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Research Article
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Effects of ACTH and Acute Heat Stress on Oxidative Stress in an Early Environmentally Enriched Broilers

Erken Yaşta Zenginleştirilmiş Çevrede Yetiştirilen Etlik Piliçlerde ACTH ve Akut Isı Stresinin Oksidatif Stres Üzerine Etkileri

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ABSTRACT

Objective: In this study, the effects of early (≤21. d) environmental enrichment and acute heat stress onş oxidative stress were investigated and the ability of broilers to cope with later heat stress and adrenocorticotropic hormone (ACTH) treatments were determined.
Material and Methods: Six hundred day-old chicks were randomly assigned to 3 experimental groups as a control (C), environmental enrichment (EE) and environmental enrichment plus heat stress (EE+HS). At 21 d of age, broilers in EE+HS group were exposed to acute heat stress at 38±1 ˚C for 3 h. On the day 42nd, C and EE groups were divided into 2 subgroups as well Control, Control+ACTH, EE, EE+ACTH. While 50 IU ACTH/kg body weight was injected to Control+ACTH and EE+ACTH groups intramuscularly, broilers in C, EE and EE+HS groups were exposed to heat stress and oxidative stress responses of birds were evaluated.
Results: Environmental enrichment did not affect blood corticosterone (CORT), malondialdehyde (MDA) and liver superoxide dismutase (SOD) levels of broilers at 21 d of age. ACTH treatment caused a significant decrease in CORT and MDA concentrations in EE broilers compared to the control group. Exposing birds to heat stress (42nd day) significantly increased CORT, MDA and decreased liver SOD levels in control as compared to EE and EE+HS groups. No significant differences were found in the SOD serum levels between groups. ACTH treatment caused more stress reactions than heat stress.
Conclusion: The results obtained from this study show that exposure of broilers to acute heat stress or treatment of experimental adrenocorticotropic hormone causes preferable reactions in oxidative metabolism. It was concluded that rearing in an enrichment environment beginning from early ages can be recommended as a useful method for adaptation to stress.

ÖZ

Bu çalışmada, erken yaşta uygulanan çevresel zenginleştirme ve akut ısı stresinin (21. gün) oksidatif stres üzerindeki etkileri araştırılmış ve etlik piliçlerin daha sonraki ısı stresi ve adrenokortikotropik hormon (ACTH) uygulamaları ile baş edebilme yetenekleri belirlenmiştir.
Materyal ve Metot: 600 adet günlük yaşta civciv kontrol (C), çevresel zenginleştirme (EE), ve çevresel zenginleştirme+ısı stresi (EE+HS) olmak üzere rastgele 3 deneme grubuna ayrılmıştır. EE+HS grubundaki civcivler denemenin 21. gününde 3 saat süre ile 38±1 ºC ısı stresine maruz bırakılmışlardır. Denemenin 42. gününde C ve EE grupları Kontrol, Kontrol+ACTH, EE ve EE+ACTH olmak üzere 2 alt gruba bölünmüşlerdir. Kontrol+ACTH ve EE+ACTH gruplarındaki piliçlere kas içi 50 IU ACTH/kg enjekte edilirken, C, EE ve EE+HS grupları ısı stresine maruz bırakılmış ve oksidatif strese karşı tepkileri değerlendirilmiştir.
Bulgular: Çevresel zenginleştirme etlik piliçlerin 21. gün kan kortikosteron (CORT), malondialdehid (MDA) ve karaciğer süperoksit dismutas (SOD) düzeylerini etkilememiştir. EE grubu piliçlerde ACTH enjeksiyonu CORT ve MDA konsantrasyonlarında kontrol grubu ile karşılaştırıldığında önemli düzeyde azalmaya neden olmuştur. Piliçlerin ısı stresine maruz bırakılmaları (42. gün) EE ve EE+HS gruplarına kıyasla kontrol grubunda CORT ve MDA düzeylerini önemli ölçüde arttırmış, karaciğer SOD düzeyini düşürmüştür. Gruplar arasında serum SOD seviyeleri bakımından önemli bir farklılık saptanmamıştır. ACTH uygulaması, ısı stresinden daha fazla stres reaksiyonuna sebep olmuştur.
Sonuç: Bu çalışmada elde edilen sonuçlar göstermektedir ki etlik piliçlerin ısı stresine maruz bırakılmaları veya deneysel adenokortikotropik hormon uygulaması oksidatif metabolizmada önemli tepkilere neden olmaktadır. Sonuç olarak, erken yaşlardan itibaren zenginleştirilmiş çevrede büyütme strese adaptasyonun sağlanması amacıyla yararlanılabilecek bir yöntem olarak önerilebilir.

INTRODUCTION

Rapid growth rate, selection of feeding and management procedures led to stress (European Commission Report, 2000) in modern broiler genotypes and therefore welfare problems (Bessei, 2006). Environmental stress is an important problem both for producers in regions where high summer temperatures are a problem, as well as for breeder businesses located in cooler regions, marketing genetic material to surrounding areas. It can be clearly seen that the main welfare problems mentioned in the last 30 years are associated with rapid growth at an early age, which is the result of selection studies. Since future broiler chicks will also be chosen for faster development and feed utilization, they will be more affected by environmental stress in addition to problems such as ascites, sudden death syndrome, fat deposition and also skeletal disorders due to low locomotor activity (Seremet, 2007).

Environmental enrichment can be defined as the modification of the environment of animals to improve biological functions and increase behavioral opportunities (Newberry, 1995). Broiler chickens are grown in a stable and uniform environment with no stimuli during their short lives of 42 days. The environment becomes highly predictable in the absence of change such as new sources (Newberry, 1999). The enriched environment is a more natural environment than standard poultry house conditions and environmental enrichment is widely recommended to improve animal welfare (Jones, 2002; Seremet Tugalay et al., 2019). Various methods used to enrich the environment have shown positive effects on the physical condition and behavior of broiler chickens (Bessei, 2006). Environmental enrichment methods that successfully stimulate activity (perches or elevated platforms, barriers, toys, lighting schedules, feeding strategies, etc.) improve leg condition and hence the welfare of broiler chickens (Balog et al., 1997; Bessei, 2006; Riber et al., 2018).

Corticosteroid concentrations in blood have been used as a measure of environmental stress in birds (Altan et al., 2000a). However, another indicator of oxidative metabolism against environmental stress in poultry was the antioxidant system responses (Lin et al., 2006; Surai, 2016; Jing et al., 2017; Surai et al., 2019). It has been well recognized that glucocorticoid hormones (de Guia and Herzig, 2015) and stress metabolism influences lipid peroxidation. Besides, lipid peroxidation leads to decreased antioxidant capacity. Environmental stress has been reported to increase blood corticosterone levels, MDA concentrations in liver and blood, and decrease liver and blood SOD levels in poultry. It is reported that environmental stress (heat, cold, noise, crating, feed restriction, etc.) in poultry enhanced blood corticosterone levels (Ismail et al., 2014; Kang et al., 2016; Scanes, 2016), increased liver and blood MDA concentrations (Sahin et al., 2001; Altan et al., 2003; Lin et al., 2006; Tatlı Seven et al., 2009; Selvam et al., 2017), reduced liver and blood SOD levels (Altan et al., 2003; Tatlı Seven et al., 2009; Yang et al., 2010; Ismail et al., 2014).

Living organisms can adapt to oxidative stress by stimulating the synthesis of damage-repair enzymes and antioxidant enzymes (Davies, 1995). The relationship between glucocorticoids and lipid peroxidation has been widely examined (Belge et al., 2003; Lin et al., 2004a,b; Lv et al., 2018; Allen and Sharma, 2019). The adrenal cortex is one of the organs most affected by lipid peroxidation due to its high levels of unsaturated fatty acids (Hornsby, 1989).

The aim of the present study was to assess the effects of ACTH injection and heat stress on blood corticosterone levels and oxidative stress in early environmental enrichment broilers.

MATERIAL and METHODS

One-day-old broiler chicks (600) were obtained from a local hatchery and placed in an environmentally controlled room. All chicks were reared under a standard management program. Feed and water were given *ad libitum*. All chicks were fed a standard broiler diet (12.8 MJ/kg, 23% CP) from 1 to 13 days, standard grower diet (13.0 MJ/kg, 22% CP) from 14 to 24 days and a standard finisher diet (13.4 MJ/kg, 20% CP) from 25 to 42 days. The lighting regimen was 23 h light: 1 h dark. Ambient temperature was gradually decreased from 32 ˚C at day-old to reach 22 ˚C at 3 wk of birds of all treatment groups were weighted, wing-banded and placed in floor pens covered with wood shavings (15 chicks/m^2).

Experimental design: The experiment consisted of stress treatments with two stages. In stage 1, chicks were divided into 3 groups as an Environmental Enrichment-EE and Environmental Enrichment plus Heat Stress-EE+HS and Control-C with 4 replicates (n=50) for each group. Chicks in EE and EE+HS groups were kept in separate pens, where a variety of colorful objects plastic bottles, balls, toys, and mirrors. While every three days, objects in the pens were fully changed with each other periodically to avoid adaptation and increase stimulation, control chicks were reared in standard floor pens.

In the first stage, on the day 21st, broilers in EE+HS group were exposed to acute heat stress at 38±1 ˚C for 3 h. In stage 2, except that the EE+HS, all groups were divided into 2 subgroups as well Control, Control+ACTH, EE, EE+ACTH at 42 days of age. While 50 IU ACTH/kg body weight (Sigma-Aldrich St. Louis, MO) was injected to (pectoralis major muscle) Control+ACTH and EE+ACTH groups (totally 16 birds) intramuscularly, C, EE and EE+HS groups were exposed to acute heat stress at 38±1 ˚C for 3 h. Blood samples were collected at 21 and 42 days of age. On the day 42 nd, blood was taken again 4 hours after ACTH injection (Martrenchar, 2001). At 21 and 42 days of age, 8 birds in each group were slaughtered and liver tissues were collected. Liver tissues were dissected from the periphery tissues after euthanasia.

Physiological parameters: Blood was drawn from a wing vein with the syringe and centrifuged at 4000 rpm for 10 min at 4 ˚C and the separated blood samples were stored at -80 ˚C for MDA, SOD and corticosterone analysis.

MDA analysis measured by the TBA method (Ohkawa et al., 1978). MDA forms a colored complex in the presence of thiobarbituric acid, which is detectable by measurement of absorbance at 532 nm. Absorbance was measured with Shimadzu UV-160 spectrophotometer. 1,1′,3,3′ Tetraethoxypropane was used as a standard and the results were expressed as nmol/ml. This method evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress (Bayraktar et al., 2011).

SOD activity in erythrocytes was measured by the method of Sun et al. (1988). The principle of this assay based on using xanthine-xanthine oxidase to generate superoxyde radical (O2•–). Nitroblue tetrazolium (NBT) reduction is used as an indicator of O2•– production. SOD will compete with NBT for O2•–; the percent inhibition of NBT reduction is a measure of the amount of SOD present. The production of formazan was determined at 560 nm using Shimadzu UV-160 spectrophotometer. Under these conditions, the absorbance at 560 nm of the Blanc tube was about 0.25. The percentage of inhibition was calculated as follows: inhibition% = (Ablank − Asample)/Ablank × 100. Cu, Zn-SOD activity was calculated via using a standard curve. The calculated SOD activity was expressed as U/g of hemoglobin (Hb) (Halliwell and Gutteridge, 1999). Hb concentration was determined with a (Beckman Coulter gen S-System 2, Miami, USA) hematological analyzer. The same colorimetric assay was used for SOD activity in tissues.

Corticosterone levels were determined using a commercially available kit (Rat/Chicken CORT (Corticosterone) ELISA Kit, Elabscience). This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with CORT. During the reaction, CORT in the sample or standard competes with a fixed amount of CORT on the solid phase supporter for sites on the Biotinylated Detection Ab specific to CORT. Excess conjugate and unbound sample or standard are washed away, and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of CORT in tested samples can be calculated by comparing the OD of the samples to the standard curve. The results expressed as ng/ml.

Statistical analysis

Data were subjected to one-way ANOVA using the General Linear Model Procedure of SAS (1999). Means were compared using the Duncan test ($\alpha = 0.05$) and all data were presented as means and standard error of means (SEM).

Results and Discussion

The effects of enriched environment and enriched environment+heat stress on CORT, MDA and SOD concentrations at 21 d of age are shown in Table 1. CORT, MDA and SOD levels significantly increased in the EE+HS group compared to the EE group on day 21 whereas MDA and SOD levels were like the control group.

To determine the effects of ACTH injection and heat practices on control, environmental enrichment and environmental enrichment plus heat stress groups, CORT, serum MDA, serum and liver SOD values were measured (Table 2). At the end of the experiment, after ACTH and heat stress treatments, a 2-fold increase in corticosterone levels of the control group occurred in comparison with EE groups ($P<0.05$). In order to adapt to the heat stress, the animals that were applied heat (38 ± 1 °C) on the 21st day of the experiment had significantly lower CORT responses (3.823 ng/ml) to the heat stress on the 42nd day ($P<0.05$). In agreement with our 21. d findings, Adeniji (2012) in a thesis investigating the effects of environmental enrichment and heat stress on behavior and production performance found that the CORT levels were significantly increased compared with those in control birds. Similarly, Martrenchar et al. (2001) in a study investigating the influence of enriched environment on corticosterone after ACTH injection in young male and female turkeys reported that an increase in corticosterone concentrations in the control group than enriched ones. Moreover, injection of ACTH increases serum glucocorticoid concentrations by direct stimulation of adrenocortical cells (Trout and Mashaly, 1994).

Table 1. The effects of early environmental enrichment and acute heat stress (21. d) on blood CORT, MDA and liver SOD levels in broilers ($\bar{x}$±SE)

Çizelge 1. *Etlik piliçlerde erken yaşta uygulanan çevresel zenginleştirme ve akut ısı stresinin (21. gün) kan CORT, MDA ve karaciğer SOD düzeylerine etkileri ($\bar{x}$±SH)*

Groups	CORT* (ng/ml)	MDA* serum (nmol/ml)	SOD* liver (U/ml)
Control	0.494 ± 0.067^{b}	0.555 ± 0.029^{ab}	7.473 ± 0.207^{ab}
EE	0.339 ± 0.095^{b}	0.506 ± 0.029^{b}	6.940 ± 0.207^{b}
EE+HS	0.868 ± 0.087^{a}	0.633 ± 0.029^{a}	7.880 ± 0.207^{a}
Source of variation	Probabilities (*P* values)		
Group	0.0017	0.0139	0.0099

*Mean ± SE

^{a,b}Means within columns with no common superscript differ significantly *(P<0.05)*

CORT – corticosterone; MDA – malondialdehyde; SOD – superoxide dismutase

EE – environmental enrichment; EE + HS – environmental enrichment plus heat stress

Table 2. Effects of ACTH and acute heat stress (42. day) on blood CORT, MDA, SOD and liver SOD levels in early environmental enrichment broilers ($\bar{x}$±SE)

Çizelge 2. *Erken yaşta çevresel zenginleştirme uygulanan etlik piliçlerde ACTH ve akut ısı stresinin (42. gün) kan CORT, MDA, SOD ve karaciğer SOD düzeylerine etkileri ($\bar{x}$±SH)*

Treatments	Groups	CORT* (ng/ml)	MDA* serum (nmol/ml)	SOD* serum (U/gHB)	SOD liver (U/ml)
ACTH	Control	23.076±2.525^{a}	0.596±0.041^{a}	3.546±0.952	7.525±0.330
	EE	14.600±2.728^{b}	0.390±0.041^{b}	4.550±1.099	7.300±0.353
	Source of variation	Probabilities (*P* values)			
	Group	0.0435	0.0056	0.5034	0.6498
HEAT STRESS	Control	10.842±1.230^{a}	0.674±0.041^{a}	4.625±0.872	6.262±0.347^{b}
	EE	5.173±1.329^{b}	0.544±0.032^{b}	5.142±0.932	7.572±0.296^{a}
	EE+HS	3.823±1.151^{b}	0.544±0.032^{b}	3.218±0.822	7.291±0.283^{a}
	Source of variation	Probabilities (*P* values)			
	Group	0.0016	0.0386	0.2842	0.0219

*Mean ± SE

^{a,b}Means within columns with no common superscript differ significantly *(P<0.05)*

CORT – corticosterone; MDA – malondialdehyde; SOD – superoxide dismutase

EE – environmental enrichment; EE + HS – environmental enrichment plus heat stress

However, there was a positive relationship between blood corticosterone and lipid peroxidation in broilers exposed to high environmental temperatures (Lin et al., 2004b). Corticosterone administration in feeding led to lipid peroxidation in muscle and liver (Eid et al., 2003) and therefore oxidative stress in broiler chickens (Taniguchi et al., 1999; Lv et al., 2018). As known, one of the most important indicators of lipid peroxidation is Malondialdehyde (MDA). It was determined that the effects of both ACTH and heat treatments applied on the 42nd day of the study on serum MDA concentrations were significant (P<0.05) (Table 2). Exposure of the broilers to 38 ± 1 ℃ or ACTH injection significantly increased MDA levels (respectively, 0.674 and 0.596 nmol/ml) in the control group. Acute heat stress on 42nd day did not affect MDA levels in EE and EE+HS groups. This finding supports the idea that the increase in MDA concentration may be related to the heat stress response. Because changes in MDA concentrations in response to heat stress are an important indicator for poultry resisting heat stress. In a study that broilers exposed to heat stress at an early age, MDA concentrations have been determined higher in the control group. These changes in MDA concentrations have been considered as the reaction of broilers to heat stress (Altan et al., 2000b).

It has been demonstrated that environmental stressors such as high stocking density (Ismail et al., 2014), feed restriction (Yang et al., 2010), cold (Zhao et al., 2014) and heat stress (Altan et al., 2000b) increased lipid peroxidation. In poultry, lipid peroxidation leads to a decrease in antioxidant enzyme capacity (SOD, CAT, GPx) (Altan et al., 2003). As seen in Table 2, when the effects of ACTH injection and heat stress on serum SOD levels were evaluated, there were no significant differences among groups (P>0.05). In addition, ACTH injection did not affect liver SOD concentrations in control and EE groups. However, liver SOD levels were higher in the EE group that was no adapted heat stress on 21 days of age. These increases in antioxidant enzyme activity are considered as a protective response against oxidative stress (Mates et

al., 1999). Pereira et al. (1999) reported that in Dexamethasone-treated mice, a type of glucocorticoid, decreased antioxidant enzyme activity in the liver and skeletal muscle to result in lipid peroxidation. Similarly, corticosterone administration in feeding led to lipid peroxidation in muscle and liver (Eid et al., 2003).

No studies have been carried out on both environmental enrichment and lipid peroxidation effects in poultry. In a study evaluating the effects of resveratrol and environmental enrichment on oxidative stress in young healthy rats, MDA levels were found to be significantly higher compared to control, while SOD activity did not change (Muhammad et al., 2017). Moreover, working with male and female rats, Marmol et al. (2015) reported that environmental enrichment had beneficial effects in the hippocampus; they showed that EE rats had higher values on the antioxidant measures and lower values on the oxidative stress parameters as compared with controls.

CONCLUSION

These results demonstrated that the environmental enrichment method has a great potential coping with different stressors in broilers with differing environmental provisions and supplies them better welfare. Also, it is shown that exposure of broilers to heat stress or application of experimental adrenocorticotropic hormone causes important reactions in oxidative metabolisms. Even oxidative damage could be minimized as well by antioxidant defense mechanisms, which are repairing systems that protect the cell against cellular oxidants and prevent the accumulation of oxidatively damaged molecules. Although there is some knowledge in the scientific literature, needs to be further studies to use of environmental enrichment in commercial practice and the effects on broiler's behavior and welfare.

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