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ANIMAL WELL-BEING AND BEHAVIOR

Effect of cooled perches on performance, plumage condition, and foot health of caged White Leghorn hens exposed to cyclic heat

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ABSTRACT We examined the effects of water-chilled perches as cooling devices on hen performance during 2 summers using daily cyclic heat. White Leghorns, 17 wk of age, were assigned to 36 cages arranged into 6 banks. The banks were assigned to cooled perches, air perches, and no perches resulting in 2 replicate banks and 12 cages per treatment. Chilled water (10°C) was circulated through the cooled perches during heat episodes. Daily cyclic heat of 35°C was applied from 0600 to1800 h with a lowering of temperature to 28°C from 1800 to 0600 h during the 2014 and 2015 summers when hens were 21 to 35 and 73 to 80 wk of age, respectively. Mortality and egg production were recorded daily. Feed utilization, egg weight, and shell quality traits were measured at 4-wk intervals during the heat episodes and at 8-wk intervals during thermoneutrality. Body weight was determined at 17, 35, 72, and 80 wk of age and physical condition at 80 wk of age. At several ages during the heat episodes, cooled perch hens had increased egg production ($P < 0.0001$) and feed usage ($P < 0.04$) as compared to both air perch and control hens. The cooled perch hens had higher BW at 35 and 72 wk of age ($P_{\text{treatment}*\text{age}} = 0.03$) and lower cumulative mortality ($P = 0.02$) than control hens but not air perch hens. Eggs from cooled perch hens had overall heavier weights ($P < 0.0001$) and higher breaking force ($P < 0.0001$) than eggs from the other two group hens. Greater eggshell percentage ($P_{\text{treatment}*\text{age}} = 0.03$) and eggshell thickness ($P_{\text{treatment}*\text{age}} = 0.01$) occurred at some ages during the 2 heat episodes as compared to the other 2 treatments. Nail length, feet hyperkeratosis, and overall feather score were similar among treatments. These results indicate that cooled perch ameliorates the negative effects of heat stress on egg traits and performance without influencing the physical condition of hens.

Key words: cooled perch, heat stress, laying hen, egg production, egg quality

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INTRODUCTION

High environmental temperature is one of the most detrimental problems facing the commercial egg industry during hot summers. Numerous egg production facilities are located in tropical or subtropical regions causing hens to be exposed to heat year round. Climate change in the last 50 yr has resulted in more hot days with more frequent and unexpected heat waves (Russo et al., 2017). These conditions prevent animals from dissipating heat to the surrounding environment to maintain body temperature within a physiological range resulting in heat stress (**HS**). Heat stress suppresses feed consumption in order to reduce metabolic heat production (Etches et al., 2008) in various domesticated species including cows (Kadzere et al., 2002), pigs (Collin et al., 2001), sheep (Marai et al., 2007), broiler chickens (Quinteiro et al., 2010), and laying hens (Mashaly et al., 2004). Laying hens exposed to both acute and chronic HS have impaired immunity and oxidative damage (Panda and Cherian, 2014), negatively affecting livability, egg production, egg weight, and eggshell quality (Lin et al., 2006; Rozenboim et al., 2007; Ajakaiye et al., 2011; Yoshida et al., 2011; Lara and Rostagno, 2013); by which HS leads to welfare damage and profound economic loss (Hristov et al., 2018).

In order to alleviate the effect of HS on laying hens, a variety of strategies have been employed such as feed manipulation, genetic selection, and physical cooling (Lin et al., 2006). Evaporative cooling pads

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and fogging are the most common methods used in hen housing that effectively lower ambient temperature (Gates and Timmons, 1986; Timmons and Gates, 1988; Lin et al., 2006). However, these methods increase humidity of housing facilities, which leads to wet manure and litter, overgrowth of bacteria, and excessive ammonia levels (Chepete and Xin, 2000; Dawkins et al., 2004). The increased humidity from evaporative cooling pads and fogging ultimately interferes with the bird's ability to dissipate heat through panting, a thermoregulatory behavior releasing body heat through accelerated respiration (Yahav et al., 1995).

Laying hens are highly motivated to perform roosting behavior when they have the opportunity to access perches (Olsson and Keeling, 2000, 2002). Perches installed in both cage and cage-free environments increase the comfort level of hens, which not only provides places for roosting but also improves skeletal strength (Appleby et al., 2002; Hester, 2014). Installed perches could also serve as a cooling device to reduce the negative effects of HS on laying hens via the transfer of body heat to the cooled perches during roosting. Approximately 25% of the body heat of chickens can be released via their feet through the unique feature of the arteriovenous anastomose system (Hillman and Scott, 1989). Cooled perches improved broiler welfare and growth performance including decreased mortality, improved feed efficiency, and increase BW under HS (Pettit-Riley and Estevez, 2001; Reilly et al., 1991; Estevez et al., 2002; Okelo et al., 2003; Zhao et al., 2012, 2013). Limited studies, however, have been conducted in laying hens. In our previous study, under natural weather, the mild summer temperature (averaged 24°C) was not sufficient to influence egg production and most measured physiological parameters, but birds used the cooled perches more frequently during the single 4-h heat exposure at 33.5°C, as an acute heat wave, and several other hot days during that summer. (Strong et al., 2015; Hu et al., 2016). The objective of the present experiment was to investigate if water-chilled perches improve production performance and the physical condition of caged laying hens under more severe elevated temperatures using daily cyclic heat episodes during 2 summers.

MATERIALS AND METHODS

Chickens, Management, and Cyclic Heat

A total of 390 day-old Hy-Line W-36 White Leghorn female chicks were randomly distributed to 30 cages at the Grower Research Unit of the Purdue University Poultry Research Farm. Chicks were infrared beak trimmed at the hatchery. Among the cages, 20 pullet cages were furnished with 2 perches. Cage dimensions and perch location within the pullet cage are described by Enneking et al. (2012). All pullets were reared following standard management guidelines and vaccination schedule (Hy-Line, 2016).

At 17 wk of age, 324 hens were transferred to the Layer Research Unit. The hens were assigned randomly to 36 cages of 9 birds per cage within 6 banks, each bank had 3 decks, and each deck had 2 cages. The banks were semirandomly assigned into 1 of 3 treatments (Figure 1): thermally cooled perch (Figure 1a), air perches containing ambient air (Figure 1b), and no perch (Figure 1c). There were 12 cages per treatment. The pullets assigned to the treatments were based on their rearing condition, i.e., the ones with or without perches were randomly assigned to perch groups (cooled perch or air perch) or non-perch group (control), respectively. The layer cage dimension and the perch location have been described by Hester et al. (2013). The perches of each cage provided 16.9 cm of perch space/hen, which was adequate for all 9 hens to roost simultaneously (United Egg Producers, 2017). Day-old female chicks consumed a starter diet with 20% CP, 1.0% Ca, and 0.45% non-phytate phosphorus to 3.9 wk of age, and a grower diet from 4 to 15.9 wk with 18.6% CP, 1.0% Ca, and 0.40% non-phytate phosphorus. A pre-lay diet with 18.4% CP, 2.50% Ca, and 0.35% non-phytate phosphorus was fed from 16 to 17 wk of age followed by a laying diet with 18.3% CP, 4.2% Ca, and 0.3% non-phytate phosphorus. Food and water were provided for ad libitum. The lighting schedule was gradually stepped up from 12L: 12D beginning at 17 wk of age to 16L: 8D, which was achieved at 30 wk of age (Hu et al., 2016). In the perch groups, the 2 perches were connected to form a continuous loop for each deck. Each loop of the cooled perch banks was controlled independently by a water pump connected to a water reservoir where water was cooled to approximately 10°C using a water chiller (ELKAY Manufacturing Co., Oak Brook, IL; Figure 1a; Gates et al., 2014). Pumps were turned on by a central controller when the cage temperature rose above 25°C so as to circulate chilled water through the round, galvanized metal perch pipes (Big Dutchman, Holland, MI). Two temperature sensors were installed in each cooled perch loop of each deck to measure the supply and return water temperatures. A single point for the ambient air perch temperature was also measured. Room relative humidity and the temperatures of the room, cage, as well as the supply and return of the water within the cooled perch at each deck level were monitored independently at 1-min intervals throughout the entire experiment using HOBO data loggers (model ZW-007 for cages with perches and model ZW-003 for cages without perches, Onset Computer Co., Bourne, MA; Gates et al., 2014; Xiong et al., 2015).

Hens were subjected to daily cyclic heat of 35°C from 6:00 am to 6:00 pm. The ambient temperature was lowered to 28°C from 6:00 pm to 6:00 am. This daily cyclic heat was applied using furnaces from 21 to 35 wk of age (2014 summer) and from 73 to 80 wk of age (2015 summer). From 36 to 39 wk of age, the ambient temperature was stepped down from 35°C (6:00 am to 6:00 pm) and 28°C (6:00 pm to 6:00 am) by 2°C per week until 20 to 25°C. At all other ages, hens were kept at 20 to 25°C. The protocol was

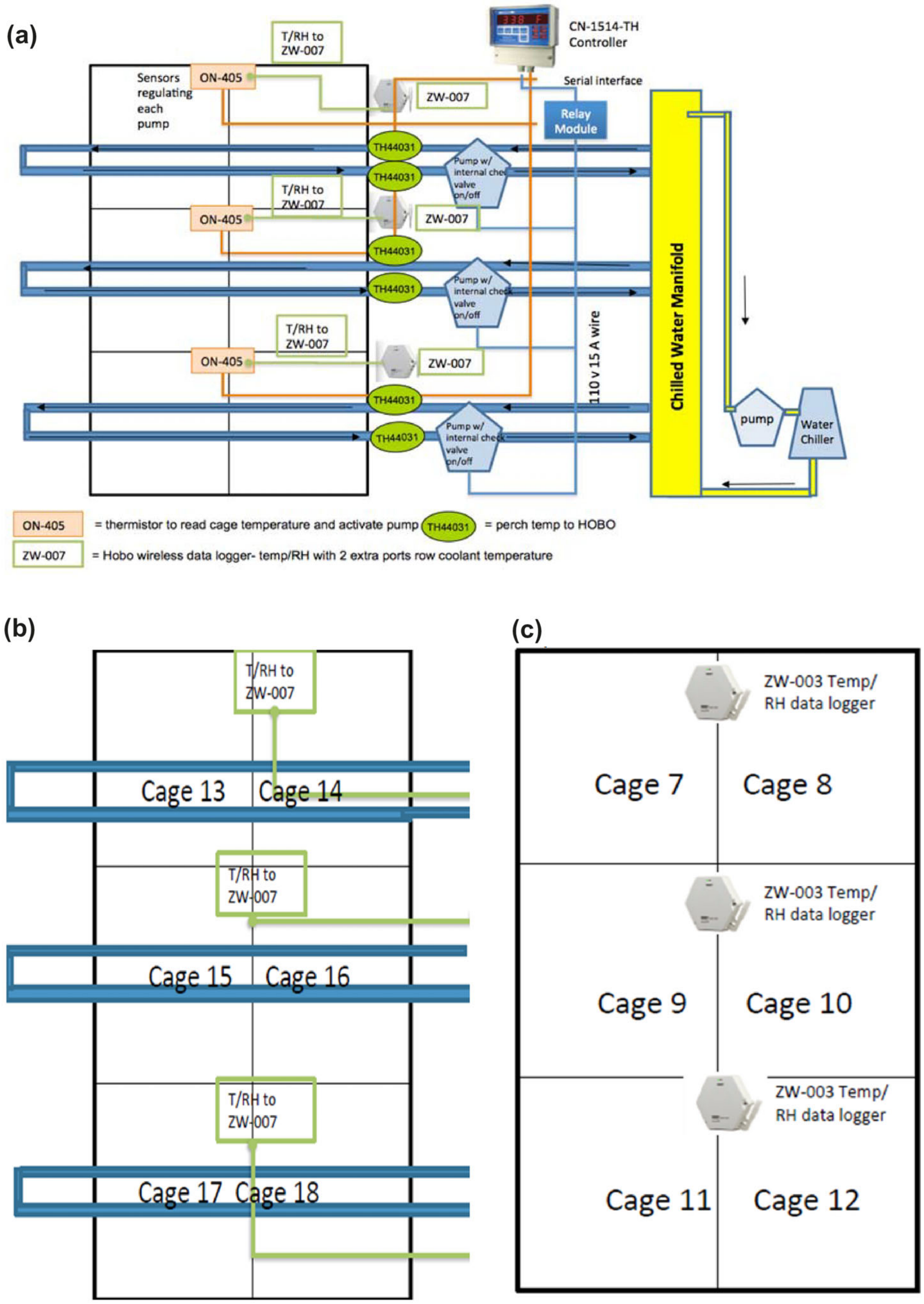

Figure 1. Cage bank design for the 3 treatments of cooled perch cages (a), air perch cages (b), and control cages with no perches (c). Two perches were installed parallel with each other in each row of a cage bank. For the cooled perch cages, a manifold was used to supply each loop with chilled water (10°C). Each loop was independently controlled by a water pump. Pumps for cooled perch cages were turned on when thermal sensors detected an air temperature exceeding 25°C. Printed with permission from the American Society of Agricultural and Biological Engineers (Gates et al., 2014) and Poultry Science (Hu et al., 2016).

approved by the Purdue University Animal Care and Use Committee (PACUC#: 1302000813).

Mortality, Feed Consumption, Egg Production, and Egg Quality

Mortality was recorded daily. The amount of feed used per cage over a 7-d period was determined at 4-wk intervals during the heat episodes and at 8-wk intervals during thermoneutral conditions, i.e., at week 24, 28, 32, 36, 40, 48, 56, 64, 72, 76, and 80. Average daily feed utilization per hen was calculated as total feed used (g)/7 d × number of live hens (Hester et al., 2013). Feed efficiency was calculated on per cage basis as the ratio of weekly feed utilized per dozen eggs produced.

Eggs were collected and recorded daily. Hen-day egg production per cage was calculated at 4-wk intervals as the total number of eggs produced/average number of live hens × 100%. Hen-house production per cage was calculated at 4-wk intervals as total number of eggs produced/number of hens housed × 100% (Bell, 2002).

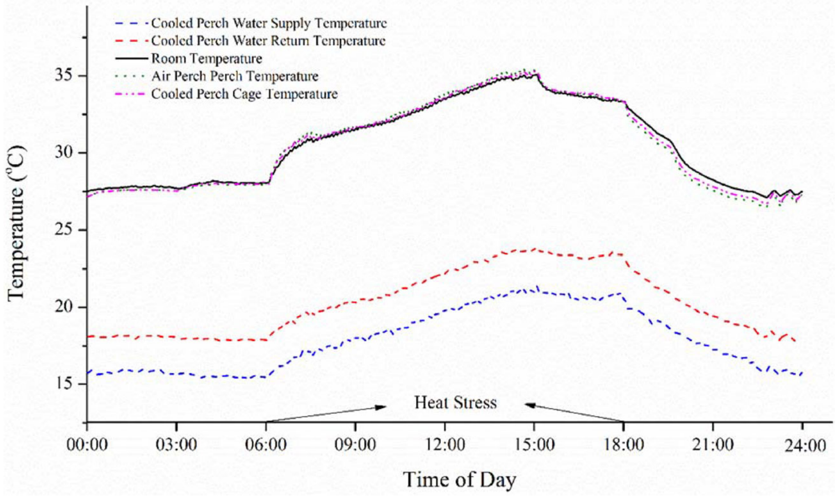

Figure 2. An example of temperatures recorded for 24 h during a heat episode (6:00 am to 6:00 pm) for the water in both the supply and return loop of a cooled perch, the room, and within air perch and cooled perch cages.

The numbers of cracked and dirty eggs (eggs with blood and or feces) were counted per cage and expressed as a percentage.

For eggshell quality, 10 intact hard-shelled eggs were collected from each cage during 2 consecutive days (120 eggs/treatment) during the same week when feed usage was determined. Each egg was weighed individually and measured for breaking force (ORKA Technology LLC, Bountiful, UT). A higher number for breaking force indicates a lower risk of shell breakage during egg handling and transportation (Hamilton et al., 1979). The proportions of eggshell and eggshell thickness were determined as described by Klingensmith and Hester (1985).

BW and Physical Condition

Hen BW was determined at the ages of 17 (transfer from pullet to laying cages), 35 (end of the first heat episode), 72 (1 wk prior to the second heat episode), and 80 wk (end of the second heat episode). Plumage condition and foot health were measured when the hens were 80-wk-old. Hen feather score was determined at 5 different regions of the body (breast, back, wings, vent, and tail areas) using a 4-point scoring system. A score of 1 represented the worst condition (severe feather damage and loss) and 4 represented the best plumage (Tauson et al., 2005). Feather score for each hen was averaged for an overall score.

Both foot pads and all toes were examined and scored for hyperkeratosis using a scoring system of 1 to 4 (Tauson, 1984a). A score of 4 represented healthy foot pads and toes with no lesions, whereas a score of 1 indicated deep and large epithelial lesions. The 8 nail lengths of each hen were measured using a flexible measuring tape. Numbers of broken claws for both feet were recorded. All foot condition data were averaged per hen for statistical analysis.

Statistical Analysis

Data from the randomized design were subjected to an ANOVA using the MIXED method of SAS (SAS Institute, 2013). Repeated measures were used for performance traits. The bank of cages was the experimental unit. Each of the 3 decks of a bank was a subsample. Each of the 2 cages within a deck was a sub-subsample. Fixed effects were treatment and age of the hens. Error terms included hens within cages, cages within a deck, and decks within a bank. The statistic model used in this study was $Y_{ijklm} = \mu + A_i + B_j + (AB)_{ij} + R_{ijklm} + (BR)_{ijklm} + \varepsilon_{ijklm}$, ε i.i.d $\sim (0, \sigma^2)$, where i and j referred to fixed effectors, treatment, and age of the hens; k, l, and m represented random effectors: bank, deck, and cage, respectively. Pooling of error terms occurred when $P > 0.25$. A 1-way ANOVA was used for the remaining data not measured over the age of the chicken. If data lacked homogenous variances, BOXCOX was used for transformation, and the data were re-analyzed (Box and Cox, 1964). Egg production data were arcsine square root transformed. Because statistical trends were similar for both transformed and untransformed data, the untransformed results were presented. The Tukey–Kramer test was used to partition differences among the means due to a significant treatment effect (Steel et al., 1997). The SLICE option was used for the 2-way interaction of treatment and age (Winer et al., 1991). Significant statistical differences were reported when $P \leq 0.05$.

RESULTS

Validation of the Thermal Perch System

The cooled perch successfully provided a resource for cooling hens as indicated by lower water supply and return temperatures as compared to room and cage ambient temperatures (Figure 2a). During a typical heat

Table 1. The effect of cooled perches on hen-day and hen-house egg production as well as the proportion of cracked and dirty eggs of caged laying hens from 17 to 80 wk of age.

Treatment	Hen-day egg production[1] (%)	Hen-house egg production[1] (%)	Cracked eggs[1] (%)	Dirty eggs[1] (%)
Cooled perch	77.6[a]	76.1[a]	1.47	0.27
Air perch	74.9[b]	73.7[b]	1.95	0.25
Control	72.6[c]	69.0[c]	1.91	0.42
n[2]	384	384	384	384
SEM	0.5	0.6	0.34	0.11
P-value				
$P_{\text{treatment}}$	<0.0001	<0.0001	0.55	0.47
P_{age}	<0.0001	<0.0001	<0.0001	0.001
$P_{\text{treatment*age}}$	<0.0001	<0.0001	0.17	0.61

[a–c]Least squares means within a column for the 3 treatments lacking a common superscript differ ($P < 0.05$).

[1]Values within a column represent the least squares means averaged over 16 mo of egg production (17 to 80 wk of age).

[2]Average number of observations per least squares mean.

episode, the ambient temperatures of cages containing air perch and cooled perch were parallel to room temperature. Temperatures went up when the heater was turned on (6:00 am to 6:00 pm) and went down when the heater was off (6:00 pm to 6:00 am). Due to the conductive transfer of hen body heat to the chilled water, the water temperature in the return loop was higher than the supply loop of the cooled perch. The temperatures of the chilled water within the cooled perch, regardless of supply or return loop, were lower than ambient air (Figure 2).

Egg Production and Eggshell Quality

Both hen-day and hen-house egg productions were increased by cooled perch installation. Among treatments, cooled perch hens had the highest overall hen-day and hen-house egg production than both air perch and control hens. The air perch hens had higher egg production than control hens ($P < 0.0001$, Table 1). The beneficial effect of cooled perch was even more distinct toward the end of the laying cycle from 57 to 80 wk of age ($P_{\text{treatment}\times\text{age}} < 0.0001$, Figure 3a). The proportions of cracked and dirty eggs were similar among treatments (Table 1). Hens laid a greater proportion of cracked eggs toward the end of the laying cycle ($P_{\text{age}} < 0.0001$, data not presented). The proportion of dirty eggs fluctuated with age showing no obvious trend ($P_{\text{age}} = 0.001$, data not presented).

The cooled perch hens laid heavier eggs ($P < 0.0001$) with greater eggshell breaking force ($P < 0.0001$) than both air perch and control hens, whereas there were no differences between the latter 2 groups (Table 2). The greater egg weight ($P_{\text{treatment}\times\text{age}} < 0.0001$, Figure 4a) and eggshell breaking force ($P_{\text{treatment}\times\text{age}} = 0.01$, Figure 4b) were mostly during the 2 summers (24 to 36 and 76 to 80 wk of age), especially when the cyclic heat episodes were applied. Hens laid heavier eggs ($P < 0.0001$, Figure 4a) with lower breaking force ($P < 0.0001$, Figure 4b) as they aged.

The treatment effects on the proportion of shell and eggshell thickness were revealed mostly during the 2 summer heat episodes (Figure 5). Compared with air perch and sometimes the control hens, cooled perch hens had higher proportions of eggshell at 24 wk of age during the first heat episode and at 76 and 80 wk of age during the second heat episode (Figure 5a) and higher eggshell thickness at 24 and 28 wk of age during the first heat episode and 76 and 80 wk of age during the second heat episode (Figure 5b). However, the other ages during the time heat was applied and the majority of the ages under thermoneutral condition showed no differences in the proportions of eggshell and eggshell thickness among treatments.

Mortality, Feed Usage and Efficiency, and BW

Cumulative mortality from 17 to 80 wk of age was lower for cooled perch as compared to control but not air perch hens ($P = 0.02$, Table 3). Hens with access to cooled perch had higher overall feed utilization than the other 2 treatments. The treatment by age interaction was due to greater feed utilization for the cooled perch hens during the 2 heat episodes. At other ages (an exception occurred at 64 wk of age) when hens were not exposed to daily cyclic heat and in their thermoneutral zone, feed utilization was similar among treatments ($P_{\text{treatment*age}} = 0.04$, Figure 6). Feed efficiency was not affected by treatment ($P = 0.23$) but became worse as hens aged ($P < 0.0001$, Table 3). Hen BW in response to provision of cooled perch was inconsistent with age ($P_{\text{treatment*age}} = 0.03$, Table 3). As compared to control hens, the cooled perch hens had higher BW at 35 wk of age, which was at the end of the first heat episode, and at 72 wk of age which was the onset on the second heat episode. No differences due to treatment occurred at the other ages of 17 and 80 wk (Figure 7).

Physical Measurements

Overall feather score, hyperkeratosis score, and nail length were unaffected by treatment (Table 4). Breast feather scores were worse ($P = 0.002$), but tail feather scores were improved ($P = 0.05$, Figure 8) in both perch groups as compared to control hens. The other plumage areas of wing, back, and vent were not affected by treatment. Hens with access to cooled perch had fewer broken toe nails than air perch hens. The number of nails broken in control hens was intermediate between the other 2 treatments ($P = 0.04$, Table 4).

DISCUSSION

Water-chilled perches ameliorate the negative effects of cyclic heat on egg production, mortality, BW, egg

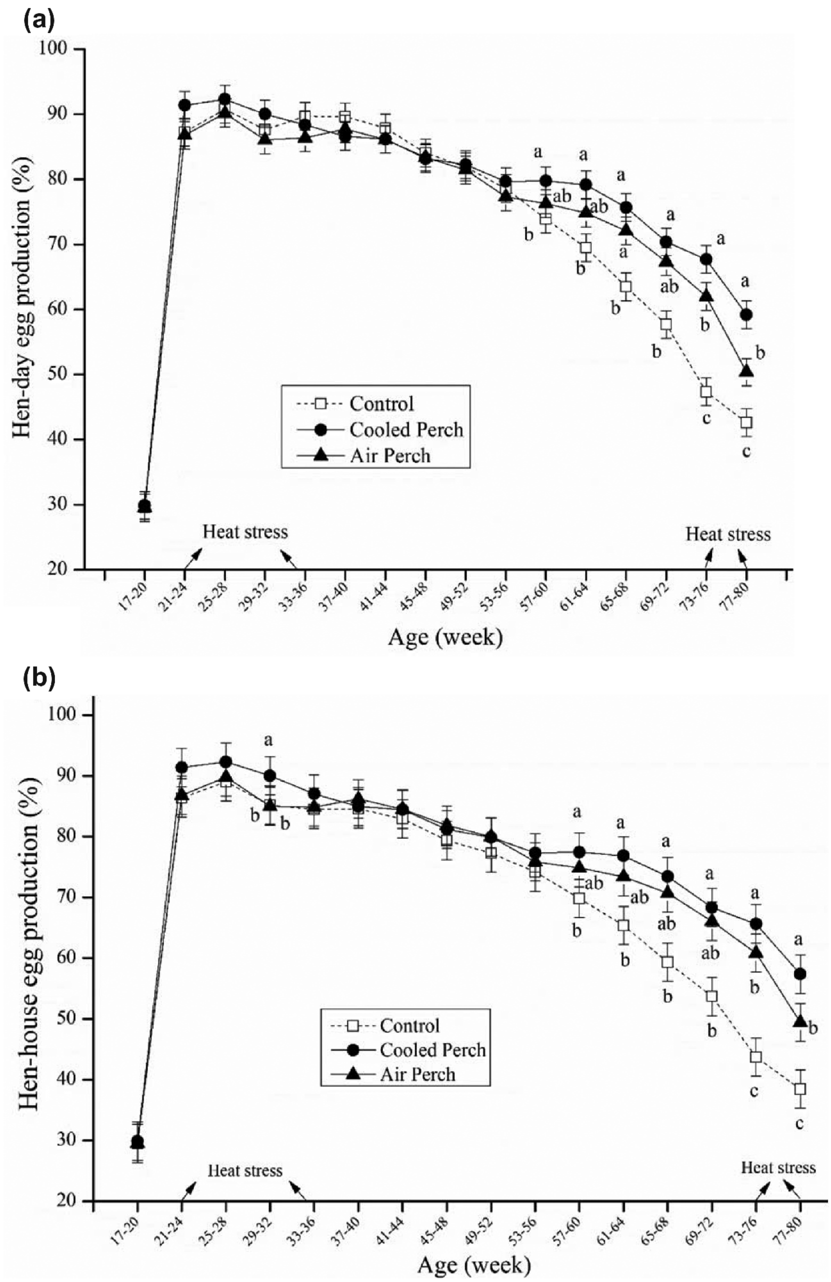

Figure 3. Monthly hen-day (a) and hen-house egg production (b) from 17 to 80 wk of age (□ = control, • = cooled perch, ▲ = air perch). [a–c]Within a month, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P < 0.0001$).

weight, and shell quality traits without influencing the percentage of dirty and cracked eggs, overall plumage condition, and foot health of caged laying hens. Provision of water-chilled perches likely assists hens in conducting body heat to the perch through their feet or other body parts when the ambient temperature is above the upper critical boundary of the thermoneutral zone. Furthermore, compared to air perch and control hens, cooled perch hens exhibited fewer HS-induced behaviors, such as panting and wing spreading, under both acute heat (Hu et al., 2016) and daily cyclic heat during summer (Makagon et al., 2015). Less effort is needed by cooled perch hens to maintain thermal homeostasis through other methods such as limiting energy intake from feed and increasing evaporative heat loss through panting, and thus production is improved.

Mortality, Feed Usage and Efficiency, and BW

The control hens had higher cumulative mortality than cooled perch hens with intermediate mortality for air perch hens. The higher death of control chickens is perhaps an indicator of these hens' inability to acclimate to elevated temperatures. When temperature is consistently higher or suddenly climb above the upper limit of the thermoneutral zone, osmolality and protein structure are disturbed resulting in multiple

Table 2. The effect of cooled perches on weight and shell traits of eggs from caged laying hens.

Treatment	Egg weight[1] (g)	Breaking force[1] (N)	Proportion of eggshell[1] (%)	Eggshell thickness[1] (mm)
Cooled perch	61.1[a]	36.3[a]	8.69	0.34
Air perch	60.0[b]	34.9[b]	8.66	0.33
Control	59.6[b]	35.0[b]	8.71	0.33
n[2]	1320	1320	1320	1320
SEM	0.1	0.3	0.06	0.002
P-value				
$P_{treatment}$	<0.0001	<0.0001	0.82	0.44
P_{age}	<0.0001	<0.0001	<0.0001	<0.0001
$P_{treatment*age}$	<0.0001	0.01	0.03	0.01

[a,b]Least squares means within a column for the 3 treatments lacking a common superscript differ ($P < 0.05$).

[1]Values within a column represent the least squares means of 10 eggs from each of the 3 treatments averaged over 11 ages of 24, 28, 32, 36, 40, 48, 56, 64, 72, 76, and 80 wk.

[2]Average number of observations per least squares mean.

organ failure, including heart, kidney, liver, etc. (Jardine, 2007; Hansen, 2009). In 2011, 50,000 chickens died at a farm after the power went off for less than an hour when the ambient temperature exceeded 37°C (Hegeman, 2011). The immune system is compromised with high-temperature exposure (Mashaly et al., 2004). Both humoral and cell-mediated immunity of birds were depressed under HS (Ogle et al., 1997; Zulkifli et al., 2000a), which make the birds more prone to infectious diseases that can lead to higher mortality. In the present study, the mortality observed in controls was most likely due to organ failures rather than disease as necropsy of dead birds showed only 1 case of *Escherichia coli* infection. Similar to our study, elevated mortality was reported in broiler chickens and laying hens housed under an ambient temperature of 35°C compared to a thermoneutral environment (Deaton et al., 1978; Mashaly et al., 2004). Installation of cooled perch should greatly facilitate hen survival under conditions of elevated temperatures.

Elevated temperatures depress appetite causing poultry to lose weight (Tanor et al., 1984; Zulkifli et al., 2000b; Sahin et al., 2002; Mashaly et al., 2004; Çiftçi et al., 2005). For temperatures that range from 5 to 35°C, a 1.5% decrease in feed intake per 1°C increase was observed in laying hens under chronic or cyclic temperature regimens, with baseline control at 20 to 21°C (National Research Council, 1981). Our results on feed usage showed that these negative effects of HS were ameliorated by providing hens with access to cooled perch. Likewise, broiler chickens responded favorably to water-chilled perches when exposed to high environmental temperature (Muiruri and Harrison, 1991; Reilly et al., 1991; Estevez et al., 2002; Zhao et al., 2013). Specifically, broilers subjected to HS of 32 to 35°C with access to cooled perch had increased BW, feed utilization, and improved feed efficiency. In the present study, BW was higher in cooled perch hens than control after the first heat exposure (35 wk of age) and 1 wk before the second heat period (72 wk of age), but no differences were found at the beginning of the laying phase (17 wk of age), which was expected because heat episodes had not been initiated, and at the end of the second heat episode (80 wk of age). It is unknown why differences in BW dissipated among treatments at the end of the study as 80-wk-old cooled perch hens utilized more feed than air perch and control hens (Figure 6) and, therefore, they should have weighed more. The increased feed utilization is an indicator of better thermoregulation of hens with access to cooled perch as hens have greater ability to match heat production with heat loss to the environment without suppressing energy intake (Lin et al., 2006; Slimen et al., 2016).

Feed efficiency was not improved as a result of providing cooled perch to laying hens unlike other studies with broiler chickens (Reilly et al., 1991; Estevez et al., 2002; Zhao et al., 2013). Hens with cooled perch did produce more eggs (Table 1), but at the same time, they used more feed than the other 2 treatments (Table 3).

Egg Production

Reproductive diminishment is a well-known phenomenon in both mammals and birds exposed to high environmental temperature (Hansen, 2009; Sahin et al., 2018). Providing cooled perch reduced the negative effects of HS on overall egg production (Table 1), especially near end of lay (Figure 3). In addition, the air perch hens had higher production performance than control hens when exposed to 2 cyclic heat episodes. Previous studies dealing with perch effects on egg production were conducted on hens under thermoneutral conditions, and they reported no beneficial effect of perch availability on egg laying (Tauson, 1984b; Duncan et al., 1992; Abrahamsson and Tauson, 1993; Hester et al., 2013; Hester, 2014). In the current study, access to perches, whether cooled or not, was most likely an effective way for hens to increase space availability when standing on the perch, thereby increasing airflow rate to the hens and avoiding heat accumulation and transfer among cage mates (Pettit-Riley and Estevez, 2001). However, under conditions of daily cyclic heat, egg production of hens with cooled perch benefited greatly from thermal cooling offering an enhanced advantage over air perch hens. Heat stress in laying hens inhibits nutrient intake, digestibility, and intestinal absorption due to heat-induced intestinal damage, which limits the availability of circulating nutrients in the blood that are essential for egg formation (Jeurissen et al., 2002; Etches et al., 2008). In addition, the nutrient deprivation under elevated temperatures is most likely associated with redistribution of blood inside the body. Under HS, blood flow is redistributed mostly to the peripheral tissues in order to dissipate more heat to the environment, which results in reduced blood flow and motility in the gastrointestinal tract, thus

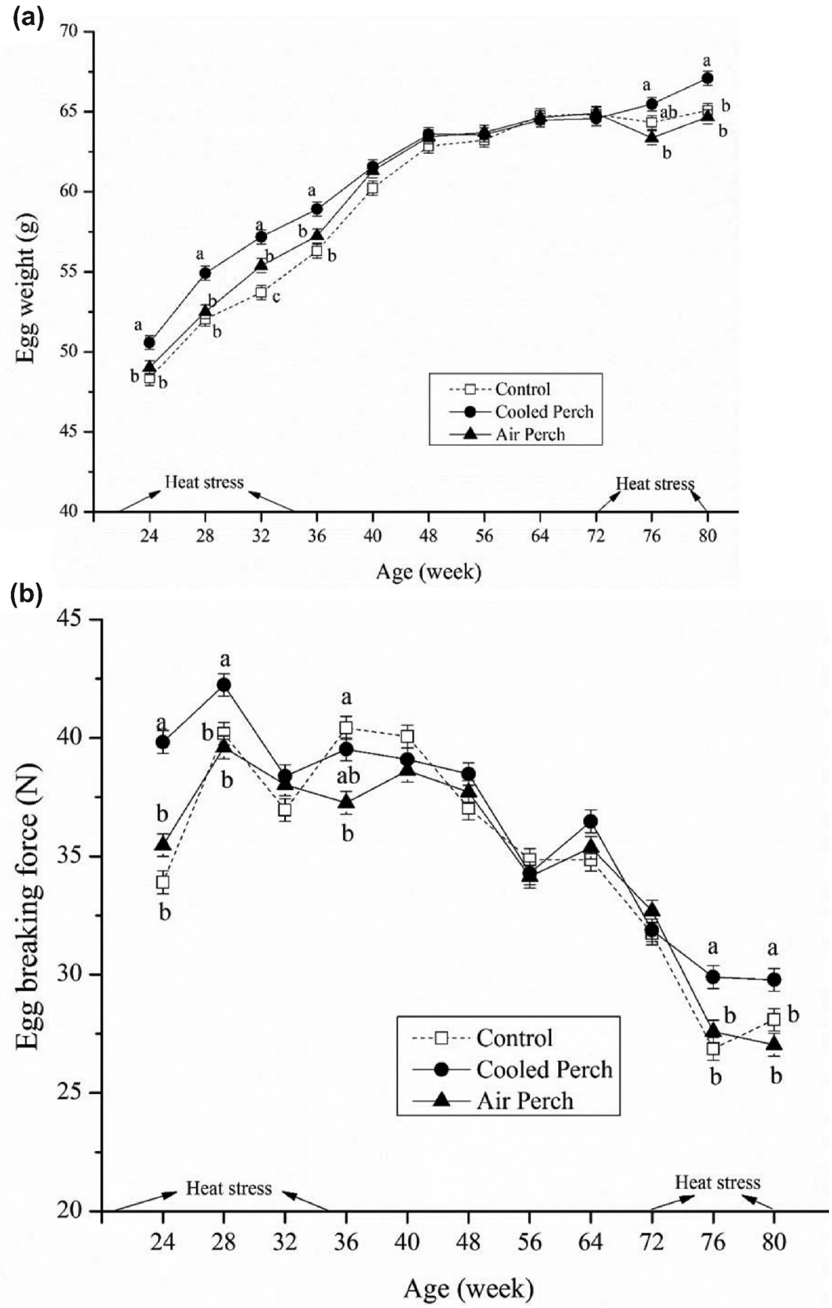

Figure 4. The weight (a) and breaking force (b) of eggs from White Leghorns submitted to 1 of 3 treatments (□ = control, • = cooled perch, ▲ = air perch) between 24 and 80 wk of age. [a–c]Within an age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P < 0.0001$ and $P = 0.01$ for weight and breaking force, respectively). Means represent 120 eggs collected from 12 cages/treatment per age.

affecting intestinal function (Wolfenson et al., 1981; Mitchell and Carlisle, 1992). Diminished egg laying under HS could also be partially influenced by depression of reproductive hormones (Rozenboim et al., 2004, 2007). The higher reproductive performance of hens with access to cooled perch indicates that this cooling system has the potential to ameliorate the negative effect of HS by conductively transferring heat from hens to the cooled water.

The cooled perch mitigated the negative effects of HS on egg production without increasing the incidence of unmarketable eggs as the percentages of cracked and dirty eggs did not differ among the 3 treatments. Previous studies have reported an increased incidence of cracked eggs when perches were placed in cages compared to conventional cages, as birds may lay eggs while standing on the perch (Tauson, 1984b; Glatz and Barnett, 1996; De Reu et al., 2009). The low perch height (8.9 cm) used in the current study minimized the chance of shell breakage when eggs were laid by perching hens, agreeing with the results from a previous study with the same perch height (Hester et al., 2013). Tuyttens et al. (2013) suggested that a lower perch could be an effective remedy for the high prevalence

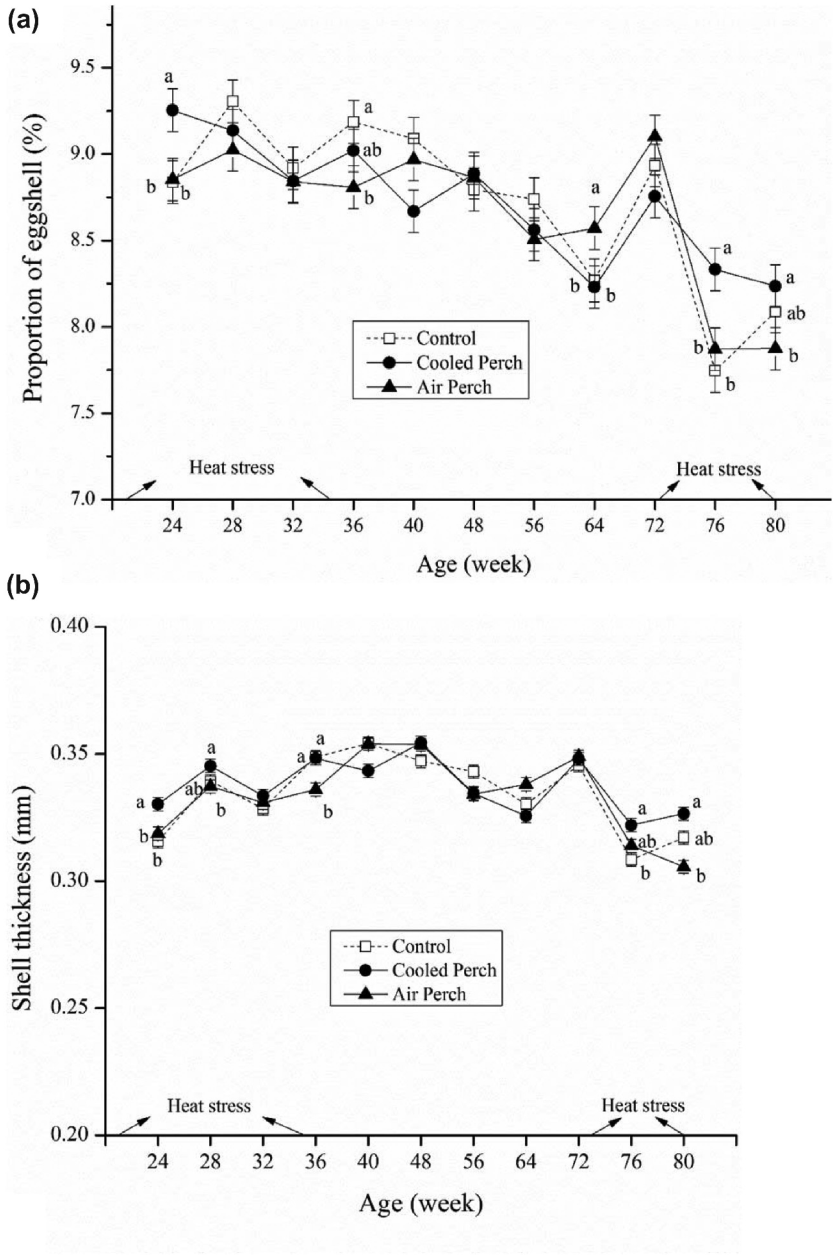

Figure 5. The proportion of shell (a) and shell thickness (b) of eggs collected from White Leghorns submitted to 1 of 3 treatments (☐ = control, • = cooled perch, ▲ = air perch) between 24 and 80 wk of age. [a,b]Within an age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P = 0.03$ and 0.01 for % shell and thickness, respectively). Means represent 120 eggs collected from 12 cages/treatment per age.

of broken eggs in furnished cages with elevated perches and at the same time meet the hen's behavioral needs. Under thermoneutral conditions, laying hens with access to perches as compared to conventionally cage hens without perches showed an increase in the proportion of dirty eggs (Nakaue et al., 1984; Hester et al., 2013). Other studies reported no perch effect on the incidence of dirty eggs (Tauson, 1984b; Appleby et al., 1992). The installation of perches in cages causes chickens to spend less time walking on the bottom of the cage floor which can interfere with eggs rolling to the collection area due to less vibration (Hester, 2014). Heavy perch use can also cause manure accumulation directly under the perch leading to dirty eggs (Nakaue et al., 1984).

Egg Weight and Shell Qualityquality

Egg traits, including egg weight and shell breaking force, were improved with the presence of cooled perch compared to the other 2 treatments. Eggs from hens exposed to elevated temperatures, either constant or cyclic, weighed less than eggs from hens housed within their thermoneutral zone (Kirunda et al., 2001; Mashaly et al., 2004). The increased weight of eggs laid by hens with access to cooled perch is likely related to increased protein and amino acid consumption due to higher feed utilization. Increased egg size and weight occur in broiler breeder hens (Joseph et al., 2000) and laying hens (Summers and Leeson, 1994; Keshavarz and

Table 3. The effect of cooled perches on hen mortality, feed usage and efficiency, and BW.

Treatment	Cumulative hen mortality (%)	Feed utilization[1] (g/hen/d)	Feed efficiency[1] (kg of feed/dozen eggs)	Hen BW (kg)[2]
Cooled perch	2.78[b]	103.02[a]	1.57	1.44
Air perch	3.70[a,b]	98.28[b]	1.58	1.40
Control	10.19[a]	100.56[b]	1.84	1.40
n[3]	24	264	264	96
SEM	1.93	0.82	0.12	0.02
P-value				
$P_{treatment}$	0.02	0.0002	0.23	0.27
P_{age}	–	<0.0001	<0.0001	<0.0001
$P_{treatment*age}$	–	0.04	0.19	0.03

[a,b]Least squares means within a column for the 3 treatments lacking a common superscript differ ($P < 0.05$).
[1]Values within a column represent the least squares means averaged over 11 ages of 24, 28, 32, 36, 40, 48, 56, 64, 72, 76, and 80 wk.
[2]Values within a column represent the least squares means averaged over 4 ages of 17, 35, 72, and 80 wk.
[3]Average number of observations per least squares mean.

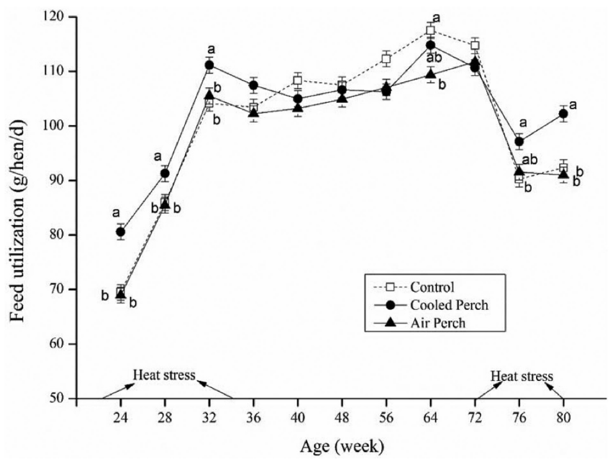

Figure 6. Feed utilization of White Leghorns submitted to 1 of 3 treatments (□ = control, • = cooled perch, ▲ = air perch) between 24 and 80 wk of age. [a,b]Within an age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P = 0.04$).

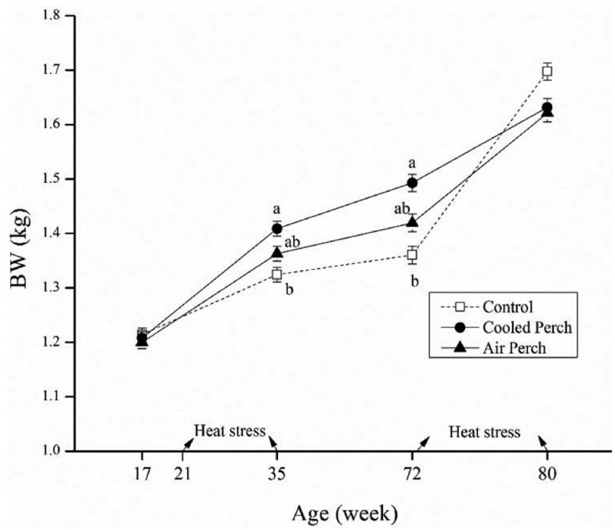

Figure 7. The BW of White Leghorns submitted to 1 of 3 treatments (□ = control, • = cooled perch, ▲ = air perch) at 17, 35, 72, and 80 wk of age. [a,b]Within an age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P = 0.03$). Means represent 2 hens weighed from each cage for a total of 12 cages/age.

Table 4. The effect of cooled perches on feather score and foot health at 80 wk of age.

Treatment	Mean feather score[1]	Hyperkeratosis score[2]	Mean nail length (cm)	Number of broken toenails
Cooled perch	2.02	3.82	2.46	0.96[b]
Air perch	2.46	3.91	2.16	1.58[a]
Control	1.95	3.83	1.80	1.13[a,b]
n[3]	24	24	24	24
SEM	0.27	0.24	0.23	0.18
P-value	0.36	0.96	0.13	0.04

[a,b]Least squares means within a column for the 3 treatments lacking a common superscript differ ($P < 0.05$).
[1]Scores for feather condition ranged from 1 to 4, with 4 representing no damage to the feathers and 1 representing severe damage.
[2]Scores for hyperkeratosis ranged from 1 to 4 with 4 representing normal feet and 1 representing severe hyperkeratosis.
[3]Average number of observations per least squares mean.

Nakajima, 1995) fed increased CP levels in the pre-lay and early lay diets. Higher breaking force numbers of eggs from cooled perch hens suggest that these eggs are less likely to crack or break during handling and transportation (Hamilton et al., 1979), leading to more marketable eggs and economic profits for producers. The increase in egg weight (Figure 4a) and the decline in egg breaking force as hens age (Figure 4b) are in agreement with previous studies (Minvielle et al., 1994; Rodriguez-Navarro et al., 2002; Mitrovic et al., 2010; Tumova et al., 2017).

In addition to shell breaking force, proportions of shell and eggshell thickness are indicators of shell quality. These shell traits are all positively correlated to each other with low numbers indicating a greater probability for shells to crack (Ar et al., 1979; Sun et al., 2012). Similar to our results on shell breaking force, these shell traits were mostly improved in cooled perch hens as compared to air perch and control hens during the 2 summer cyclic heat episodes with little to no differences in these shell traits when hens were kept in their thermoneutral zone. Lowered calcium consumption critically affects shell quality (Scott et al., 1971; Creger et al., 1976). Under conditions of homeostasis, calcium used for eggshell formation mostly come from the diet (Taylor, 1970; Keshavarz and Nakajima, 1993; Roberts, 2004). Under normal temperatures when hens are in their thermoneutral zone, they will overconsume feed if they are provided with a low calcium diet in order to have enough calcium for eggshell formation (Roland et al., 1985; Clunies et al., 1992). During HS, appetite is depressed to minimize metabolic heat production causing inadequate calcium intake leading to poor shell quality. In addition, continuous panting that

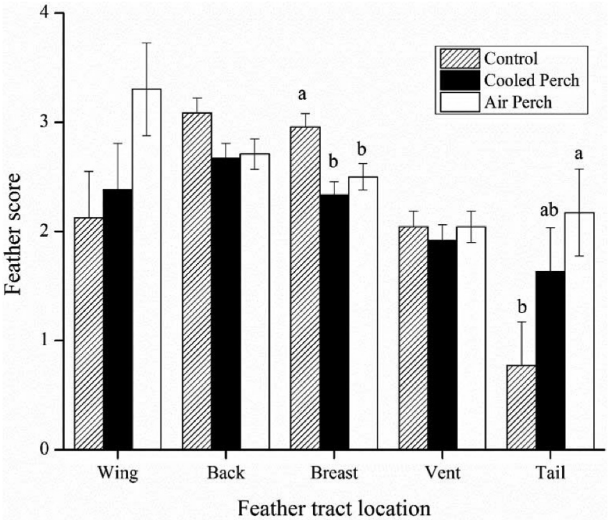

Figure 8. The effect of cooled perch as compared to air perch and controls on the feather scores of the wing, back, breast, vent, and tail of caged White Leghorn hens at 80 wk of age. Scores for feather condition ranged from 1 to 4, with 4 representing non-damaged feather and 1 indicative of severe damage. Values represent the least squares means ± SEM. Within a region of the hen's body, scores lacking a common letter (a, b) differ ($P \leq 0.05$).

could lead to hyperventilation under extreme HS conditions causes respiratory alkalosis. Arterial blood pH increases during alkalosis, decreasing the availability of circulating bicarbonate and calcium ions, 2 major components of eggshell (Balnave and Muheereza, 1997; Nys, 1999). These changes further decrease eggshell quality (Odom et al., 1986; Koelkebeck and Odom, 1994; Ruzal et al., 2011). Reduced panting in the cooled perch as compared to air perch and control hens was observed in our first study dealing with cooled perch (Hu et al., 2016) and was confirmed in our current study (Makagon et al., 2015). Broiler chickens also show reduced panting when given access to cooled perch (Zhao et al., 2012). A reduction in panting that avoids deep breathing, and subsequently respiratory alkalosis, could also contribute to improved shells of eggs from cooled perch hens.

The lack of an effect of cooled perch on shell breaking force at 32 wk of age, the proportion of shell at 28 and 32 wk of age, and shell thickness at 32 wk of age during the first summer cyclic heat episode is perplexing as hens used more feed at these ages which would be indicative of greater calcium intake.

The higher proportion of shell post HS in eggs from control hens at 36 wk of age and air perch hens at 64 wk of age can be explained in part by small egg size or lower rate of lay. Compared to large eggs, hens laying lighter eggs place the same amount of shell around the yolk and albumen contents leading to thicker shells. Lower egg production creates less demand for calcium. Thus, the proportion of shell based on egg weight could increase (Mazzuco and Hester, 2005) under circumstances of lower egg weight and production. In addition, heat habituation and acclimation might be achieved after repeated or chronic HS. Adjustments in systematic functioning in response to HS could help the hens to develop a new homeostasis which may improve thermotolerance and their ability to lay eggs with better shell quality (Yahav et al., 2009).

Physical Condition

Feather condition, when averaged across all of the examined feather tracts, was not affected by provision of perches (Table 4), similar to results reported by Barnett et al. (1997) in hens not exposed to HS. In contrast, hens maintained in their thermoneutral zone had poorer overall feather scores due to the presence of metal (Hester et al., 2013) or wooden (Tauson, 1984b) perches. Caged hens with perches had worse feather scores for the neck, breast, wings, and tail as compared to these same feather tracts of hens in conventional cages without perches (Tauson 1984b). Hester et al. (2013) observed poorer breast and tail feather scores but better back feather scores in hens with perch access as compared to those hens with no perches under thermoneutraily. The poorer scores for breast feathers of hens with access to either cooled perch or air perch in the current study were most likely due to the rubbing of the hen's breast when sitting on the metal perches. The reason for better tail feather scores of hens with access to perches, in particular the air perch as compared to control, is less clear as tail feathers of cooled perch hens were intermediate in response. The better tail feather scores of air perch as compared to control hens could be due to the reduction of HS-induced aggressive behavior. Clauer (2009) identified excessive heat as one of the factors causing cannibalism in laying hens, and the tail area is a favorite location for cannibalism (cloaca) and feather pecking (Gunnarsson, 1999)

Foot health for all hens in this study was excellent because the average hyperkeratosis score of 3.85 (1 to 4 scale) was near to perfect resulting in no treatment effect. Broiler chickens raised on littered floor and given access to cooled perch had better foot condition including improved burn scores for the hock and footpad as compared to control without perches (Zhao et al., 2012). Floor housing with wet litter leads to a higher incidence of foot damage in both broilers (Dunlop et al., 2016) and laying hens (Wang et al., 1998). Although water condensation was observed on the surface of the cooled perch due to the large temperature gap between perch (10°C) and ambient temperature (35°C), foot health was not affected by the wet roosting surface in the current study.

The presence of metal perches serves as an abrasive to trim toenails of caged hens (Hester et al., 2013), but different results have also been reported with no effect of perch installation on claw length (Tauson, 1984b; Appleby et al., 1992), which is similar to the current finding. It is important to keep toe nails trimmed as long claws, which are especially prevalent in aging hens, can break off more easily leading to bleeding and open wounds that could cause pain and infection (Lay et al., 2011). Generally, in perch equipped cages, hens jumping on and off the perches have a higher risk of breaking toenails (Hester et al., 2013). Interestingly, we found more broken toenails in the air perch hens than cooled perch hens but not control. During daily cyclic heat, hens with cooled perch may have spent longer periods of time remaining on the perches to stay cool, whereas the air perch hens were more restless. The air perch hens may have jumped on and off the perches, continuously switching to different locations in the cage in their attempt to find a cooler area during heat exposure. Further behavior data, however, are needed to prove this hypothesis.

CONCLUSIONS

Thermally chilled perches facilitated hen thermoregulation during daily cyclic heat episodes of 35°C. The presence of cooled perch in cages improved egg production, egg weight, shell traits, and livability of laying hens during exposure to elevated temperatures. The provision of cooled perch did not compromise overall plumage conditions and foot health. Our results indicate that the cooled perch could be an effective alternative cooling method for caged laying hens to ameliorate the deleterious effects of high ambient temperature, thus improving the production performance of hens during hot weather.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

REFERENCES

Abrahamsson, P., and R. Tauson. 1993. Effect of perches at different positions in conventional cages for laying hens of 2 different strains. Acta Agric. Scand. Sect. A-Anim. Sci. 43:228–235.

Ajakaiye, J. J., A. Perez-Bello, and A. Mollineda-Trujillo. 2011. Impact of heat stress on egg quality in layer hens supplemented with l-ascorbic acid and dl-tocopherol acetate. Vet. Archiv. 81:119–132.

Appleby, M. C., S. F. Smith, and B. O. Hughes. 1992. Individual perching behaviour of laying hens and its effects in cages. Br. Poult. Sci. 33:227–238.

Appleby, M. C., A. W. Walker, C. J. Nicol, A. C. Lindberg, R. Freire, B. O. Hughes, and H. A. Elson. 2002. Development of furnished cages for laying hens. Br. Poult. Sci. 43:489–500.

Ar, A., H. Rahn, and C. V. Paganelli. 1979. The avian egg: mass and strength. The Condor 81:331–337.

Balnave, D., and S. K. Muheereza. 1997. Improving eggshell quality at high temperatures with dietary sodium bicarbonate. Poult. Sci. 76:588–593.

Barnett, J. L., P. C. Glatz, E. A. Newman, and G. M. Cronin. 1997. Effects of modifying layer cages with perches on stress physiology, plumage, pecking and bone strength of hens. Aust. J. Exp. Agric. 37:523–529.

Bell, D. D. 2002. Chapter 55: Egg production and egg weight standards for table-egg layers. in Pages 1079–1090 Commercial Chicken Meat and Egg Production. 5th ed. D. D. Bell, and W. D. Weaver, Jr., ed. Kluwer Academic Publishers, Norwell, MA.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. J. R. Stat. Soc. Ser. B. Stat. Methodol. 26:211–252.

Chepete, H. J., and H. Xin. 2000. Cooling laying hens by intermittent partial surface sprinkling. Trans. ASAE. 43:965–971.

Çiftçi, M., O. N. Ertas, and T. Güler. 2005. Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Rev. Med. Vet. 156:107–111.

Clauer, P. J. 2009. Cannibalism: Prevention and Treatment. Publ. No. 2902-1095. Virginia Cooperative Extension. Virginia Tech University, Blacksburg,VA.

Clunies, M., D. Parks, and S. Leeson. 1992. Calcium and phosphorus metabolism and eggshell formation of hens fed different amounts of calcium. Poult. Sci. 71:482–489.

Collin, A., J. van Milgen, S. Dubois, and J. Noblet. 2001. Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. Br. J. Nutr. 86:63–70.

Creger, C. R., H. Phillips, and J. T. Scott. 1976. Formation of an egg shell. Poult. Sci. 55:1717–1723.

Dawkins, M. S., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342–344.

De Reu, K., T. B. Rodenburg, K. Grijspeerdt, W. Messens, M. Heyndrickx, F. A. M. Tuyttens, B. Sonck, J. Zoons, and L. Herman. 2009. Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: an international on-farm comparison. Poult. Sci. 88:2442–2448.

Deaton, J. W., F. N. Reece, and J. L. McNaughton. 1978. The effect of temperature during the growing period on broiler performance. Poult. Sci. 57:1070–1074.

Duncan, E. T., M. C. Appleby, and B. O. Hughes. 1992. Effect of perches in laying cages on welfare and production of hens. Br. Poult. Sci. 33:25–35.

Dunlop, M. W., A. F. Moss, P. J. Groves, S. J. Wilkinson, R. M. Stuetz, and P. H. Selle. 2016. The multidimensional causal factors of 'wet litter' in chicken-meat production. Sci. Total Environ. 562:766–776.

Enneking, S. A., H. W. Cheng, K. Y. Jefferson-Moore, M. E. Einstein, D. A. Rubin, and P. Y. Hester. 2012. Early access to perches in caged White Leghorn pullets. Poult. Sci. 91:2114–2120.

Estevez, I., N. Tablante, R. L. Pettit-Riley, and L. Carr. 2002. Use of cool perches by broiler chickens. Poult. Sci. 81:62–69.

Etches, R. J., T. M. John, and A. M. V. Gibbins. 2008. Behavioral, physiological, neuroendocrine and molecular responses to heat stress. In Pages 48–79 Poultry Production in Hot Climates. 2nd ed. N. J. Daghir, ed. CABI, Wallingford, UK.

Gates, R. S., S. A. Enneking, Y. Xiong, P. Y. Hester, M. M. Makagon, and H. W. Cheng. 2014. Design and performance of cooled perches for alternative egg laying production systems, Paper No. 141901235, American Society of Agricultural and Biological Engineers, ASABE and CSBE/SCGAB Annual International Meeting, Montreal, Quebec, Canada, 13–16 July. St. Joseph, MI.

Gates, R. S., and M. B. Timmons. 1986. Stochastic and deterministic analysis of evaporative cooling benefits for laying hens. Trans. ASAE. 31:904–909.

Glatz, P. C., and J. L. Barnett. 1996. Effect of perches and solid sides on production, plumage and foot condition of laying hens housed in conventional cages in a naturally ventilated shed. Aust. J. Exp. Agric. 36:269–275.

Gunnarsson, S. 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. Br. Poult. Sci. 40:12–18.

Hamilton, R. M. G., K. G. Hollands, P. W. Voisey, and A. A. Grunder. 1979. Relationship between egg shell quality and shell breakage and factors that affect shell breakage in the field–a review. Worlds Poult. Sci. J. 35:177–190.

Hansen, P. J. 2009. Effects of heat stress on mammalian reproduction. Phil. Trans. R. Soc. B 364:3341–3350.

Hegeman, R. 2011. Heat wave kills thousands of poultry. Huffington Post. Accessed May 2018. https://www.huffingtonpost.com/2011/07/13/heat-wave-poultry_n_896812.html.

Hester, P. Y. 2014. The effect of perches installed in cages on laying hens. Worlds Poult. Sci. J. 70:247–264.

Hester, P. Y., S. A. Enneking, K. Y. Jefferson-Moore, M. E. Einstein, H. W. Cheng, and D. A. Rubin. 2013. The effect of perches in cages during pullet rearing and egg laying on hen performance, foot health, and plumage. Poult. Sci. 92:310–320.

Hillman, P. E., and N. R. Scott. 1989. Energy budget of the chicken foot. J. Therm. Biol 14:205–217.

Hristov, A. N., A. T. Degaetano, C. A. Rotz, E. Hoberg, R. H. Skinner, T. Felix, H. Li, P. H. Patterson, G. Roth, M. Hall, T. L. Ott, L. H. Baumgard, W. Staniar, R. M. Hulet, C. J. Dell, A. F. Brito, and D. Y. Hollinger. 2018. Climate change effects on livestock in the Northeast US and strategies for adaptation. Clim. Change 146:33–45.

Hu, J. Y., P. Y. Hester, M. M. Makagon, G. Vezzoli, R. S. Gates, Y. J. Xiong, and H. W. Cheng. 2016. Cooled perch effects on performance and well-being traits in caged White Leghorn hens. Poult. Sci. 95:2737–2746.

Hy-Line. 2016. Hy-Line W-36 commercial layers management guide. Accessed May 2018. http://www.hyline.com/UserDocs/Pages/36_COM_ENG.pdf.

Jardine, D. S. 2007. Heat illness and heat stroke. Pediatr. Rev. 28:249–258.

Jeurissen, S. H., F. Lewis, J. D. van der Klis, Z. Mroz, J. M. Rebel, and A. A. ter Huurne. 2002. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr. Issues Mol. Biol. 3:1–14.

Joseph, N. S., F. E. Robinson, D. R. Korver, and R. A. Renema. 2000. Effect of dietary protein intake during the pullet-to-breeder transition period on early egg weight and production in broiler breeders. Poult. Sci. 79:1790–1796.

Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. 77:59–91.

Keshavarz, K., and S. Nakajima. 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poult. Sci. 72:144–153.

Keshavarz, K., and S. Nakajima. 1995. The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. Poult. Sci. 74:50–61.

Kirunda, D. F. K., S. E. Scheideler, and S. R. McKee. 2001. The efficacy of vitamin E (DL-alpha-tocopheryl acetate) supplementation in hen diets to alleviate egg quality deterioration associated with high temperature exposure. Poult. Sci. 80:1378–1383.

Klingensmith, P. M., and P. Y. Hester. 1985. Effects of an induced molt and shell quality on the physical dimensions and mineral composition of eggs and intrauterine pH. Poult. Sci. 64:2368–2376.

Koelkebeck, K. W., and T. W. Odom. 1994. Laying hen responses to acute heat stress and carbon dioxide supplementation: I. Blood gas changes and plasma lactate accumulation. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 107:603–606.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356–369.

Lay, D. C., R. M. Fulton, P. Y. Hester, D. M. Karcher, J. B. Kjaer, J. A. Mench, B. A. Mullens, R. C. Newberry, C. J. Nicol, N. P. O'Sullivan, and R. E. Porter. 2011. Hen welfare in different housing systems. Poult. Sci. 90:278–294.

Lin, H., H. C. Jiao, J. Buyse, and E. Decuypere. 2006. Strategies for preventing heat stress in poultry. Worlds Poult. Sci. J. 62:71–86.

Makagon, M. M., V. A. Cussen, R. S. Gates, P. Y. Hester, and H. W. Cheng. 2015. Effects of cooled perch access during chronic heat stress on the behavior of White Leghorn hens. Poult. Sci. 94(E-Suppl.1):79. (Abstr.).

Marai, I. F. M., A. A. El-Darawany, A. Fadiel, and M. A. M. Abdel-Hafez. 2007. Physiological traits as affected by heat stress in sheep—a review. Small Rumin. Res. 71:1–12.

Mashaly, M. M., G. L. Hendricks, M. A. Kalama, A. E. Gehad, A. O. Abbas, and P. H. Patterson. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83:889–894.

Mazzuco, H., and P. Y. Hester. 2005. The effect of an induced molt using a nonfasting program on bone mineralization of White Leghorns. Poult. Sci. 84:1483–1490.

Minvielle, F., P. Mérat, J. L. Monvoisin, G. Coquerelle, and A. Bordas. 1994. Increase of egg weight with age in normal and dwarf, purebred and crossbred laying hens. Genet. Sel. Evol. 26:453–462.

Mitchell, M. A., and A. J. Carlisle. 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 101:137–142.

Mitrovic, S., T. Pandurevic, V. Milic, V. Djekic, and V. Djermanovic. 2010. Weight and egg quality correlation relationship on different age laying hens. J. Food. Agric. Environ. 8:580–583.

Muiruri, H. K., and P. C. Harrison. 1991. Effect of roost temperature on performance of chickens in hot ambient environments. Poult. Sci. 70:2253–2258.

Nakaue, H. S., M. P. Goeger, and G. H. Arscott. 1984. Effect of feed trough heights and perches on the performance of dwarf Single Comb White Leghorn layers housed in cages. Poult. Sci. 63:447–449.

National Research Council. 1981. Effect of environment on nutrient requirements of domestic animals. Washington, DC.

Nys, Y. 1999. Nutritional factors affecting eggshell quality. Czech J. Anim. Sci. 44:135–143.

Odom, T. W., P. C. Harrison, and W. G. Bottje. 1986. Effects of thermal-induced respiratory alkalosis on blood ionized calcium levels in the domestic hen. Poult. Sci. 65:570–573.

Ogle, C. K., J. F. Valente, X. Guo, B. G. Li, J. D. Ogle, and J. W. Alexander. 1997. Thermal injury induces the development of inflammatory macrophages from nonadherent bone marrow cells. Inflammation. 21:569–582.

Okelo, R. O., L. E. Carr, P. C. Harrison, L. W. Douglass, V. E. Byrd, C. W. Wabeck, P. D. Schreuders, F. W. Wheaton, and N. G. Zimmermann. 2003. Effectiveness of a novel method to reduce heat stress in broilers: a cool roost system. Trans. ASAE. 46:1675–1683.

Olsson, I. A. S., and L. J. Keeling. 2000. Night-time roosting in laying hens and the effect of thwarting access to perches. Appl. Anim. Behav. Sci. 68:243–256.

Olsson, I. A. S., and L. J. Keeling. 2002. The push-door for measuring motivation in hens: laying hens are motivated to perch at night. Anim. Welf. 11:11–19.

Panda, A. K., and G. Cherian. 2014. Role of vitamin E in counteracting oxidative stress in poultry. J. Poult. Sci. 51:109–117.

Pettit-Riley, R., and I. Estevez. 2001. Effects of density on perching behavior of broiler chickens. Appl. Anim. Behav. Sci. 71:127–140.

Quinteiro, W. M., A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, M. Sakai, L. R. M. Sa, A. J. P. Ferreira, and J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult. Sci. 89:1905–1914.

Reilly, W. M., K. W. Koelkebeck, and P. C. Harrison. 1991. Performance evaluation of heat-stressed commercial broilers provided water-cooled floor perches. Poult. Sci. 70:1699–1703.

Roberts, J. R. 2004. Factors affecting egg internal quality and egg shell quality in laying hens. Jpn. Poult. Sci. 41:161–177.

Rodriguez-Navarro, A., O. Kalin, Y. Nys, and J. M. Garcia-Ruiz. 2002. Influence of the microstructure on the shell strength of eggs laid by hens of different ages. Br. Poult. Sci. 43:395–403.

Roland, D. A., M. Farmer, and D. Marple. 1985. Calcium and its relationship to excess feed consumption, body weight, egg size, fat deposition, shell quality, and fatty liver hemorrhagic syndrome. Poult. Sci. 64:2341–2350.

Rozenboim, I., N. Mobarky, R. Heiblum, Y. Chaiseha, S. W. Kang, I. Biran, A. Rosenstrauch, D. Sklan, and M. E. El Halawani. 2004. The role of prolactin in reproductive failure associated with heat stress in the domestic turkey. Biol. Reprod. 71:1208–1213.

Rozenboim, I., E. Tako, O. Gal-Garber, J. A. Proudman, and Z. Uni. 2007. The effect of heat stress on ovarian function of laying hens. Poult. Sci. 86:1760–1765.

Russo, S., J. Sillmann, and A. Sterl. 2017. Humid heat waves at different warming levels. Sci. Rep. 7:7477.

Ruzal, M., D. Shinder, I. Malka, and S. Yahav. 2011. Ventilation plays an important role in hens' egg production at high ambient temperature. Poult. Sci. 90:856–862.

Sahin, N., A. Hayirli, C. Orhan, M. Tuzcu, J. R. Komorowski, and K. Sahin. 2018. Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poult. Sci. 97:1298–1305.

Sahin, K., N. Sahin, M. Onderci, F. Gursu, and G. Cikim. 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. Biol. Trace Elem. Res. 89:53–64.

SAS Institute. 2013. SAS User's Guide: Statistics Version 9.4. SAS Inst. Inc., Cary, NC.

Scott, M. L., S. J. Hull, and P. A. Mullenhoff. 1971. The calcium requirements of laying hens and effects of dietary oyster shell upon egg shell quality. Poult. Sci. 50:1055–1063.

Slimen, I. B., T. Najar, A. Ghram, and M. Abdrrabba. 2016. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. J. Anim. Physiol. Anim. Nutr. 100:401–412.

Steel, R. G. D., J. H. Torrie, and D. J. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw Hill Book Co., New York, NY.

Strong, R. A., P. Y. Hester, S. D. Eicher, J. Hu, and H. W. Cheng. 2015. The effect of cooled perches on immunological parameters of caged White Leghorn hens during the hot summer months. PLoS One 10:e0141215.

Summers, J. D., and S. Leeson. 1994. Laying hen performance as influenced by protein intake to sixteen weeks of age and body weight at point of lay. Poult. Sci. 73:495–501.

Sun, C. J., S. R. Chen, G. Y. Xu, X. M. Liu, and N. Yang. 2012. Global variation and uniformity of eggshell thickness for chicken eggs. Poult. Sci. 91:2718–2721.

Tanor, M. A., S. Leeson, and J. D. Summers. 1984. Effect of heat stress and diet composition on performance of White Leghorn hens. Poult. Sci. 63:304–310.

Tauson, R. 1984a. Plumage condition in SCWL laying hens kept in conventional cages of different designs. Acta. Agric. Scand. 34:221–230.

Tauson, R. 1984b. Effects of a perch in conventional cages for laying hens. Acta Agric. Scand. 34:193–209.

Tauson, R., J. Kjaer, G. A. Maria, R. Cepero, and K. E. Holm. 2005. Applied scoring of integument and health in laying hens. Anim. Sci. Pap. Rep. 23:153–159.

Taylor, T. G. 1970. How an eggshell is made. Sci. Am. 222:88–95.

Timmons, M. B., and R. S. Gates. 1988. Predictive model of laying hen performance to air temperature and evaporative cooling. Trans. ASABE 31:1496–1502.

Tumova, L., L. Uhlirova, R. Tuma, D. Chodova, and L. Machal. 2017. Age related changes in laying pattern and egg weight of different laying hen genotypes. Anim. Reprod. Sci. 183:21–26.

Tuyttens, F. A. M., E. Struelens, and B. Ampe. 2013. Remedies for a high incidence of broken eggs in furnished cages: effectiveness of increasing nest attractiveness and lowering perch height. Poult. Sci. 92:19–25.

United Egg Producers. 2017. Animal Husbandry Guidelines for U.S. Egg Laying-Flocks. United Egg Producers, Johns Creek, GA. Accessed May 2018. https://uepcertified.com/wp-content/uploads/2017/11/2017-UEP-Animal-Welfare-Complete-Guidelines-11.01.17-FINAL.pdf.

Wang, G., C. Ekstrand, and J. Svedberg. 1998. Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens. Br. Poult. Sci. 39:191–197.

Winer, B. J., D. R. Brown, and K. M. Michels. 1991. Statistical Principles in Experimental Design. 3rd ed. McGraw-Hill, Inc. New York. NY.

Wolfenson, D., Y. F. Frei, N. Snapir, and A. Berman. 1981. Heat-stress effects on capillary blood-flow and its redistribution in the laying hen. Physiol. Biochem. Zool. 390:86–93.

Xiong, Y., R. S. Gates, J. Hu, K. S. O. Rocha, M. M. Makagon, P. Y. Hester, and H. W. Cheng. 2015. Performance assessment of cooled perch system for heat stress trials in egg laying production: Year 1. ASABE Papers: 152183776. New Orleans, LA.

Yahav, S., S. Goldfeld, I. Plavnik, and S. Hurwitz. 1995. Physiological-responses of chickens and turkeys to relative-humidity during exposure to high ambient-temperature. J. Therm. Biol. 20:245–253.

Yahav, S., D. Shinder, M. Ruzal, M. Giloh, and Y. Piestun. 2009. Controlling body temperature–the opportunities for highly productive domestic fowl. in Pages 65–98 Body Temperature Regulation. A. B. Cisneros, and B. L. Gions, eds. Nova Science, Hauppauge, NY.

Yoshida, N., M. Fujita, M. Nakahara, T. Kuwahara, S. I. Kawakami, and T. Bungo. 2011. Effect of high environmental temperature on egg production, serum lipoproteins and follicle steroid hormones in laying hens. J. Poult. Sci. 48:207–211.

Zhao, J. P., H. C. Jiao, Y. B. Jiang, Z. G. Song, X. J. Wang, and H. Lin. 2012. Cool perch availability improves the performance and welfare status of broiler chickens in hot weather. Poult. Sci. 91:1775–1784.

Zhao, J. P., H. C. Jiao, Y. B. Jiang, Z. G. Song, X. J. Wang, and H. Lin. 2013. Cool perches improve the growth performance and welfare status of broiler chickens reared at different stocking densities and high temperatures. Poult. Sci. 92:1962–1971.

Zulkifli, I., N. Abdulllah, N. M. Azrin, and Y. W. Ho. 2000a. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci. 41:593–597.

Zulkifli, I., M. T. Che Norma, D. A. Israf, and A. R. Omar. 2000b. The effect of early age feed restriction on subsequent response to high environmental temperatures in female broiler chickens. Poult. Sci. 79:1401–1407.