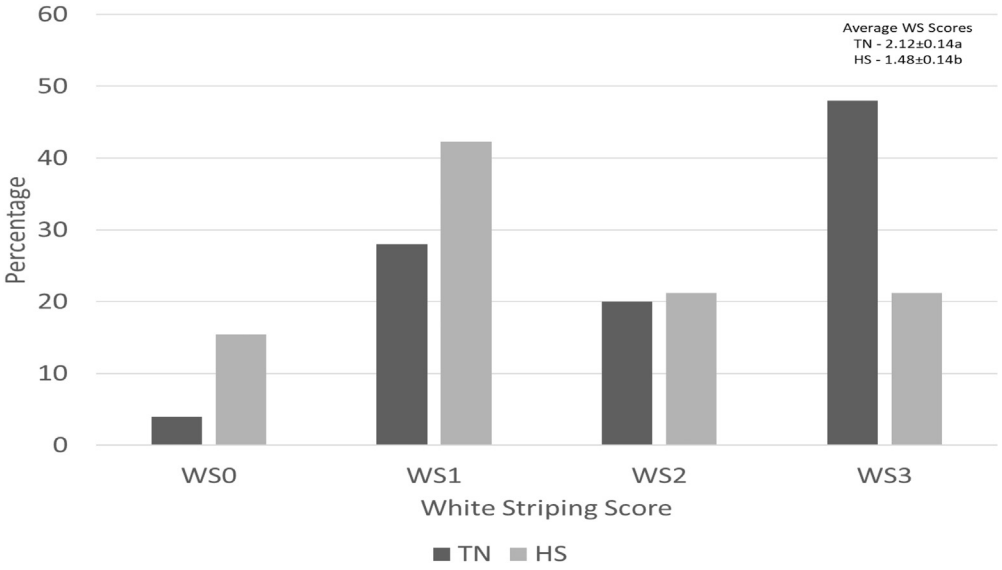

Figure 2. Percent of breast fillets within each white striping score for the MRB line in HS and TN conditions at 55 D of age. A subjective scoring scale was used for white striping (WS) with WS0 being no signs of white striping, and WS3 being severe white striping. Abbreviations: MRB, modern random bred line from 2015; TN, thermal neutral; HS, heat stress.

recorded at 15 min postmortem is highest in the MRB line and lowest in the JF line. Through genetic selection of the broiler over the past 70 yr, initial pH and possibly physiological pH of the modern broiler has been altered. The same trend is present when considering the initial decline in pH seen between 15 min and 4 h postmortem as well as the ultimate pH that the breast fillet reaches. Overall, genetic selection has increased initial pH and ultimate pH in broiler breast fillets. While breast pH and breast color have been shown to be highly correlated (Barbut, 1997), L* values appear erratic. The MRB and JF line has the highest L*, or palest fillet but considering their differences in ultimate pH, this result was not expected. Inadequate breast muscle size and thickness in both the JF and ACRB lines may have contributed to the differences in breast color observed in this study.

When considering the environmental treatment, periods of heat stress in production systems have been shown to alter postmortem pH (McKee and Sams, 1997). In general, heat stress reduces the rate of pH decline and the ultimate pH achieved after rigor mortis. The TN groups for all lines in this study showed a higher initial pH at 15 min and higher 4 h pH than the HS group, as was expected. However, the ultimate pH at 24 h was not different between the groups. While the rate of pH decline was decreased by pH, overall pH was not affected. Heat stress has also been shown to increase the incidence of pale meat,

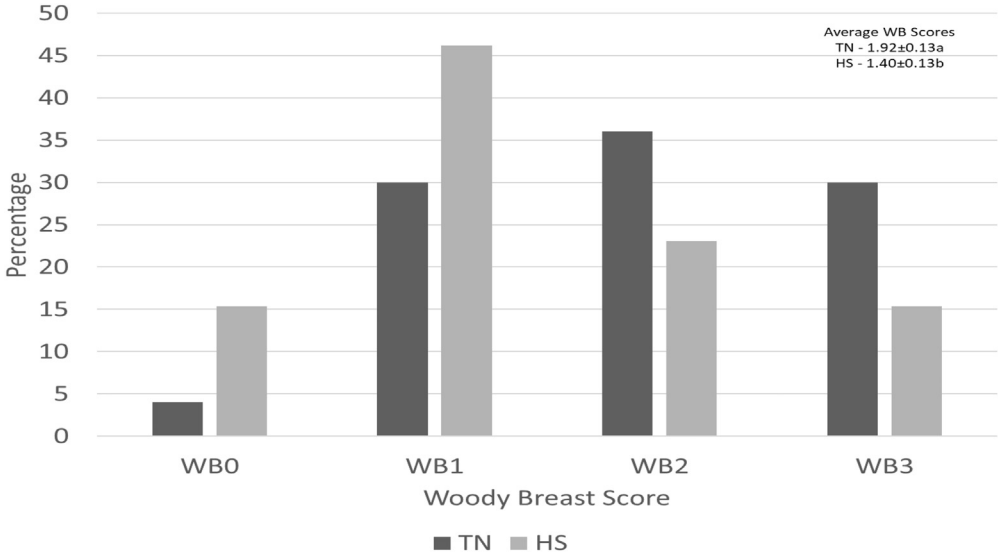

Figure 3. Percent of breast fillets within each woody breast score for the MRB line in HS and TN conditions at 55 D of age. A subjective scoring scale was used for woody breast (WB) with WB0 being no signs of white striping, and WB3 being severe white striping. Abbreviations: MRB, modern random bred line from 2015; TN, thermal neutral; HS, heat stress.

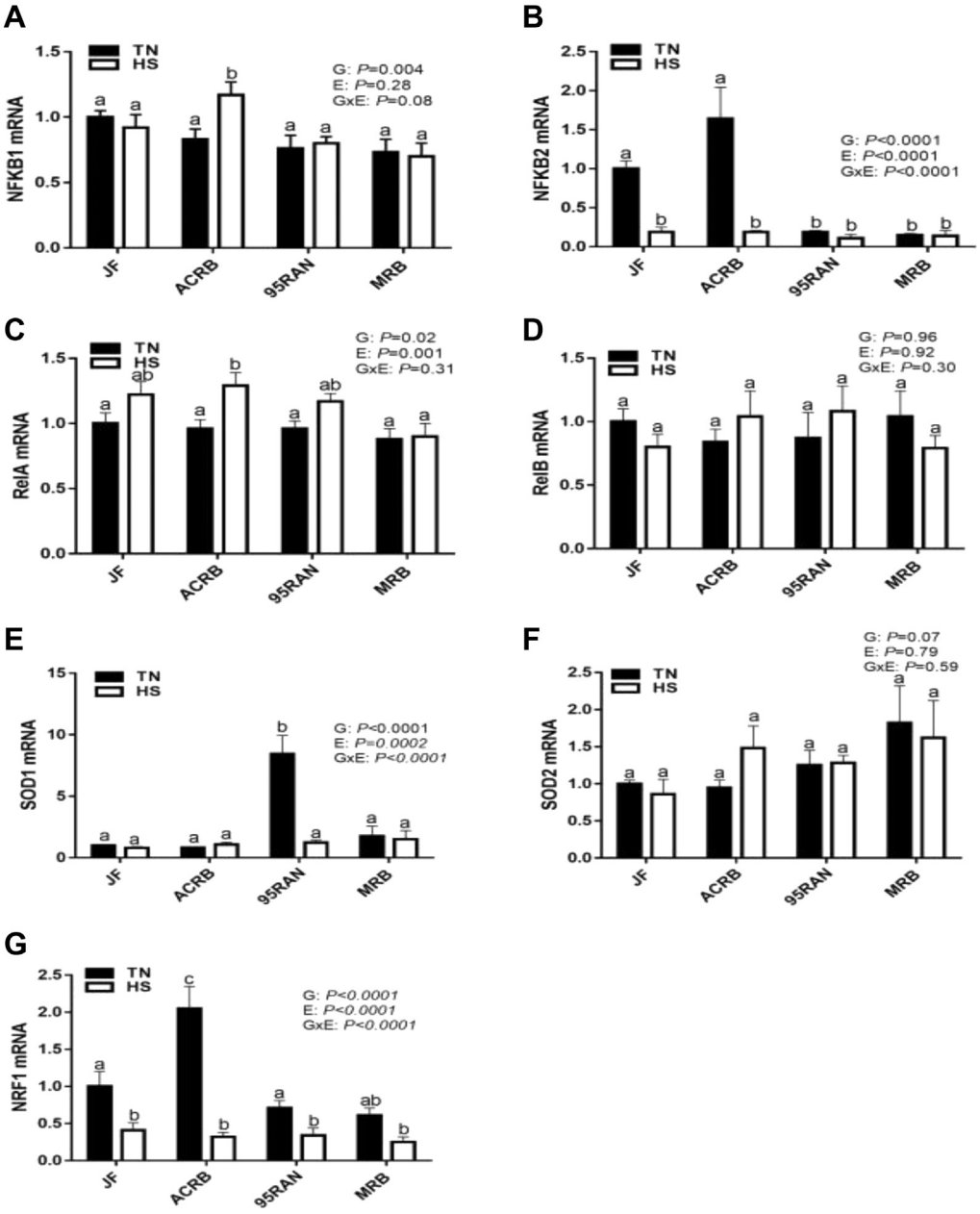

Figure 4. Effect of heat stress on the expression of stress- and antioxidant-related genes in the breast muscle of modern broilers and their ancestors. NFKB1 (A), NFKB2 (B), RelA (C), RelB (D), SOD1 (E), SOD2 (F), and NRF1 (G). mRNA abundances were measured by qPCR. Data are presented as mean ± SEM (n = 8/group). Abbreviations: MRB, modern random bred line (2015); 95RAN, 1995 random bred; ACRB, Athens Canadian random bred; JF, jungle fowl; TN, thermal neutral; HS, heat stress.

especially in turkeys (McKee and Sams, 1997) so it would be expected that the L* would be increased in the HS group. For this study, no difference was detected between the TN and HS groups for L* and it is unclear why that may be.

Woody breast (**WB**) and white striping (**WS**) are 2 conditions that have recently become a major concern to broiler producers as it directly impacts broiler meat quality. Both conditions are thought to occur more commonly and have an increased severity in birds with high growth and high breast muscle yield (Griffin et al., 2018). Unsurprisingly, only the MRB line exhibited either of the conditions so a comparison was only made between the HS and TN groups for the MRB line. The chronic period of heat stress significantly reduced the body weights in the HS group of the MRB line. This reduction in body weight and growth rate had a direct impact on the incidence of both the WB and WS myopathies. For both WB and WS, the incidence of severe cases (score of 3) for both myopathies was higher in the TN group than in the HS group while there were more fillets without the myopathy (score of 0) in the HS group. When considering the MRB line as a whole, the average WB and WS scores were higher for the TN group than for the HS group. For both WB and WS, statistical analysis indicated that there were more unaffected fillets in the HS treatment and more affected birds in the TN treatment. These results

indicate that both myopathies may be associated with growth rate as the literature suggests (Petracci et al., 2013; Sihvo et al., 2014). The increased levels of basal NRF1 in JF and ACRB suggest that these lines had a higher mitochondrial biogenesis and function (Kiyama et al., 2018; Gureev et al., 2019) which, in turn, might explain, the lower (or even absence) incidence of woody breast and white striping myopathies (Hubert et al., 2018; Greene et al., 2019).

In conclusion, it appears that heat stress had a larger effect on the higher yielding, faster growing populations, however, which may be due to the increase in heat production that would be seen from larger birds. This provides some potential insight into differences between fast-growing commercial birds and slow-growing breeds under heat stress conditions. Understanding the effect that heat stress may have on these lines when all processed at a common body weight (physiological age) will help to better understand the interaction between genetic selection for growth rate and feed conversion, and potential issues that may be associated with heat stress. This study also creates a better visualization for understanding the great improvements that have been made in the commercial broiler as a result of genetic selection.

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