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Acute phase proteins, endocrine and productive responses of laying hens to heat stress

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Abstract: This study was designed to characterize changes in the acute phase proteins (APPs), hormones parallel to performance, and egg quality traits in laying hens exposed to heat stress (HS). A total of 120 sixteen-week-old Lohmann hens was allowed an adaptation period of 5 weeks in 3 different rooms at 21 °C. The hens were then exposed to one of three climatic thermal treatments over the next 6 weeks; thermoneutrality (TN, 21 °C), constant (C-HS, 28 °C), and cyclic HS (HS, 20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1 °C). Blood samples were weekly obtained, and serum levels of APPs [serum amyloid A (SAA), transferrin, and ovotransferrin], and hormones [leptin, triiodothyronine (T3), thyroxine (T4), and corticosterone] were measured. In addition, core body temperature (T_{core}), hen's body weight (BW), and egg quality parameters were recorded. Results indicated that SAA, transferrin, and ovotransferrin were not significantly ($p > 0.05$) different among the three thermal treatments in comparison with control; HS-hens had numerical increases of 2.46%, 3.46%, and 5.05% of the three APPs, respectively. Leptin levels were higher ($p < 0.05$), and T4 levels were lower ($p < 0.05$) in the HS than TN, meanwhile both C-HS and HS suppressed ($p < 0.05$) T3 levels in comparison with the TN. Furthermore, HS induced a significant variation ($p < 0.05$) in T_{core} patterns, BW, eggs' number and weight, albumen height, and Haugh unit among the three thermal treatments. In conclusion, HS induced multiple changes in the APPs, hormones, performance, and egg quantity and quality parameters tested, and can potentially be used in future research as early biomarkers for stress in layers.

Key words: Heat stress, acute phase proteins, hormones, laying hens, performance, egg quality

1. Introduction

The poultry industry has occupied a leading role among agricultural industries in much of the world [1]. Heat-waves can cause great losses in poultry sector especially in tropical, subtropical, arid, and semiarid regions of the world [2,3,4]. Heat stress (HS) is the most concerning issue nowadays in the ever-changing climatic scenario due to global warming [5,6]. The intergovernmental panels on climate change expect that 20%–30% of animals will be at risk of extinction due to detrimental climatic changes [7]. Laying hens are more susceptible to HS because their metabolic heat production is high [8], and there is little heat dissipation by convection, and radiation [9]. As a result, laying hens reduce their feed intake to limit metabolic heat production; resulting in a huge compromise in performance and productivity [10,11]. These economic losses by HS not only threaten chicken welfare [12,13], but also negatively influence body weight (BW), egg quantity and quality, fertility, hatchability, and survival of chickens [14,15]. Furthermore, under HS conditions, birds generate several behavioral, physiological, and immunological responses seeking thermoregulation [16], and thereby decrease their core body temperature (T_{core}) [2,12]. Thus, improving chicken performance is still a challenging task in animal production, especially under HS conditions.

The acute phase proteins (APPs) are a group of blood proteins that change in concentrations in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma, or stress [17,18]. Understanding the harmony among different APPs is a useful tool in the assessment of health status and prediction of diseases of animals, and acts as a valuable biomarker system of inflammation in veterinary medicine [4,19]. Thus, APPs as diagnostic markers may be of significant value in identifying chicken health problems, and hence allowing early and rapid screening of chicken health and welfare [20,21]. In chickens, alterations in APPs are still much less characterized as compared to ruminants, and in recent years more studies are being published on chicken APPs' changes in association with inflammation, infection, diseases, and HS [22]. In chickens, APPs have been described so far, with more attention focused on serum amyloid A (SAA) [23], transferrin and ovotransferrin [19]. Chicken transferrin and ovotransferrin levels increase to

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trigger inflammatory and metabolic responses to stress [24,25]. Some hormones, such as leptin, triiodothyronine (T3), thyroxine (T4), and corticosterone are already involved in thermal adaptation, and could act as vital indicators for stress assessment in animals [26].

In Jordan, the climate is particularly characterized as being hot and dry in nature where the air temperature ranges between 19 °C and 41 °C [27], comprising recurrent HS waves to a great portion of chickens reared under less environmentally controlled conditions. To date, there were no previous studies on APPs in laying hens, and we herein reported for the first time the impact of HS on certain APPs in laying hens. Furthermore, there has been very limited work on the impact of HS on hormones in laying hens. Thus, in view of the above considerations, the current study was designed to investigate certain APPs, hormones, performance, and eggs' quality traits in laying hens during exposure to different HS protocols, emphasizing the necessity to objectively assess the levels of stress in laying hens in an attempt to establish baseline information for improving their welfare.

2. Materials and methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) protocol at Jordan University of Science and Technology (JUST) (5/2013), Irbid, Jordan, which is in line with the international guidelines of animal use in scientific research procedures.

2.1. Animal housing, feeding and management

The study was set up at the Animal House Facility at JUST, Irbid, Jordan between July, and September, 2019. A total of 120 sixteen-week-old Lohmann hens (Lohmann LSL-Classic White) with similar body weights was purchased from an authorized agent (commercial hatchery). The hens had free access to fresh drinking water as well as a commercial grower ration ad libitum, on a daily basis. The ingredient compositions (%) of the standard basal diet were corn (62.07%), soybean meal (23%), corn gluten meal (6%), soybean oil (4%), limestone (1.2%), dicalcium phosphate (2%), L-lysine (0.35%), DL-methionine (0.08%), common salt (0.3%), and a vitamin/mineral premix (1%). Apparently, healthy hens were used in the study, and the routine management procedures, i.e. cleaning of housing pens, feeders, drinkers, and faeces were regularly carried out. No clinically relevant health problems occurred throughout the entire experimental period. When the experiment began, the laying hens were subjected to a photoperiod of 7 h light: 17 h dark, and it was increased to 16 h light: 8 h dark (04:00 a.m. to 08:00 p.m., 24 h light/dark cycle).

2.2. Experimental design and blood sampling

Hens (n = 120) were allocated to 3 different climatically controlled condition rooms (40 hens/room) of identical size, ventilation, humidity, temperature, and light intensity, and scheduled in a completely randomized designed experiment. Density of 10 birds/m^2 was precisely followed. All hens were allowed to an adaptation period of 5 weeks in the three different rooms at a constant temperature of 21 ± 1 °C (thermoneutrality, TN). Once the hens reached 21 weeks of age, they were exposed to one of three different thermal treatments over the next 6 weeks. In the 1st room (TN), hens (n = 40) continued to be exposed to TN of 21 ± 1 °C, while hens (n = 40) in the 2nd room (constant HS, C-HS) were exposed to a relatively stable temperature of 28 ± 1 °C. Hens (n = 40) in the 3rd room, however, were exposed to cyclic HS (HS) such that ambient temperature was cycled monophasically between a peak of 33 ± 1 °C (20 h/d), and a nadir of 28 ± 1 °C (4 h/d) using a thermostatically controlled heater. It is to mentioned that the sample size is determined using the equation: $n = 8 \times (C.V.)^2 / (d\%)^2$, where n = sample size, C.V. = coefficient of variation, and d = significant level ($p < 0.05$), assuming that C.V. (%) = 10%. Thus, $n = 8 \times (10)^2 / (5\%)^2 = 32$ hens. Based on this, we located 40 hens per each treatment (> 32) hens/treatment).

Blood samples from hens (n = 10) of each thermal treatment were weekly obtained from the wing vein using a heparinized syringe, and collected in plain tubes at 09:00 h until the end of the experimental period. All collected blood samples were centrifuged (10 min, 3000 × g, 4 °C; Centrific TM Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA), and the obtained serums were stored at –20 °C until analyzed.

2.3. Analyses of APPs and hormones

Serum levels of SAA, transferrin, and leptin were measured by a sandwich ELISA double antibody technique using chicken commercial ELISA kits (Wuhan fine Biotech Co., Ltd., China) according to the manufacturer's instructions. Meanwhile, serum levels of ovotransferrin, T3, T4, and corticosterone were measured by a competitive inhibition enzyme immunoassay technique using chicken commercial ELISA kits (T3, T4, and corticosterone: Wuhan fine Biotech Co., Ltd., China; ovotransferrin: MyBiosource Inc., San Diego, CA, USA) according to the manufacturer's instructions. The analyses were conducted in the Biotechnology Laboratory at JUST, Irbid, Jordan. The final absorbance was measured in a microtiter plate reader at 450 nm wavelength. The intra- and interassay coefficients of variations were < 8% and < 10% for SAA, transferrin, leptin, T3, T4, and corticosterone, as well as < 8% and < 12% for ovotransferrin.

2.4. Performance and egg quality traits

Intraperitoneal temperature (IP) was recorded twice weekly at 09:00 h and 12:30 h starting from the first experimental week until the end of the experimental period. The IP measurements were read using a minuscule electronic

thermal chip system using a special hand-held reader. An inert thermochromic microchip (Desetron LifeChip, USA) was subcutaneously injected below the right wing under aseptic conditions (iodine/alcohol disinfection) as described by the manufacturer's manual. The skin under the right wing was firstly rubbed with medical grade alcohol to disinfect the implantation site. Then, the chip was inserted subdermally to a caudal distance of 4 cm at a 45 º angle (to the vertical standing plane) with the bevel of the injection needle pointing out. Hereafter, the injected site was then disinfected with a gentamycin spray. Twenty hens from each thermal treatment were ID-marked using a color spray (Gentian Violet) at a distinct body location for individual T_{core} monitoring. No signs of irritation or inflammation were noticed on any of the implanted hens throughout the trial. Also, to rule out the potential interference of this procedure, performance parameters of the implanted hens were compared to unimplanted counterparts throughout the study, and were found to be homogeneously indifferent. T_{core} readings from 60 hens (20 hens/thermal treatments) were collected using handheld scanners (DTR5-60 and Portable Global Pocket Reader, Desetron Fearing, Dallas, TX, USA).

After the 5-week adaptation period, hen's BW was measured using a digital balance at the beginning of the experiment (pre-BW; weight obtained at 21 weeks of age was considered the experimental initial BW), and at the end of the experiment (post-BW; weight obtained at 27 weeks of age was considered the experimental final BW) in the three different thermal treatments (40 hens/thermal treatment). The hens' egg production was daily gathered in the morning, and mean egg production was calculated as number of daily laid eggs by all hens (n = 40) in each thermal treatment. For egg weight determination, 840 eggs were randomly selected from each thermal treatment and weighted by a scale with a sensitivity of 0.01 g, and the mean egg weight was calculated. To measure the egg quality (albumen height, yolk color, and Haugh unit), 87 eggs were randomly selected from each thermal treatment, and analyzed using egg multitester (EMT-5200, Robotmation Co., Ltd.). Means of albumen height, yolk color, and Haugh unit were calculated. The egg quality analyses were performed at the Veterinary College, JUST, Irbid, Jordan.

2.5. Statistical analysis

All statistical analyses were performed using the Proc General Linear Model (GLM) (SPSS 19.0, SPSS Inc., Chicago, IL, USA). The experiment was factorial arrangement within complete randomization design (CRD). One-factor analysis of variance (ANOVA) was conducted to detect differences among means at the level of < 0.05 [28]. Least significant difference test (LSD) was used to compare among several means [29]. Thermophysiological data were analyzed as split plot in time using mixed procedure of SAS, in which treatment and time were plugged in the main plots, while their interaction was used in the subplot as described by Littell et al. [30]. All data were herein presented as means ± standard error of the mean (SEM).

3. Results

3.1. Effect of HS on AAPs' levels

The time-course changes of serum levels of the APPs; SAA, transferrin, and ovotransferrin are presented in Figure 1. Mean total SAA level was not significantly (p = 0.721) different among the three treatments, where it was 0.541 ± 0.09, 0.540 ± 0.08, and 0.528 ± 0.09 ng/mL in HS, C-HS, and TN, respectively. The SAA levels were significantly altered over time (time simple effect) similarly at the three thermal treatments tested (p = 0.000–0.001) (Figure 1A). No significant (p = 0.570) differences were detected among the three different thermal treatments in mean total transferrin level, with means of 3.33 ± 0.84, 3.26 ± 0.67, and 3.18 ± 0.70 ng/mL in HS, C-HS, and TN treatments, respectively. A significant time effect was detected on transferrin levels in all the three thermal treatments (p = 0.001–0.019) (Figure 1B). In addition, mean total level of ovotransferrin was also not significantly different (p = 0.534) among the three treatments (70.27 ± 13.78, 67.30 ± 14.55, and 67.93 ± 14.64 pg/mL in HS, C-HS, and TN treatments, respectively). The time had a significant effect on ovotransferrin level at the three thermal treatments tested (p = 0.003–0.050) (Figure 1C).

3.2. Hormone responses to HS

The time-course changes of serum levels of the hormones; leptin, T3, T4, and corticosterone are shown in Figure 2. Mean circulating levels of leptin were significantly (p = 0.049) higher in HS-hens than the other two treatments. In leptin level, the time had a significant effect in all three thermal treatments tested (p = 0.000–0.002, Figure 2A). Significant suppressions by HS and C-HS in means of T3 (p = 0.003), and by HS in means of T4 (p = 0.042, Figures 2B and 2C) were detected. The time had a significant effect on T3 level in HS and C-HS treatments (p = 0.000 and 0.041, respectively) (Figure 2B), while for T4 the time had a significant effect only in HS treatment (p = 0.001) (Figure 2C). Mean total levels of corticosterone were not significantly (p = 0.270) different among the three treatments, where they were 94.28 ± 36.11, 93.82 ± 42.36, and 82.92 ± 43.88 ng/mL in HS, C-HS, and TN, respectively. The time had a significant effect on corticosterone levels in C-HS and TN treatments (p = 0.026 and 0.036, respectively) (Figure 2D). The HS-hens had an increase of 13.70% in corticosterone levels as compared to TN-hens.

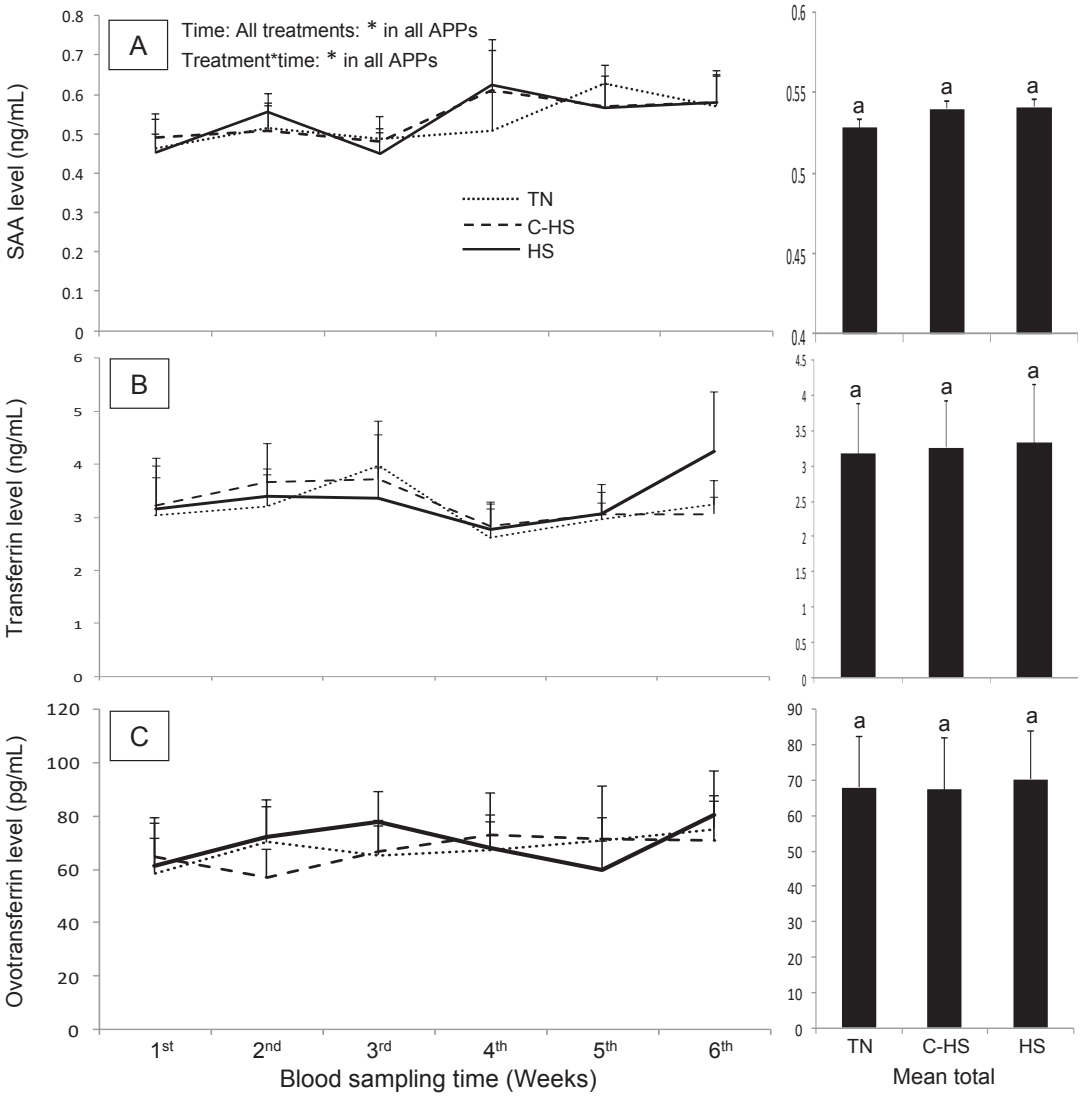

Figure 1. Time course changes in mean (± SEM) serum levels of the APPs; amyloid A (SAA) (A), transferrin (B) and ovotransferrin (C) in laying hens submitted to heat stress (n = 10). *: Indicates significant differences among the different times within the same treatment at $p < 0.05$. Bars on right side indicate mean total (± SEM) serum levels of all APPs sampling times within each protein at the three temperatures tested. TN: thermoneutral, 21 ± 1 °C; C-HS: constant 28 ± 1 °C; HS: cyclic HS (20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1°C).

3.3. Performance and egg quality traits

3.3.1. Body core temperature

Mean (± SEM) T_{core} (°C) in laying hens submitted to HS are presented in Figure 3. Mean T_{core} was significantly different ($p = 0.002$) being 40.81 ± 0.27 °C, 40.82 ± 0.31 °C, and 40.42 ± 0.32 °C at 09:00 h, as well as 40.81 ± 0.40 °C, 40.93 ± 0.24 °C, and 41.29 ± 0.35 °C at 12:30 h in TN, C-HS, and HS, respectively. However, at 12:30 h, T_{core} was significantly ($p = 0.017$) higher in the HS than in both C-HS and TN treatments.

3.3.2. Body weight

The pre- and posttreatment BWs in laying hens submitted to HS are shown in Figure 4. Mean pre-BW was not significantly different ($p = 0.941$) among the three thermal treatments. In contrast, mean post-BW of laying hens was significantly ($p = 0.002$) lower in the HS (1505.2 ± 130.52 g/bird) and C-HS (1542.48 ± 103.46 g/bird) compared to TN treatment (1593.55 ± 93.84 g/bird). There was 3.21% and 5.55% reduction in post-BW of laying hens subjected to C-HS and HS, respectively, as compared to post-BW of laying hens in the TN treatment.

3.3.3. Egg quality traits

The egg quality traits (eggs' production and weight, albumen height, yolk color, and Haugh unit) are presented in Table 1. Mean number of laid eggs/40 hens/day was significantly ($p = 0.032$) lower in HS (30.79 ± 5.74) and

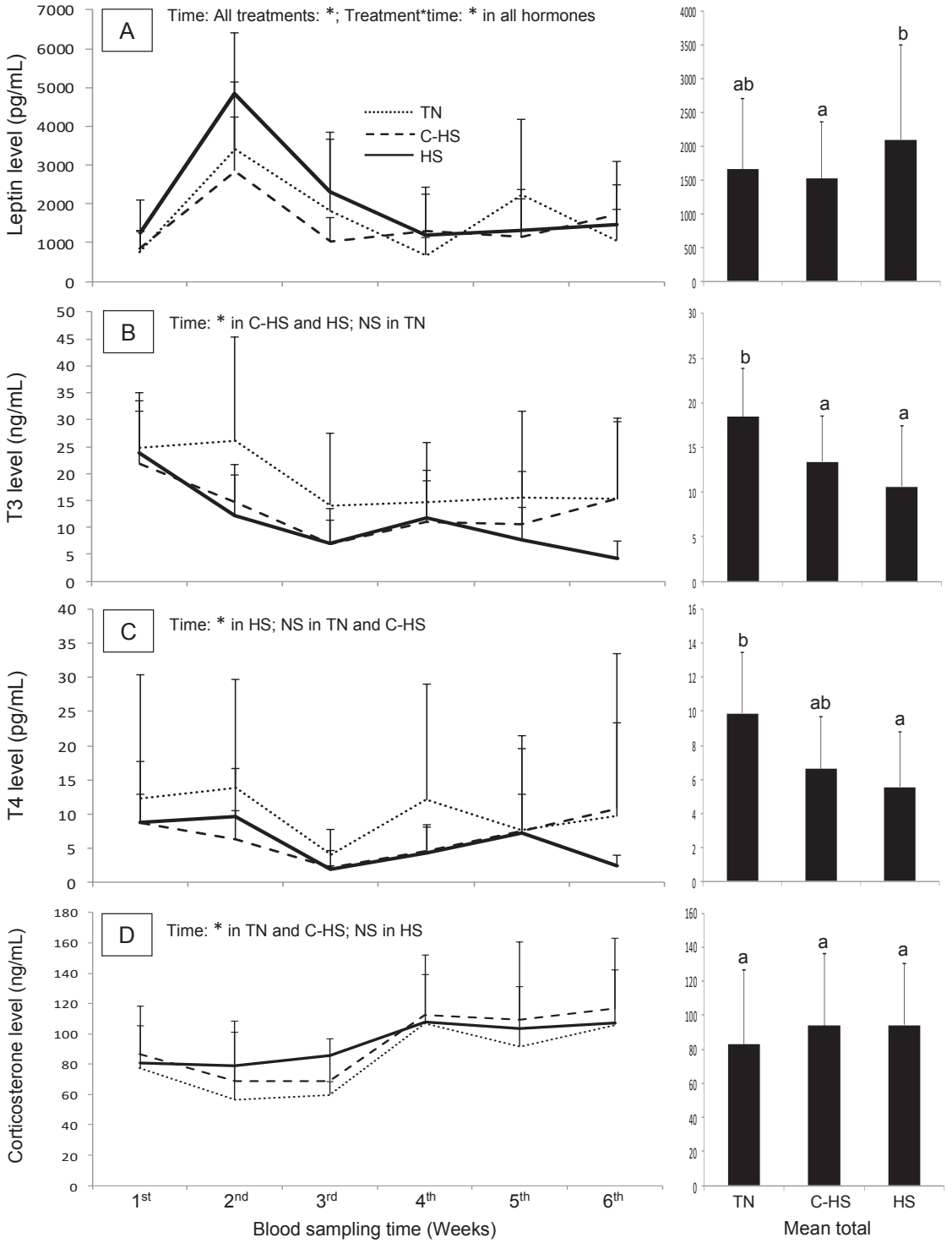

Figure 2. Time course changes in mean (± SEM) serum levels of the hormones; leptin (A), triiodothyronine (T3) (B), thyroxine (T4) (C) and corticosterone (D) in laying hens submitted to heat stress (n = 10). *: Indicates significant differences among the different times within the same treatment at $p < 0.05$. NS: Not significant. Bars on right side indicate mean total (± SEM) serum levels of all sampling times within each hormone at the three temperatures tested. TN: thermoneutral, 21 ± 1 °C; C-HS: constant 28 ± 1 °C; HS: cyclic HS (20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1 °C).

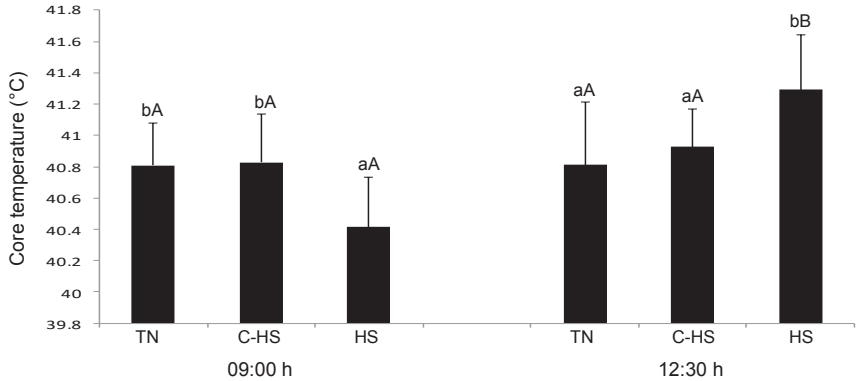

Figure 3. Mean (± SEM) core body temperature (T_{core}; °C) in laying hens submitted to heat stress (n = 20). Different small letters above bars indicated significant differences among the different treatments within the same hour (09:00 or 12:30) at $p < 0.05$, while capital letters indicated significant differences between the two different hours (09:00 and 12:30) within the same treatment (TN, C-HS or HS) at $p < 0.05$. TN: thermoneutral, 21 ± 1 °C; C-HS: constant 28 ± 1 °C; HS: cyclic HS (20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1 °C).

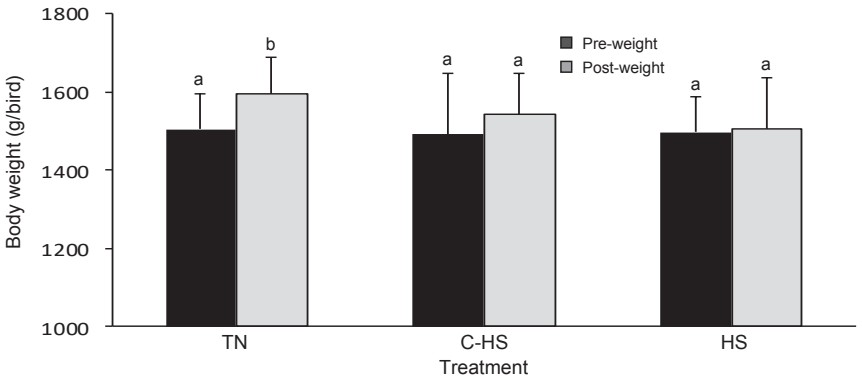

Figure 4. Mean (± SEM) pre- and posttreatment body weight (g/bird) in laying hens submitted to heat stress (n = 40). Different small letters above bars indicated significant differences among the different temperatures within the same pre- or posttreatment body weight at $p < 0.05$. TN: thermoneutral, 21 ± 1 °C; C-HS: constant 28 ± 1 °C; HS: cyclic HS (20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1 °C).

Table 1. Egg quality and quantity traits in laying hens submitted to heat stress.

Parameter	Unit	Treatment group		
		TN	C-HS	HS
Egg production	Mean no. of laid eggs/40 hens/day	33.08 ± 4.73 b	30.69 ± 3.91 a	30.79 ± 5.74 a
Egg weight (n = 840/each group)	g/egg	56.26 ± 4.43 b	55.32 ± 3.53 a	55.21 ± 3.13 a
Albumen height (n = 87/each group)	mm	6.56 ± 1.33 a	7.07 ± 1.32 b	6.70 ± 1.122 ab
Yolk color (n = 87/each group)	Scale 12	9.66 ± 1.56 a	9.86 ± 1.31 a	9.58 ± 1.05 a
Haugh unit (n = 87/each group)	HU	81.50 ± 8.84 a	84.88 ± 8.10 b	82.68 ± 6.98 ab

Different letters within each row indicate significant differences among the different treatment groups at $p < 0.05$. TN: thermoneutral, 21 ± 1 °C; C-HS: constant 28 ± 1 °C; HS: cyclic HS (20 h/d at 28 ± 1 °C and 4 h/d at 33 ± 1 °C).

C-HS (30.69 ± 3.91) than in the TN (33.08 ± 4.73). Mean egg weight was also significantly ($p = 0.000$) lower in HS (55.21 ± 3.13 g/egg) and C-HS (55.32 ± 3.53 g/egg) than in TN treatment (56.26 ± 4.43 g/egg). Albumen height and Haugh unit were significantly ($p < 0.023$ and 0.019) the highest in C-HS, followed by HS, and then TN treatments. There were no significant differences ($p = 0.339$) in yolk color among the three thermal treatments tested.

4. Discussion

4.1. Effect of HS on APPs' levels

Herein, we reported for the first time the impact of HS on certain APPs in laying hens. However, the current findings indicated that the levels of APPs; SAA, transferrin, and ovotransferrin were not significantly affected by HS, although in comparison with the TN and HS-hens had numeric increases of 2.46%, 3.46%, and 5.05% in serum level of SAA, transferrin, and ovotransferrin, respectively. In accordance with the present results, SAA and ovotransferrin levels were found to be elevated in Silkie fowls subjected to chronic HS at 35 °C when compared with fowls raised under thermoneutral condition [31]. In addition, ovotransferrin level was greater in HS-broiler birds than in their nonheat stressed counterparts [32]. Under HS conditions, SAA level was elevated in goat [33], and in cows [34]. It is worth mentioning that there was no hens' mortality under the HS conditions in the current study. This may indicate that none of the HS protocols was severe enough to trigger a clear acute phase response. In this regard, Melesse et al. [35] reported that Lohmann White breed proved to be highly heat tolerant chickens compared to other genotypes.

4.2. Hormone responses to HS

The present findings demonstrated that HS elevated leptin level, which is in agreement with the findings of previous studies in broilers [36], and in other animal species, i.e. goats [33], cows [37], mice [38], and pigeons [39]. Chicken leptin is expressed in the hypothalamus and in various tissues including pancreas, muscles, liver, and adipose tissues [40,36]. The hepatic leptin and muscle uncoupling proteins gene expression increases in broiler chickens under an extreme ambient temperature (32 °C) [36]. In contrast to mammals, different mechanisms for leptin regulation of feed intake and energy expenditure have been reported. The leptin hepatic expression attributed to the avian lipid metabolism, in which the liver is the primary site of lipogenesis [40]. Under HS conditions, this change in leptin level could be considered as a metabolic adaptive response to HS, a way to increase heat loss and reduce heat production to remain euthermic.

The current results showed that HS suppressed significantly T3 and T4 levels. Our results are in accordance with the findings of Maak et al. [41], and Melesse et al. [35] who stated that HS reduces T3 in laying hens, and Hosseini et al. [42] who also reported a significant suppression of T3 and T4 in broilers. The noticeable decline in T3 seen in HS-hens prospectively supports the reduction in internal overall heat load, as a vital tool of adaptation in poultry. It could be concluded that both T3 and T4 could be considered as reliable indicators of HS, thus, both hormones' level in blood could be used as a criterion to select thermo-tolerant commercial chicken breeds in hot regions.

Corticosterone is a common hormone that is released under numerous stress situations by the hypothalamus-pituitary-adrenal axis. Furthermore, corticosterone is a sensitive indicator of HS response, which involves in metabolic rate and heat production of broilers [43,44]. Corticosterone level is elevated by stressors due to increased secretion of adrenocorticotropic hormone and corticotropin releasing hormone [45]. In this study, corticosterone level was not significantly affected by HS, although HS-hens had an increase of 13.7% in corticosterone level as compared to TN-hens. In consistent with our results, He et al. [44] and Gu et al. [46] reported no significant elevation in serum corticosterone under HS. The nonsignificant change of measured corticosterone levels in laying hens might suggest that hens have the capability to adapt to HS after certain period of time [47].

4.3. Performance and egg quality traits

HS induced a significant variation in T_{core} (especially those taken at 12:30 h; Figure 3) among the three temperatures tested. Our present data are in agreement with previous studies which showed that exposure of broilers [48], Japanese quail [49], and layers [50] to acute cyclical HS (33–36 °C; ranging from 1–4 h/day) resulted in more detrimental effects (hyperthermia and hindered productivity) when compared to constant and mild HS (< 30 °C). This is corroborated by the T_{core} results as well as circulating T3 (Figure 2B), as evidenced by the treatment by time interactions ($p < 0.05$) observed in both parameters.

Interestingly, the HS treatment displayed significantly ($p < 0.05$) lower T_{core} than the TN treatment (especially those taken at 09:00 h; Figure 3), despite the fact that the temperature was actually still lower in the latter group. This adaptive response was likely attained by two strategies: 1) a decline in thermogenic potential, as supported by the steeper decline in circulating T3 levels of the HS, especially towards the end of the trial. 2) Changing the feeding behavior, HS-chickens were often observed to switch from regular/typical major feeding bouts (at the time of feed provision in the morning; at 08:00 h) to nighttime feeding, which coincided with the cooling phase of the climatic thermal conditions (after 16:00 h). Such behavioral and metabolic acclimation means were clearly effective in minimizing the impact of HS during the daytime, and were likely responsible for the distinctive thermal liability noticed in that particular animal group (Figure 3).

The current findings showed that post-BW was significantly lower in both HS and C-HS-hens compared to TN-hens. There was 5.55% reduction in post-BW of laying hens subjected to HS compared to post-BW in the TN treatment. The literature has reported that exposed to HS induced a marked depression of BW in laying hen [10,11,51]. Reduction of BW under HS may be due to the

interaction between stress, and nutrition, which results in nutrient deficiency as HS is associated with marked reduction in appetite, feed intake, dry matter intake, and metabolic rate [33,52].

The reduction in performance associated with HS is a well-known phenomenon in domestic birds [15,53]. Eggs' number and weight were significantly lower in HS and C-HS-hens compared to TN-hens. In agreement with our findings, previous work showed that HS causes a significant decrease in egg production [11], and egg weight [54,55] of laying hens. In a study on the effect of HS (34 ± 2 °C) on laying hens (Lohmann LSL-Lite), it was found that exposure to HS caused decreases in egg production and weight [56]. Another response to elevated temperatures is to reduce egg production due to insufficient energy and nutrient intake to sustain egg production at non-HS levels [57]. This is probably due to the direct debilitating effect of high ambient temperature on ovarian function in the birds [58].

C-HS reduced ($p < 0.05$) albumen height and Haugh unit compared to TN. In line with our results, Haugh units decreased after 2 and 4 weeks of HS when hens were subjected to a daily cyclic HS (30/35 °C) [11]. Furthermore, exposing laying hens (Lohmann LSL-Lite) to HS of 34 ± 2 °C decreased Haugh unit [56]. In contrast, no significant change was noticed in either albumen height, over a 5-week cyclic or constant HS [57], or Haugh unit during a 2-week constant HS [51].

In conclusion, under HS conditions of the present study, the results suggest a possible use of hormones as potential indicators, and sensitive markers in monitoring HS in laying hens more than APPs. Furthermore, since HS was not able to induce significant rises in serum levels of APPs investigated herein, further studies on the impact of HS on laying hens with higher temperatures more than 33 °C or prolonged HS period are suggested. Overall, the outcomes of our present trial may shed some basic light on understanding relationships between the studied HS protocols and laying hen productivity, in shadows of APP's and endocrine responses, and may prospectively contribute to improved food security worldwide where eggs and white meat are major sources of calories and protein. Finally, it is expected that this study will provide guidance to animal biologists, animal husbandry farmers, researchers, veterinarians, and nutritionists for effectively managing HS, and will therefore contribute to the sustainability of agriculture, and develop management techniques to alleviate HS on hens, and highlight the need for future research on HS in poultry sector.

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Conflict of interests
The authors declare no conflict interests.

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