Effect of Heat Stress on Production Parameters and Immune Responses of Commercial Laying Hens[1]

M. M. Mashaly,†,[2] G. L. Hendricks, III,† M. A. Kalama,*
A. E. Gehad,‡ A. O. Abbas,† and P. H. Patterson†

*Department of Poultry Production, Minofiya University, Egypt; †Department of Poultry Science, The Pennsylvania State University, University Park, Pennsylvania 16802; and ‡National Research Center, Giza, Egypt

ABSTRACT The present study was conducted to determine the adverse effects of high temperature and humidity not only on live performance and egg quality but also on immune function in commercial laying hens. One hundred eighty 31-wk-old laying hens at peak production were used in this study. Hens were housed in cages (15 cages of 4 birds/cage) in each of 3 environmental chambers and received 1 of 3 treatments. The 3 treatments were control (average temperature and relative humidity), cyclic (daily cyclic temperature and humidity), and heat stress (constant heat and humidity) for 5 wk. Different production and immune parameters were measured. Body weight and feed consumption were significantly reduced in hens in the heat stress group. Egg production, egg weight, shell weight, shell thickness, and specific gravity were significantly inhibited among hens in the heat stress group. Likewise, total white blood cell (WBC) counts and antibody production were significantly inhibited in hens in the heat stress group. In addition, mortality was higher in the heat stress group compared to the cyclic and control groups. Even though T- and B-lymphocyte activities were not significantly affected by any of the treatments, lymphocytes from hens in the heat stress group had the least activity at 1 wk following treatment. These results indicate that heat stress not only adversely affects production performance but also inhibits immune function.

(*Key words*: egg production and quality, heat stress, immunity, laying hen)

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INTRODUCTION

For many years, researchers have been investigating the effect of high environmental temperature on the performance of different poultry species, including turkeys (Kohne and Jones, 1976; McKee and Sams, 1997), young chickens (Henken et al., 1983), broilers (Cooper and Washburn, 1998), broiler breeders (McDaniel et al., 1995), and laying hens (Emery et al., 1984; Muiruri and Harrison, 1991; Whitehead et al., 1998), and have found that high environmental temperatures have deleterious effects on productive performance. In laying hens, heat stress depresses body weight (Scott and Balnave, 1988), egg production (Muiruri and Harrison, 1991; Whitehead et al., 1998), egg weight (Balnave and Muheereza, 1997), and shell quality (Emery et al., 1984; Mahmoud et al., 1996) and is generally accompanied by suppression of feed intake, which could be the cause of the decline in production. In addition, Larbier et al. (1993) found that chronic heat exposure significantly decreased protein digestion and Bonnet et al. (1997) reported that the feed digestibility of the different components of the diet (proteins, fats, starch) decreased with exposure of broiler chickens to high temperatures.

On the other hand, Emery et al. (1984) reported that high temperature did not affect egg production. Furthermore, Muiruri and Harrison (1991) found that heat stress did not significantly affect egg weight or feed conversion. In addition, heat exposure during the night did not significantly affect egg or albumen weights (Wolfenson et al., 1979). Koelkebeck et al. (1998) indicated that acute heat stress had no adverse effects on dietary amino acid digestibility in laying hens. The differences in the above results could be due to differences in heat stress treatments or the type of birds used.

Regarding the decline in the reproductive performance of acutely heat-stressed hens, Mahmoud et al. (1996) suggested that alterations in acid-base balance, the status of Ca^{2+}, and diminished ability of duodenal cells to transport calcium could be critical factors in the detrimental effects

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[1]This work was partially supported by the PA Egg Check-Off Program.
[2]To whom correspondence should be addressed: hxk@psu.edu.

Abbreviation Key: H/L ratio = heterophil/lymphocyte ratio; WBC = white blood cell.

of heat stress on egg production, egg shell characteristics, and skeletal integrity often documented in laying hens.

Several studies have been conducted on the effects of high temperature on the immune responses of chickens, with variable results. Thaxton et al. (1968) were the first to demonstrate that high environmental temperatures (44.4 to 47.8°C) affect the development of specific immune responses in young chickens. These effects include the suppression of circulating white blood cells (WBC) (Nathan et al., 1976; Heller et al., 1979) and an increase in the heterophil/lymphocyte ratio (H/L ratio) (Mogenet and Youbicier-Simo, 1998), which is an indicator of stress (Gross and Siegel, 1983). Heat stress was also reported to cause a reduction in antibody production in young chickens (Zulkifi et al., 2000). On the other hand, Donker et al. (1990) found that heat exposure did not reduce antibody production to SRBC and Heller et al. (1979) even found significantly increased antibody titers to SRBC following heat exposure. The difference in these findings could be associated with age and type of bird used or due to the experimental methodology that was applied. Regnier et al. (1980) suggested that heat-induced immunosuppression may depend on breed of bird and Kelley (1983) reported that effects on immune responses may depend on the length and intensity of the heat exposure.

Few studies are available that address the effects of heat stress and humidity on production and immune parameters. Therefore, the present study was conducted in order to determine the effects of heat stress and humidity on production performance and the immune responses of laying hens.

MATERIALS AND METHODS

One-day-old pullets from a commercial laying strain were housed in pullet batteries at the Poultry Education and Research Center (PERC) of the Pennsylvania State University and were provided with feed and water ad libitum. The lighting program was a step-down, step-up program; when the pullets were 18 wk of age, they were housed 4 per cage ($0.53 \times 0.30 \times 0.50$ m) and received 14 h of light per day. When the hens were 26 wk of age, they were moved to 1 of 3 environmental chambers (of identical size, ventilation, humidity, temperature, light intensity, and light schedule), with 1 cage battery per chamber; 60 hens per battery (15 cages with 4 hens per cage) and cages measuring the same as above. Hens were allowed to adapt to the environmental chambers for 5 wk at a constant temperature of 24°C and RH of 50% and a photoperiod of 16L:8D at 5.4 lx. When they reached 31 wk of age (peak production), hens in each of the chambers received 1 of 3 different heat treatments for 5 wk. In the first chamber, hens were exposed to 23.9°C and 50% RH with a heat index of 25°C, representing an average heat index throughout different seasons (control group). The hens in the second chamber were exposed to cyclic daily temperatures and humidity ranging from 23.9 to 35°C and from 50 to 15% RH, representing natural cyclic temperatures during hot summer months (cyclic group). Normal temperatures with high humidity (23.9°C and 50% RH) were maintained approximately 8 h/d; hot and dry conditions (35°C and 15% RH) were maintained approximately 4 h/d. The other 12 h/d were spent in temperature and RH transition. Hot and dry conditions started increasing at 0800 h and began decreasing at 1800 h. The hens in the third chamber were exposed to constant 35°C and 50% RH with heat index of 41.1°C, representing severe heat stress (heat stress group). Birds were hand fed hen mash daily from containers stored outside the environmental chambers (23.9°C). The hen mash was a corn and soybean meal based diet containing 19.7% CP, 2,855 kcal of ME/kg, 4.45% Ca, and 0.49% available P. Eggs were gathered daily and stored in a 12.8°C cooler. All animal care procedures were carried out as described in the protocol approved by the Pennsylvania State University Institutional Animal Care and Use Committee (98R064-0).

Production Parameters

Feed consumption, hen-day egg production, and egg quality (including egg weight, shell weight, shell thickness, specific gravity, and Haugh units) were measured weekly while birds were housed in the environmental chambers. BW was measured before and after the 5-wk temperature and RH treatments. Egg quality was based on 2 d of collection per week. Egg specific gravity was measured using 9 solutions (1.100, 1.095, 1.090, 1.085, 1.080, 1.075, 1.070, 1.065, and 1.060) at room temperature. Shell weight was determined after cleaning adhering egg yolk and albumen and drying to a constant weight at room temperature.

Immunological Parameters

Blood Sampling. Ten birds from each treatment were used on wk 1 and 4 following the start of treatments. Four milliliters of blood was drawn from the brachial vein using heparinized syringes. Whole blood was used to prepare smears for H/L ratio, to count total white blood cells (WBC), and to measure the activities of T- and B-lymphocytes. Another 2 mL of blood was drawn from the brachial vein using plain syringes; blood was allowed to clot, and the serum was used to measure antibody levels. Blood samples were taken in the middle of wk 1 and 4 following the start of treatments; birds were randomly selected from each group using pens at identical locations in all 3 rooms.

Total WBC Count. The total WBC was measured using brilliant Cresyl blue stain.[3]

H/L Ratio. Using Hema 3 stain,[4] the different types of WBC were counted, and percentages of heterophils and lymphocytes were used to calculate H/L ratio.

Antibody Measurements. Antibody production against SRBC, a thymus-dependent antigen, was measured using microtiter technique (Trout et al., 1996).

[3]Sigma Chemical Co., St. Louis, MO.
[4]Fisher Scientific, Hanover Park, IL.

T- and B-Lymphocyte Activities. T- and B-lymphocyte activities were measured using standard mitogen proliferation assay described previously (Gehad et al., 1999). Briefly, peripheral blood leukocytes were isolated from the blood using histopaque-1077,[3] plated at 5×10^5 cells per well, and stimulated with 50 μL of concanavalin A (Con A)[3] (a T-cell mitogen) at 12.5 μg/mL or 50 μL of pokeweed[3] (a B-cell mitogen) at 25 μg/mL. Cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 for 48 h. One microcurie of tritiated thymidine[5] was added to each well, and the plates were incubated for another 18 h under the same conditions.

Thymidine uptake, an indicator of lymphocyte activity, was measured using a scintillation counter.[6] All immunological parameters were measured at 1 and 4 wk, representing acute and chronic responses to the heat and humidity treatments, respectively.

Statistical Analysis

Data were analyzed using the GLM procedures of SAS software (1996) by analysis of variance and Duncan's multiple-range test. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Production Parameters

All production parameters were severely affected by heat stress (Table 1). The average BW for birds at the conclusion of the 5 wk study were 1,528, 1,414, and 1,233 g, respectively, for the control, cyclic, and heat stress groups. Although there was no difference in BW at the beginning of the study, each treatment group was significantly different from the others at the end of 5 wk. These findings agree with those of Scott and Balnave (1988), who found that BW of laying hens were decreased when exposed to high temperature. Furthermore, Emery et al. (1984) showed that birds under cycling temperatures, either between 15.6 and 37.7°C (mean, 26.7°C) or between 21.1 and 37.7°C (mean, 29.4°C) lost more BW than those at a constant 23.9°C. Decreased BW in our study and others may be due to a reduction in feed consumption. In the present study, feed intake was reduced in proportion to the severity and length of heat stress exposure; birds from the heat stress group ate significantly less feed than birds from the cyclic group, which in turn ate significantly less feed than birds from the control group throughout the 5 wk experiment (Table 1). The reduction in feed intake in response to heat stress confirms earlier studies (de Andrade et al., 1977; Scott and Balnave, 1988; Muiruri and Harrison, 1991; McKee et al., 1997; Kirunda et al., 2001). In addition, Hurwitz et al. (1980) showed that appetite is also decreased as a primary response to high temperature.

In addition to decreasing BW and FC, heat stress increased mortality. Mortality for the heat-stressed group (31.7%) was much higher than for the cyclic (6.7%) or control (5%) groups. This increase in mortality could be due to inhibition of some immune responses.

Egg production in this study was inversely related to high temperature. Hen-day egg production was significantly decreased through all 5 wk for hens exposed to the constant hot temperature compared with those in the cyclic or control chambers (Table 1). These findings are in agreement with those of Muiruri and Harrison (1991), Whitehead et al. (1998) and Kirunda et al., (2001), who reported that egg production in White Leghorns decreased when they were exposed to high environmental temperature. The decrease in egg production in our study was most likely due to the decrease in feed consumption, reducing the available nutrients for egg production. Daniel and Balnave (1981) indicated that feed intake is reduced prior to subsequent loss in egg production. Heat stress not only reduces feed intake but has been reported to also reduce digestibility of different components of the diet (Bonnet et al. 1997). Furthermore, it has been reported that exposure to high temperature decreased plasma protein concentration (Zhou et al., 1998) and plasma calcium concentration (Mahmoud et al., 1996), both of which are required for egg formation.

Exposure of hens to high temperatures also resulted in a significant decrease in egg quality. Egg weight, shell weight, shell thickness, and specific gravity were all significantly decreased when the birds were exposed to heat stress (Table 1). Eggs from hens housed in the hot chamber weighed significantly less than eggs from the cyclic chamber, and eggs from the cyclic chamber weighed significantly less than eggs from the control chamber throughout the 5 wk experiment. These results agree with those of de Andrade et al. (1976, 1977), Emery et al. (1984), and Kirunda et al. (2001) who found that either high environmental or cyclic temperatures decrease egg weight. This finding could be due to the reduction in feed consumption as reported by de Andrade et al. (1976). The adverse effect of high environmental temperature on eggshell quality has been well documented (de Andrade et al., 1976, 1977; Mahmoud et al. 1996; Balnave and Muheereza, 1997; Odom et al., 1986). Eggshell thickness and specific gravity of eggs from the cyclic hens were less than the control but greater than the heat-stressed hens in the first 2 wk of the experiment ($P < 0.05$). However, during wk 3, 4, and 5, no difference in shell thickness or specific gravity was observed between the control and cyclic groups, whereas eggs from the heat-stressed group were always significantly less. The decrease in shell quality in the current study may be partially due to a reduction in plasma calcium. It has been reported that plasma calcium level was significantly decreased in laying hens (Mahmoud et al., 1996) and in turkeys (Kohne and Jones, 1976) when the birds were exposed to high temperatures. In addition, it has been shown that calcium use (Odom et al., 1986) and calcium uptake by duodenal epithelial

[5]ICN Biomedicals, Irvine, CA.
[6]LKB Instruments, Gaithersburg, MD.

TABLE 1. Effect of heat stress on different production parameters in commercial laying hens

Parameter and treatment group[1]	Weeks of treatment 1	2	3	4	5	Mean
Daily feed consumption (g/bird per day)						
Control	76.3[a]	96.1[a]	93.7[a]	84.1[a]	83.2[a]	86.7[A]
Cyclic	50.9[b]	74.5[b]	62.8[b]	70.5[b]	70.8[b]	65.9[B]
Heat stress	23.2[c]	38.4[c]	54.4[c]	48.2[c]	43.8[c]	41.6[C]
Hen day egg production (%)						
Control	81.2[a]	88.3[a]	89.0[a]	91.0[a]	87.4[a]	87.4[A]
Cyclic	78.4[a]	81.9[a]	85.7[a]	83.3[a]	83.1[a]	82.5[A]
Heat stress	48.0[b]	52.9[b]	63.1[b]	61.1[b]	55.8[b]	56.2[B]
Egg weight (g)						
Control	56.4[a]	56.3[a]	56.2[a]	56.2[a]	57.1[a]	56.4[A]
Cyclic	53.2[b]	53.3[b]	53.5[b]	53.5[b]	54.0[b]	53.5[B]
Heat stress	51.1[c]	46.8[c]	46.0[c]	45.4[c]	45.0[c]	46.9[C]
Shell weight (g)						
Control	5.03[a]	5.13[a]	5.09[a]	5.10[a]	4.96[a]	5.06[A]
Cyclic	4.45[b]	4.77[b]	4.90[b]	4.87[b]	4.82[a]	4.76[B]
Heat stress	3.44[c]	3.62[c]	3.60[c]	3.51[c]	3.31[b]	3.50[C]
Shell thickness (× 0.01 mm)						
Control	35.2[a]	36.2[a]	35.8[a]	33.9[a]	33.1[a]	34.8[A]
Cyclic	32.2[b]	35.0[b]	35.8[a]	33.6[a]	33.0[a]	33.9[A]
Heat stress	26.8[c]	29.3[c]	28.3[b]	28.4[b]	28.7[b]	28.3[B]
Specific gravity (f/cm^3)						
Control	1.082[a]	1.077[a]	1.071[a]	1.070[a]	1.072[a]	1.074[A]
Cyclic	1.073[b]	1.075[b]	1.071[a]	1.070[a]	1.072[a]	1.072[A]
Heat stress	1.064[c]	1.065[c]	1.064[b]	1.064[b]	1.063[b]	1.064[B]
Albumen height (mm)						
Control	6.20[b]	5.83[a]	5.36[b]	5.01[c]	6.14[b]	5.70[A]
Cyclic	6.63[a]	5.87[a]	5.56[ab]	5.10[b]	6.14[b]	5.86[A]
Heat stress	6.98[a]	6.06[a]	5.74[a]	5.38[a]	6.47[a]	6.13[A]

[a–c]Means for the same parameter within the same column with different letters are significantly different ($P < 0.05$).

[A–C]Means for the same parameter with different letters are significantly different ($P < 0.05$).

[1]Treatment groups were: control = 23.9°C and 50% RH; cyclic = 23.9 to 35°C and 50 to 15% RH; heat stress = 35°C and 50% RH.

cells (Mahmoud et al., 1996) are decreased by exposure to high environmental temperatures.

Finally, eggs from birds housed in the hot chamber, in general, had significantly higher Haugh units than those from birds in either the control or cyclic chambers (Table 1). This finding is contrary to the findings of Kirunda et al. (2001) who reported that Haugh units of eggs from heat-stressed birds were reduced after heat exposure. An explanation could be that the reduced egg production of the heat-stressed hens imparted greater quality to fewer eggs (Patterson et al., 1988). Hens fed low energy, high wheat middling diets were observed to produce fewer eggs but with greater interior quality.

Immunological Parameters

Data on immunological parameters are presented in Table 2. Our results show that B- and T-cell proliferation was not significantly affected by heat stress, although birds from the heat stress group displayed the lowest activity at 1 wk after initiation of treatments. Kadymov and Aleskerov (1988) found that high temperature caused inhibition in the synthesis of T and B lymphocytes and suppression of phagocytic activity of blood leukocytes.

Furthermore, in our experiment total WBC of birds from the heat-stressed group, after 4 wk of heat exposure, were lower than the control group and significantly lower than the cyclic group. Our results agree with those of Nathan et al. (1976) who found that total WBC were inhibited following heat exposure. These results could indicate that exposure to heat stress can reduce both the number and activities of leukocytes.

Four weeks of heat and RH stress resulted in an increased H/L ratio. McFarlane and Curtis (1989) also reported that in broiler chicks, heat exposure resulted in an increased H/L ratio. The H/L ratio has been used as a reliable indicator of stress in birds (Gross and Siegel, 1983), indicating that, in our study, birds from the cyclic group were significantly stressed compared with control birds, whereas birds from the heat-stressed group were under significant stress compared with the cyclic and control groups.

Finally, the humoral immunity of birds from the heat stress group was depressed compared with the cyclic and control groups. We found that at 1 and 4 wk after the start of heat exposure, birds from the heat-stressed group had significantly lower antibody titers to SRBC than those from the cyclic or control chambers. These results agree with the findings of Zulkifi et al. (2000), who showed that heat stress caused a reduction in antibody synthesis. This reduction could be indirectly due to an increase in inflammatory cytokines under stress (Ogle et al., 1997), which stimulates the hypothalamic production of corticotropin releasing factor (Sapolsky et al., 1987). Corticotropin re-

TABLE 2. Effect of heat stress on different immunological parameters in commercial laying hens

	Treatment group[1]					
	Week 1			Week 4		
Parameters	Control	Cyclic	Heat stress	Control	Cyclic	Heat stress
T-cell proliferation (cpm)	11,190	8,575	7,023	5,347	7,166	5,178
B-cell proliferation (cpm)	4,369	2,737	1,575	2,329	2,812	2,874
Total WBC[2] count ($\times 10^3/mm^3$)	35,450	45,714	48,250	54,500[b]	91,350[a]	37,100[b]
H/L ratio[3]	0.39	0.56	0.42	0.12[c]	0.15[b]	0.19[a]
Antibody titer[4]	5.3[a]	4.9[a]	3.8[b]	7.1[a]	8.0[a]	5.3[b]

[a–c]Means for the same parameter within the same week with different letters are significantly different ($P < 0.05$); n = 10.

[1]Treatments were: control = 23.9°C and 50% RH; cyclic = 23.9 to 35°C and 50 to 15% RH; heat stress = 35°C and 50% RH.

[2]White blood cell.

[3]H/L ratio = heterophil/lymphocyte ratio.

[4]Titer values are $\log_2$ of the reciprocal of the last dilution.

leasing factor is known to increase adrenocorticotropic hormone from the pituitary; adrenocorticotropic hormone then stimulates corticosterone production from the adrenal gland. Corticosterone inhibits antibody production (Gross, 1992). Furthermore, heat stress is known to decrease T-helper 2 cytokines (Wang et al., 2001), which are important for antibody production (Lebman and Coffman, 1988).

In conclusion, it is clear that heat and RH stress of Leghorn hens caused poor production performance and also increased the percentage of mortality. This increase in mortality could be due to inhibition of immune responses. Birds exposed to acute heat stress (1 wk) showed no difference in WBC and H/L ratio compared with birds exposed to acute cyclic or control temperature and RH. Birds exposed to chronic heat stress (4 wk) had a lower WBC count and a higher H/L ratio compared with birds exposed to chronic cyclic or control temperatures and RH. We found that the heat stress group not only had an increase in the H/L ratio, indicating the birds were under increased stress, but also a decrease in antibody titer. Our results could be helpful in establishing guidelines for temperature control in laying hen houses, especially during the summer months when birds are most susceptible to heat stress.

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