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# Effects of Heat Stress on Acid-Base Imbalance, Plasma Calcium Concentration, Egg Production and Egg Quality in Commercial Layers

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Abstract: In high environmental temperatures poultry production is generally reduced, due to decreasing feed intake especially calcium (Ca) and try to overcome heat stress by panting. The purpose of present study was to determine the effect of heat stress on acid-base imbalance, plasma Ca concentration, egg production and egg quality( egg weight, specific gravity, egg shell thickness, egg shell weight and Haugh unit) in commercial layers. In this study two hundred laying hens (Hy-Line) 40 weeks age were divided into two groups, each group contains 100 hens (control group and purpose group). Every 4 hens were kept in a cage with water and same basal diet ad libitum. During the experiments, hen houses received 16h of light per day. Hen house's temperature was measured four times a day (06.00, 12.00, 18.00 and 24.00). The average minimum and maximum daily temperature respectively were 22°C and 36°C that recorded in hot season of year. The length of the experiment was 90 days. The control group was kept in the same experimental situation and average minimum and maximum temperature were 20°C and 26°C respectively. Results of present study showed that Ca concentration significantly decreased (p<0.05) in purpose group. PH levels in purpose group significantly increased (p<0.05). Results of present study also showed that heat stress in purpose group results in decrease egg production (p < 0.05) and egg quality (p < 0.05) that can be relation with PH increase, decreased ionized Ca of plasma. 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.

Key words: Heat Stress · Laying Hens · Calcium Concentration · PH · Egg Production · Egg Quality

### **INTRODUCTION**

Stress due to high environmental temperature is widely recognised as one of the primary problems in poultry production [1-5]. High environmental or shed temperatures may affect the feed especially calcium (Ca) intake of the bird, thus, resulting in a decreased availability of Ca for shell deposition [6] as well as heat stress in birds cause many biochemical and physiological changes such as shift in acid-base balance, hyperthermia, increased of O2 and production of CO2, increased production of free radical and corticosterone [7]. At high ambient temperature, bird's body temperature rises and respiratory rate increases to dissipate extra heat via evaporative cooling, therefore, partial pressure of CO<sub>2</sub> decreases in the blood plasma. In turn, the bicarbonate buffer system decrease the concentration of carbonic acid  $(H_2 CO_3)$  and hydrogen  $(H^+)$ , causing in rise of plasma pH

[8]. In laying hens, egg shell is affected by acid-base balance in the blood because it is a restricted factor for accumulation of  $CaCO_3$  in egg shell [9]. Arima *et al.* [10] found that the egg quality of older hens was more severely affected by increased temperature than younger hens.

The general objective of the present study was thus to assess the effect of heat stress on acid-base imbalance, plasma Ca concentration, egg production and egg quality in commercial layers.

# MATERIALS AND METHODS

This experimental study was performed in Hy-Line laying hens at the age of 40 weeks. The animals were obtained from commercial company and divided randomly into two groups (control and purpose groups), each group contains 100 hens.

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Table 1: Ingredients and chemical composition of the basal diet fed to laying hens

a/ Tong
cg/ 10lls
440
240
100
100
80
15
15
6.5
2.5
1
300g

Nutritional supplements per kg: Mn: 40 mg; Fe: 30 mg; Zn: 25 mg; Cu: 3.5 mg; Iodine:1.5 mg; Se: 0.75 mg; choline chloride: 200 mg; vitamin A: 7.750 IU; cholecalciferol: 1.250 IU; vitamin E: 7.5 IU; menadione: 1 mg; thiamin: 2.5 mg; riboflavin: 3.5 mg; d-pantothenic acid: 4 mg; pyrodixine: 1 mg; vitamin  $B_{12}$ : 0.075 mg; folic acid: 750 mg; niacin:15 mg.

Every 4 hens were kept in a cage with water and basal diet ad libitum (Table 1). During the experiments hen houses received 16h of light per day. Hen house's temperature was measured four times a day (06.00, 12.00, 18.00 and 24.00). The average minimum and maximum daily temperature respectively were 22°C and 36°C that recorded in hot season of year. The length of the experiment was 90 days. The control group was kept in the same experimental situation and average minimum and maximum temperature were 20°C and 30°C respectively.

Blood samples were obtained weekly in heparinized tubes from the brachial vein of birds and transferred to laboratory (Laboratory of Tabriz veterinary Medicine School). Blood samples were centrifuged at 3000 rpm for 15 minutes to separate clear plasma which was stored at-20°C to determine Ca concentrations (at the same time) using available commercial kits. PH values in plasma were determined by Electric PH meter. Egg production and egg quality were recorded daily and data were summarized on weekly basis. Parameters included for egg quality measurement were egg weight, specific gravity, egg shell thickness, egg shell weight and Haugh unit [11].

Egg weight determined to the nearest 0.01g using a digital scale.

Specific gravity of eggs was determined by using the saline flotation method of Hempe *et al.* [12]. Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.120.

Eggshell thickness was measured after removing the internal membranes of the eggshell. It was used a precision micrometer to the nearest 0.01mm (Mitutoyo Dial Thickness Gage, Japan). Three measurements were taken at the equatorial region of the shell and the mean was calculated.

Haugh units were calculated using the HU formula [13] based on the height of albumen determined by a micrometer (AMES S-6428) and egg weight.

Differences between groups were evaluated by t-tests. P-values <0.05 were considered statistically significant. Statistical analysis was performed using the SPSS statistics software package version 15.0.15.

# RESULTS

Results of present study showed that PH levels in purpose group were higher than control group (p<0.05). The average PH level in purpose and control groups was 7.84 and 7.23 respectively (Table 2). Plasma Ca concentration measured in current study showed a significant decrease (p<0.05) during heat stress period (Table 3).

All production parameters were severely affected by heat stress (Table 4). According to Table 4, egg weight and specific gravity decreased significantly (p<0.05) when the layers were submitted to heat stress conditions.

Table 2: Average weekly PH levels measured in purpose and control groups.

	week												
PH level	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	7.37	7.05	6.96	7.73	7.91	6.45	7.30	7.33	7.39	7.52	7.49	6.23	7.43
Purpose*	7.80	7.86	8	8.04	7.90	7.56	7.77	7.96	8.07	7.84	7.62	7.99	7.70

\*PH levels in purpose group were significantly higher than control group (p<0.05).

Table 5: Plasma Ca concentration measured in purpose and control groups												
	week											
Ca (mg/dl)	1	2	3	4	5	6	7	8	9	10	11	12
Control	17.2	16.6	16.8	16	17.3	18	18	16.6	17.5	18	18.1	17
Purpose*	15.1	12.3	15.4	12.1	12	14.7	14.6	12	15	17.5	14.2	13

\*Plasma Ca concentration in purpose group showed a significant decrease (p<0.05) during heat stress period.

Table 4: The effects of heat stress on the egg quality in laying hens (average in 13 weeks)

Parameters	Control	Purpose*
Egg weight (g)	61.5	59.5
Specific gravity (f/cm <sup>3</sup> )	1.074	1.053
Egg shell thickness (× 0.01 mm)	32.8	27.6
Egg shell weight (g)	5.07	3.71
Internal egg quality, HU**	80.3	73.07
Egg shell thickness (× 0.01 mm) Egg shell weight (g) Internal egg quality, HU**	32.8 5.07 80.3	27 3.7 73

\* All production parameters were decreased significantly (p<0.05) in purpose group.

\*\*HU = Haugh Unit



Fig. 1: The effects of heat stress on mean egg production in laying hens. \* P = 0.021

Specific gravity is closely related to eggshell quality. Specific gravity decreases together with eggshell thickness. This was also observed in the present study (Table 4).

The values of Haugh units were significantly different (p<0.05) between the two groups. Under heat stress, the value decreased significantly. This might have been due to the stress to which the birds had been subjected.

Main egg production showed significant (p < 0.05) differences among two groups (Figure 1).

#### DISCUSSION

13 17.5 17.5

According to results of this study PH levels increased and plasma Ca concentrations decreased during heat stress period that were significant and were mentioned in previous studies [14-16]. These Results may explain decrease in egg quality during heat stress. The heat stressed laying flock often shows imbalances in acidbase disturbances in the blood as result of hyperventilation. As birds pants there is excessive loss of  $Co_2$  gas from their lungs. The lowered amount  $Co_2$  in the blood causes the blood PH to elevate or become more alkaline. The higher PH reduces the amount of ionized Ca in the blood that utilized by the shell gland. Therefore the concentration of Ca reduces in plasma and these cause pear egg quality.

Egg production in this study was inversely related to high temperature. Hen-day egg production was significantly decreased through all 13 weeks for hens exposed to the constant hot temperature compared with those in the control group. These findings are in agreement with those of Muiruri and Harrison [17], Kirunda et al. [18] and Mashaly et al. [19], 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 [20] 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 [21]. Furthermore, it has been reported that exposure to high temperature decreased plasma protein concentration [22] and plasma Ca concentration [23], 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. Eggs from purpose group weighed significantly less than eggs from the control group throughout the 13 wk experiment. These results agree with those of Kirunda et al. [18], Mashaly et al. [19], Huston et al. [24], de Andrade et al. [25, 26] and Emery et al. [27] who found that either high environmental temperatures decrease egg weight. This finding could be due to the reduction in feed consumption as reported by de Andrade et al. [26]. The adverse effect of high environmental temperature on eggshell quality has been well documented [23,25,26,28,29]. Eggshell thickness and specific gravity of eggs from the heat-stressed hens were less than the control (P < 0.05). The decrease in shell quality in the current study may be partially due to a reduction in plasma Ca. It has been reported that plasma Ca level was significantly decreased in laying hens [23] and in turkeys [30] when the birds were exposed to high temperatures. In addition, it has been shown that Ca use [29] and Ca uptake by duodenal epithelial cells [23] are decreased by exposure to high environmental temperatures. Eggs from birds kept in the heat stress, in general, had significantly lower Haugh units than those from birds in control group. This finding corroborates a previous report [18,19].

### CONCLUSION

This present study was designed to assess the effect of heat stress on acid-base imbalance, plasma Ca concentration, egg production and egg quality in commercial layers. In conclusion, results showed that heat stress of laying hens caused poor production performance. 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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