Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2019 / v.21 / n.3 / 001-006

http://dx.doi.org/10.1590/1806-9061-2019-1091

Original Article

The Investigation of the Relationship Between HSP-27 Release and Oxidative DNA Damage in Broiler Chickens with Tibial Dyschondroplasia by Using Histopathological and Immunohistochemical Methods

■Author(s)

Kapakin KAT[I] https://orcid.org/0000-0002-1740-8657
Kapakin S[I] https://orcid.org/0000-0002-2482-2813
Imik H[I] https://orcid.org/0000-0001-6933-2124
Gumus R[II] https://orcid.org/0000-0002-8812-191X
Eser G[I] https://orcid.org/0000-0002-8330-3095

[I] Department of Pathology, Atatürk University, Faculty of Veterinary Medicine, Yakutiye, Erzurum, Erzurum 25240, Turkey.
[II] Faculty of Veterinary Medicine - Department of Animal Nutrition and Nutritional Disorders, Cumhuriyet University, Sivas, Turkey, Sivas 58140, Turkey.

■Mail Address

Corresponding author e-mail address
Kubra Asena Terim Kapakin
Department of Pathology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, 25100, Turkey.
Phone: 04412317222
Email: kubra.terim@atauni.edu.tr

■Keywords

HSP-27, oxidative DNA damage, tibial dyschondroplasia.

Submitted: 06/May/2019
Approved: 26/June/2019

ABSTRACT

Tibial dyschondroplasia (TD) is a skeletal disorder that occurs in the proximal metaphyses of tibiotarsus and sometimes tarsometatarsus, resulting in the development of avascularized and non-mineralized abnormal cartilage and causing significant economic loss. In this study, we aimed to show the histopathological changes and the relationship between the release of Heat-Shock Protein 27 (HSP-27) and oxidative DNA damage in broiler chickens with tibial dyschondroplasia, using histopathologic and immunohistochemical methods. Our study material consisted of totally 20 animals out of 42 days old 205 Ross 308 broiler chickens, 10 with TD lesions and 10 healthy control subjects. Tissue samples taken from animals performed necropsy was exposed to routine tissue follow-up. Macroscopically, unilateral and bilateral thickening and swelling were observed in the growth plates of tibiotarsal joints of the broiler chickens diagnosed with tibial dyscondroplasia. Histopathologic examination of the tibiotarsal joints of broiler chickens affected by TD revealed an increase in the number of immature chondrocytes, as well as deficiencies in vascularization and calcification. In the immunohistochemical study; HSP-27 and 8-OHDG release was positive in the chondrocytes located on the Proliferative Zone, Maturation Zone and Hypertrophic Zone. However, the positivity was the most profound in the PZ and MZ, while less in the HZ chondrocytes. As a result; we demonstrated by immunohistochemical methods that the increase in the HSP-27 release is parallel to the increase in 8-OHDG release in TD lesioned areas and this may be related to oxidative stress.

INTRODUCTION

Tibial dyschondroplasia (TD) is a skeletal disorder that occurs in the form of avascularized and non-mineralized abnormal cartilage growth in the proximal metaphyses of the tibiotarsus and sometimes tarsometatarsus of poultry (Dinev, 2012; Genin *et al.,* 2012; Tian *et al.,* 2013; Velada *et al.,* 2011).The disease is attributed to abnormal differentiation of chondrocytes leading to bone growth, cartilage vascularization, and mineralization (Nabi *et al,* 2016a; Shahzad *et al.,* 2014; Tian *et al.,* 2013). TD, most frequently seen more in rapidly growing poultry, is a growth plate disease, which is more common in broiler chickens and less common in turkeys and ducks. The most important cause of abnormal cartilage development has been reported to be the failure of the cartilage tissue in adapting itself to such a rate of growth due to rapid growth (Angel, 2007; Dinev, 2012; Shim *et al.,* 2012). TD is attributed to various factors such as genetics, gender, nutritional content or nutritional deficiencies, toxins, growth rate and environmental factors, although its etiology is not clearly understood (Gay *et al.,* 2007; Houshmand *et al.,* 2011).

Heat shock proteins (HSPs), also known as stress proteins, form a highly conserved family of proteins released by all prokaryotic and eukaryotic cells, protecting the organism and the cells from injury and increasing resistance. The release of these proteins is increased by the effects of various stress factors such as toxins, free radicals, temperature, infections, and nutritional inadequacy (Haslbeck & Vierling, 2015; Dökümancıoğlu *et al.,* 2018; Meher *et al.,* 2018).

Heat shock proteins are composed of six main families, such as HSP-100, HSP-90, HSP-70, HSP-60, HSP40 and small HSPs (sHsps) according to their molecular weights and functions (Meher *et al.*, 2018). Heat shock protein-27 (Hsp 27) is a member of the small HSPs, playing important roles such as cytoprotective, antiapoptotic, anti-aging, and embryogenesis (Leonardi et a.l, 2004; Rogalla *et al.*, 1999; Thompson *et al.*, 2001; Hasbeck & Vierling, 2015, Dökümancıoğlu *et al.*, 2018)

In this study, we aimed to show the histopathological changes and the relationship between the release of Heat-Shock Protein 27 (HSP-27) and oxidative DNA damage in broiler chickens with tibial dyschondroplasia, using histopathologic and immunohistochemical methods.

MATERIALS AND METHODS

Experimental animals

The experiments were performed according to the ethical conditions confirmed by the Ethics Committee of Experimental Animal Teaching and Researcher Center, Ataturk University, Erzurum, Turkey (Decision No: 25.03.2011/3/10). Our research consisted in a total of 205 42 days old Ross 308 broiler chickens, of which 20 were used for study material, being10 with TD lesions and 10 healthy control subjects. The necropsy was performed on broiler chickens sacrificed using the method of cervical dislocation. Tissue specimens taken from the tibiotarsal joints of the animals that were sacrificed by cervical dislocation were subjected to routine histopathologic and immunohistochemical examinations.

Histopathological examination

The samples were fixed in 10% formalin for 24 h. Then, the samples were decalcified in 36.8% formic acid and 6.8% sodium formate. Finally, the samples were post-fixed and embedded in paraffin. After routine procedures, sections of 5-6 µ thickness were obtained and stained routinely with haematoxylin-eosin, and examined under a light microscope (Presnell & Schreibman, 1997).

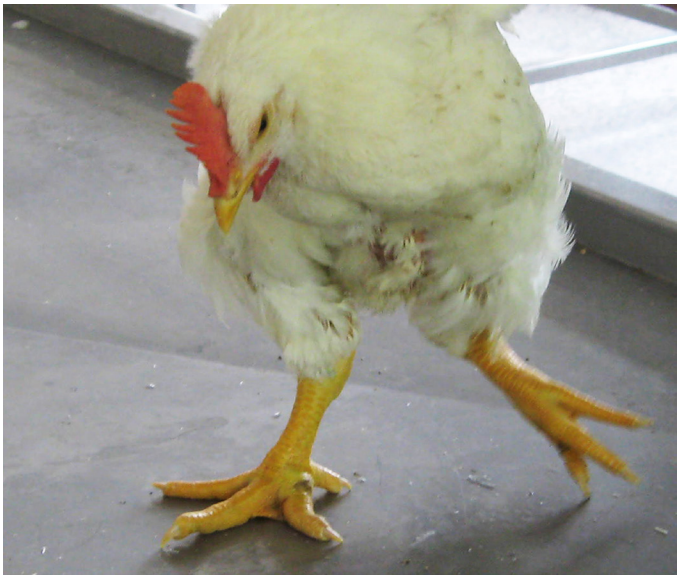


Figure 1 – Swollen of the tibiotarsal joint in TD group.

Immunohistochemical examinations

After the deparafinizing process, antigen retrieval (pH 6.0) was applied in the microwave for 15 min. Then the sections were incubated in 3% H_2O_2 for 10 min to prevent endogenous peroxidase activity. The sections washed with phosphate buffered saline (PBS) were incubated with polyclonal rabbit HSP-27 antibody (clone:ab78806, dilution 1/100; Abcam, UK) and rabbit 8-OHdG (clone:sc66036, Santa Cruz Biotecnology, dilution 1/200); at room temperature for 30 min. Sections rewashed with PBS were stained with expose mouse and rabbit specific horseradish peroxidase/ diaminobenzidine (HRP/DAB) detection IHC kit (Catalog No: ab80436, Abcam, UK) as recommen-ded by the manufacturer. 3,3-diaminobenzidine (Dako Cytomation) was used as the chromogen. Slices which were passed through alcohol xylol series following counterstaining with Mayer's hematoxylin were examined under light microscope (Dökümancıoğlu *et al.,* 2018).

RESULTS

Macroscopic findings

Macroscopically, difficulty in standing up, walking, specially lameness was observed in 10 of the–205 animals. Weight loss and hair loss were observed in these animals. Unilateral thickening in seven and bilateral thickening in eleven growth plates of the tibiotarsal joints of these animals were noticed as well as swelling (Figure 1). On the cross section, opaque cartilaginous structure was detected, varying in color from white to gray and extending from the distal end of the epiphysis plate into the metaphysis.

Histopathologic Findings

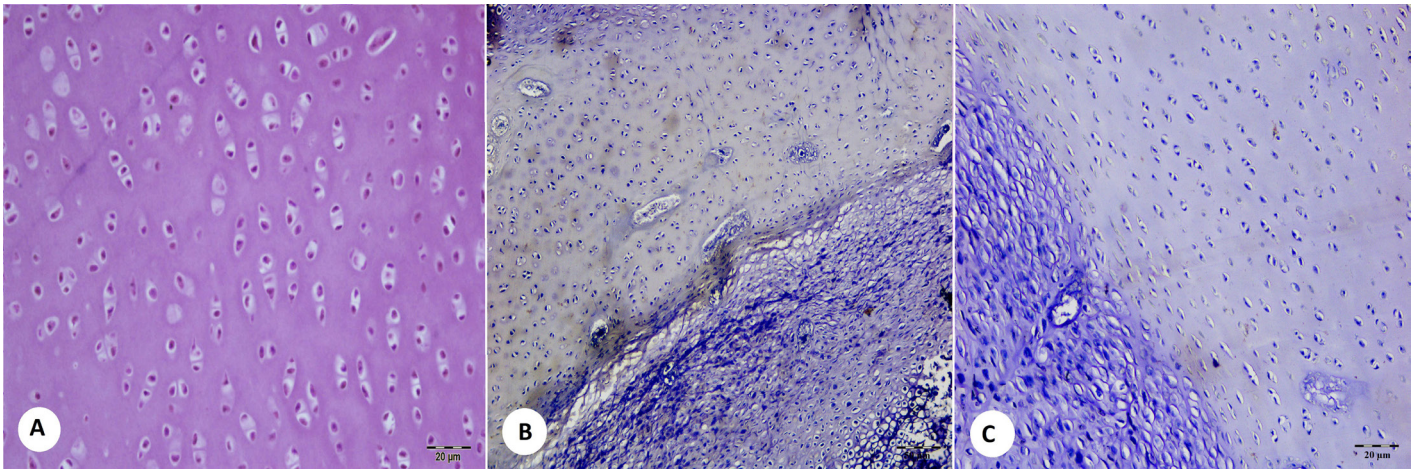
Histopathologically, no lesions were observed in the animals in the control group (Figure 2A) Histopathologic examination of the tibiotarsal joints of the broiler chickens affected by TD revealed an increase in the number of immature chondrocytes (Figures 3A, B), as well as deficiencies in vascularization and calcification. Degenerative changes were also observed in some chondrocytes and necrosis in some others.

Immunohistochemical Findings

HSP-27 release was positive in the chondrocytes located on the Proliferative Zone (PZ) Maturation Zone (MZ) and Hypertrophic Zone (HZ). However, the positivity was the most profound in the PZ and MZ, while less in the HZ chondrocytes (Figures 3C, D).The expressions of HSP27 in articular cells were weak in the Control group (Figure 2B).The expression of 8-OHdG was present in the PZ, MZ, and HZ chondrocytes (Figures 3E, F). Its expression was moderate in the MZ and PZ, and weak expression was observed in the HZ chondrocytes. Similar to HSP27 expression, in 8-OHDGexpressions in articular cells for the TD group than for the control group (Figure 2C).

DISCUSSION

Obtaining rapidly growing breeds as a result of genetic selections conducted in the production of broiler chickens led to an increase in the incidence



Figures 2A – Apperance of normal chondrocytesin control group, H&E; Bar: 20 µm B-C. Immunohistochemical localization of HSP-27and 8-OHdG in control group, Bar: 20µm.

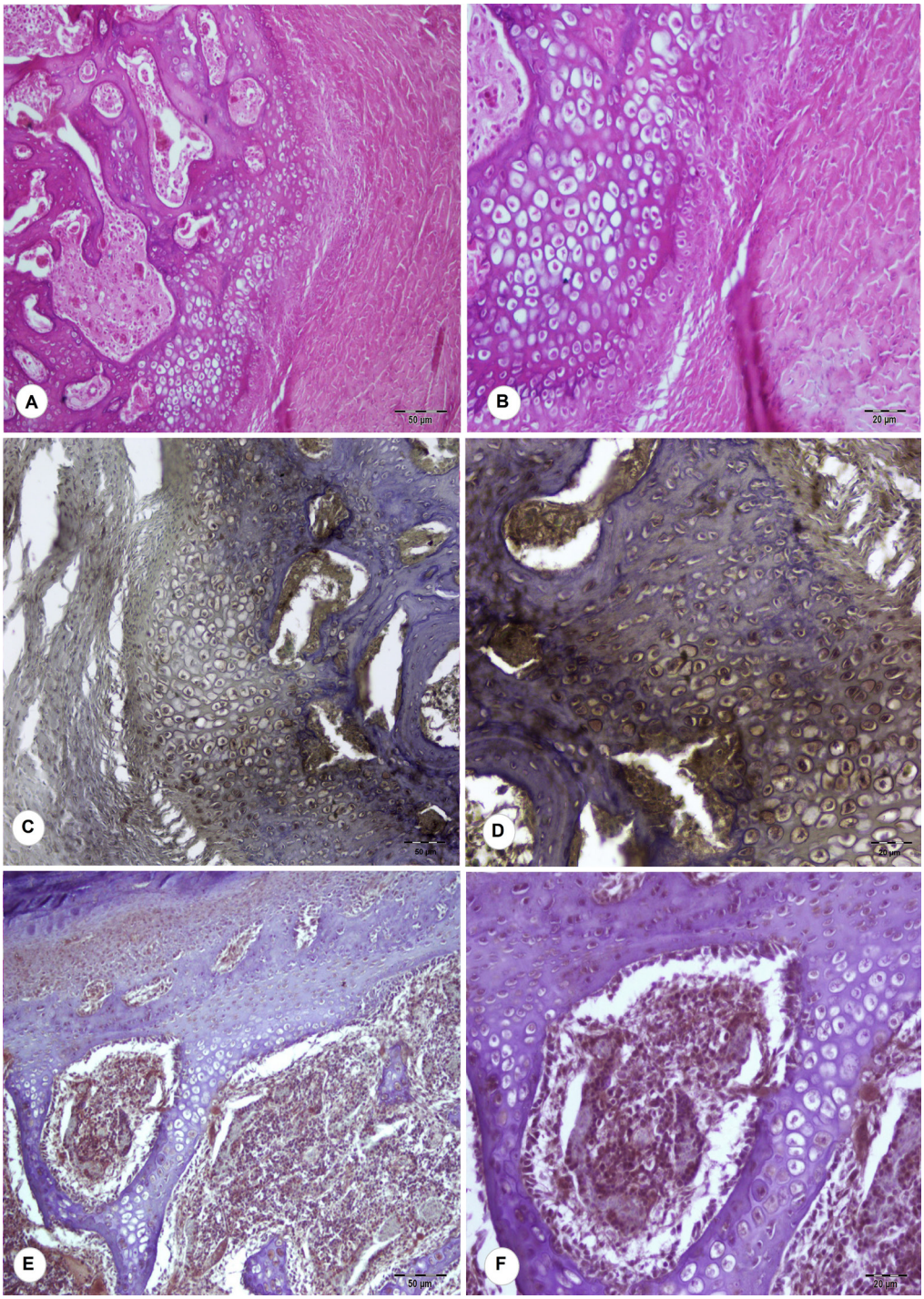
of leg problems resulted from developmental bone tissue disorders. Leg problems occur as a result of incomplete development of bone and cartilaginous tissues and primarily as a consequence of tibial dyschondroplasia. Bone formation is known to be influenced by many factors including genetic diseases, toxins, antinutritional feed, age, gender, diet, physical activity and endocrine system. Bones are composed of inorganic salts that accumulate in the organic matrix composed of collagen fibrils and glycoproteins (Angel, 2007; Gay *et al.,* 2007; Hasky-Negev *et al.,* 2008, Dinev, 2012; Shim *et al.,* 2012).

Factors such as genetics, age, gender, nutrition and flock management were reported to play an important role in the pathogenesis of TD, although its etiology is not fully known (Rath *et al.,* 2007; Houshmand *et al.,* 2011). There are studies reporting morphological, histopathological, biochemical and molecular changes observed in the tissues and cells of the animals affected by TD (Velada *et al.,* 2011; Genin *et al.,* 2008; Genin *et al.,* 2012; Imık *et al.,* 2012).

In the proximal growth plates of tibiotarsal bone in TD, lesions are usually bilateral, but occasionally they can be unilateral. Clinically, while swelling, deformity and lameness are observed in these areas at the beginning and bone fractures are formed at a later stage. For this reason, TD leads to significant economic loss worldwide, besides affecting the animal welfare negatively (Rath *et al.,* 2005; Imık *et al.,* 2012; Pelicia *et al.,* 2012).

In previous studies, thickening of the epiphyseal plate and the formation of an abnormal opaque cartilage mass extending from the distal end of the epiphyseal plate into the metaphysis was detected macroscopically (Rath *et al.,* 2005; Imık *et al.,* 2012; Pelicia *et al.,* 2012; Nabi *et al,* 2016b). In this study, clinically and macroscopically similar lesions were observed in 10 of the 205 broiler chickens.

This non-mineralized abnormal cartilage mass is devoid of blood vessels and has a soft consistency. In such cases, the most prominent histopathological lesions are the presence of hypertrophic and immature



Figures 3 A-B – Apperance of immature chondrocytesin TD group,H&E; Bar: 20µm C-D. Immunohistochemical localization of HSP-27in TD group, Bar: 20µm, 50µm E-F.Immunohistochemical localization of 8-OHdG in TD group,Bar: 20µm, 50µm.

chondrocytes in the epiphyseal plate (Rath *et al.,* 2005; Imık *et al.,* 2012). In addition, degeneration and necrosis of chondrocytes may also be seen. While extreme hypertrophy of chondrocytes increase in the number of immature chondrocytes, degeneration and necrosis of chondrocytes were observed in the tibiotarsal joint. This was accompanied by a vascularization (Rath *et al.,* 2007 ve 5; Imık *et al.,* 2012; Nabi *et al,* 2016b; Zhang *et al,* 2018). The histopathological findings observed in the broiler chickens with TD lesions in the present study were determined to be compatible with the findings of previous studies. Since the etiology of TD could not be fully clarified yet, many new studies are conducted on this issue. For this reason, the studies investigating the factors that are likely to play a role in the pathogenesis of the disease are becoming prominent recently. In these studies, Hypoxia inducible factor-1 (HIF-1α), (Genin *et al.,* 2008; Mehmood *et al.,* 2017) vascular endothelial growth factor (VEGF)(Zhang *et al.,* 2013; Mehmood *et al.,* 2018; Zhang *et al,* 2018) and HSPs (Genin *et al.,* 2012; 2018,) release were shown by several genes (Tian *et al.,* 2013, 2014), biochemical and immunohistochemical methods in poultry with TD lesions (Genin *et al.,* 2012; Rath *et al.*, 2005; Nabi *et al.,* 2016a,b; Shahzad *et al.*, 2014, 2015; Iqbal *et al.,* 2016).

Stress proteins, known to be produced by all living creatures, play important roles in all cell metabolism, including cell growth and death. The release of these proteins is increased when exposed to environmental factors such as hypoxia, reactive oxygen metabolites, heavy metals, heat, and in all conditions where cells are damaged, such as infection or tumor (Haslbeck & Vierling, 2015, Dökümancıoğlu *et al.,* 2018; Meher *et al.,* 2018).

HSPs are known to be released at a certain level in growth plates during normal bone development. HSP-27, similar to other HSPs in the group, has an important role in bone development (Leonardi *et al.,* 2004), as well as protecting and strengthening

the cell against various stress factors (Rogalla et al, 1999; Thompson *et al.*, 2001; Hasbeck & Vierling, 2015, Dökümancıoğlu *et al.,* 2018). In previous biochemical and immunohistochemical studies, HSP-90 and HSP-70 release were reported to be higher in TD-lesioned poultry than in healthy poultry. They noted that the release of HSP-90 and HSP-70 was higher in chondrocytes located on the hypertrophic zone (HZ) and the maturation zone (MZ) as compared to other zones in healthy broiler chickens. However, in broiler chickens with TD lesions, the release of these proteins was found to be higher in the proliferative zone (PZ) when compared to other zones (Genin *et al.,* 2012; Shahzad *et al.,* 2014; Iqbal *et al.,* 2016; Mehmood *et al.,* 2018).However, there was no immunohistochemical study showing HSP-27 release in broiler chickens with TD lesions in the literature reviewed. In our study, HSP-27 release was positive in the chondrocytes located on the Proliferative Zone, Maturation Zone and Hypertrophic Zone. However, the positivity was the most profound in the PZ and MZ, while less in the HZ chondrocytes.

This increase of HSP-70 and HSP-90 release in the areas with TD lesions was associated with an increase in HIF-1α in previous studies (Genin *et al.,* 2008). HIF-1α protein is an important regulator for maintaining the viability of the cells and tissues in adapting themselves to low oxygen pressure (Cheng *et al.,*2016). Under oxidative stress conditions developed as a result of hypoxia, HIF-1α protein causes an increased HSP synthesis, by binding to the promoter regions of the DNA, and thus providing transcription of the HSP gene (Huang *et al.,* 2018).

The increase in the release of HIF-1α and the release of HSP-90 and HSP-70 along with hypoxia and oxidative stress as a result of a vascularization in lesioned areas in TD in broiler chickens were shown by immunohistochemical methods in previous studies (Mehmood *et al.,* 2018).

The 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a highly sensitive biological marker that is used to detect DNA damage due to oxidative stress. Previous studies revealed a positive correlation between HIF-1α release and oxidative DNA damage in oxidative stress (Schipani *et al.,* 2001; Huang *et al.,* 2018). To the best of our knowledge, there is no immunohistochemical study showing the relationship between HSP-27 release and oxidative DNA damage in broiler chickens with TD lesions.

In this study, an increase in 8-OHdG along with an increase in HSP-27 release was remarkable in the immunohistochemical staining of TD lesions. Therefore the relationship between HSP-27 release and 8-OHdG in TD lesioned broiler chickens was demonstrated by immunohistochemical methods for the first time in the literature, in this study.

In recent years, increasing the level of HSP in order to stimulate the defense mechanisms was considered as a method in the treatment of diseases of the nervous sytem (Kampinga & Bergink, 2016), cardiovascular system (Hu *et al.*, 2017) or cancer (Chatterjee & Burns, 2017), and many studies were conducted on this topic. At the same time, there are studies currently suggesting the idea that increasing the release of HSPs to provide revascularization and reduce oxidative stress in the treatment of TD lesions would be beneficial (Huang *et al.*, 2017; Huang *et al.*, 2018, Mehmood *et al.*, 2017; Mehmood *et al.*, 2018).In accordance with the results of the present study, we agree with other researchers that HSPs proteins should not be overlooked in the development of new methods for the prevention or treatment of TD, which leads to significant economic loss regarding poultry.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This study was supported by the Coordinator of Scientific Research Projects (2012/56) at Atatürk University.

REFERENCES

Angel R. Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. The Journal of Applied Poultry Research 2007;16:138-149.

Chatterjee S, Burns TF.Targeting heat shock proteins in cancer:a promising therapeutic approach. International Journal of Molecular Sciences 2017;18:1-39.

Cheng KJ, Bao YY, Zhou SH. The role of hypoxia inducible factor in nasal inflammations. European Review for Medical and Pharmacological Sciences 2016;20:5067-5076.

Dinev I. Leg weakness pathology in broiler chickens. The Journal of Poultry Science 2012;49:63-67.

Dokumacıoglu E, Iskender H, Yenıce G, Terim Kapakin KA, Sevım C, Hayırlı A, *et al.* Effects of astaxanthin on biochemical and histopathological parameters related to oxidative stress in testes of rats on high fructose regime. Andrologia 2018;50(7):13024.

Gay CV, Gilman VR, Leach RM. Immunolocalization of vascularization factors in normal, tibial dyschondroplasia and rachitic cartilage. Avian Pathology 2007;36:445-451.

Genin O, Hasdai A, Shinder D, Pines M. Hypoxia, hypoxia-ınducible factor-1{alpha} (HIF-1{alpha}), and heat-shock proteins in tibial dyschondroplasia. Poultry Science 2008;87:1556–1564.

Genin O, Hasdai A, Shinder D, Pines M. The effect of inhibition of heat-shock proteins on thiram-induced tibial dyschondroplasia. Poultry Science 2012;91:1619-1626.

Haslbeck M, Vierling E. A first line of stress defense:Small heat shock proteins and their function in protein homeostasis. Journal Molecular Biology 2015;427:1537–1548.

Hasky-Negev M, Simsa S, Tong A, Genina O, Monsonego OE. Expression of matrix metalloproteinases during vascularization and ossification of normal and impaired avian growth plate. Journal Animals Sciences 2008;86:1306–1315.

Huang SC, Zhang L, Rehman MU, Iqbal MK, Lan Y, Mehmood K, *et al.* High altitude hypoxia as a factor that promotes tibial growth plate development in broiler chickens. PloS One 2017;12(3):e0173698

Huang S, Rehman MU, Qiu G, Luo H, Iqbal MK, Zhang H,1 *et al.* Tibial dyschondroplasia is closely related to suppression of expression of hypoxia-inducible factors 1α, 2α, and 3α in chickens. Journal Veterinary Science 2018;19:107-115.

Hu X, Van Marion DMS, Wiersma M, Zhang D, Brundel BJJM. The protective role of small heat shock proteins in cardiac diseases:key role in atrial fibrillation. Cell Stress Chaperones 2017;22:665–674.

Houshmand M, Azhar K, Zulkifli I, Bejo MH, Meimandipour A, Kamyab A. Effects of non-antibiotic feed additives on performance, tibial dyschondroplasia incidence and tibia characteristics of broilers fed low-calcium diets. Journal of Animal Physiology and Animal Nutrition 2011;95:351-358.

Iqbal MK, Liu MK, Nabi F, Rehman MU, Zhang H, Tahir AH, *et al.* Recovery of chicken growth plate by heat-shock protein 90 ınhibitors epigallocatechin-3-gallate and apigenin in thiram-ınduced tibial dyschondroplasia. Avian Diseases 2016;60:773-778.

Imik H, Terim Kapakin KA, Gümüş R, Kapakin S, Kurt A. The effect of tibial dyschondroplasia on metabolic parameters in broiler chickens. Veterinary Journal of Ankara University 2012;59:271-277.

Kampinga HH, Bergink S. Heat shock proteins as potential targets for protective strategies in neurodegeneration. Lancet Neurology 2016;15:748–759.

Leonardi R, Barbato E, Paganelli C, Lo Muzio L. Immunolocalization of heat shock protein 27 in developing jaw bones and tooth germs of human fetuses. Calcified Tissue International 2004;75:509–516.

Meher PK, Sahu TK, Gahoi S, Rao AR. ir-HSP: ımproved recognition of heat shock proteins, their families and sub-types based on g-spaced di-peptide features and support vector machine. Frontiers in Genetics2018;8:235.

Mehmood K, Zhang H, Li K, Wang L, Rehman MU, Nabi F, *et al.* Effect of tetramethylpyrazine on tibial dyschondroplasia incidence, tibial angiogenesis, performance and characteristics via HIF-1a/VEGF signaling pathway in chickens. Scientific Reports 2018;8:2495.

Mehmood K, Zhang H, Iqbal MK, Rehman MU, Shahzad M, Li K, *et al.* In Vitro effect of apigenin and danshen in tibial dyschondroplasia through inhibition of heat-shock protein 90 and vascular endothelial growth factor expressions in avian growth plate cells. Avian Disease 2017;61:372–377.

Nabi F, Li K, Shahzad M, Han ZQ, Zhang D, Liu JY, *et al.* Gambogic acid inhibits Hsp90 expressions in thiram-induced tibialdyschondroplasia. Pakistan Veterinary Journal 2016a;36:224–226.

Nabi F, Shahzad M, Liu J, Li K, Han Z, Zhang D, *et al.* Hsp90 inhibitor celastrol reinstates growth plate angiogenesis in thiram-induced tibial dyschondroplasia. Avian Pathology 2016b;45:187–193.

Pelicia K, Aparecido IM, Garcia EA, Molino AB, Santos GC, Berto DA, *et al.* Evaluation of a radiographic method to detect tibial dyschondroplasia lesions in broilers. Brazilian Journal of Poultry Science 2012;14:129-135.

Presnell J, Schreibman MP. Animal tissue techniques. 5th ed. London: The Johns Hopkins University; 1997. p.269-27,

Rath NC, Huff WE, Huff GR. Thiram-induced changes in the expression of genes relating to vascularization and tibial dyschondroplasia. Poultry Science 2007;86:2390–2395.

Rath NC, Richards MP, Huff WE, Huff GR, Balog JM. Changes in the tibial growth plates of chickens with thiram-induced dyschondroplasia. Journal of Comparative Pathology 2005;133:41-52.

Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, *et al.* Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor α by phosphorylation. Journal of Biological Chemistry 1999;274:18947-18956.

Shahzad M, Liu J, Gao J, Wang Z, Zhang D, Nabi F, *et al.* Hsp-90 inhibitor geldanamycin attenuates liver oxidative stress and toxicity in thiram-induced tibial dyschondroplasia. Pakistan Veterinary Journal 2014;34:545– 547.

Shahzad M, Liu J, Gao J, Wang Z, Zhang D, Nabi F, *et al.* Differential expression of extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) in avian tibial dyschondroplasia. Avian Pathology 2015;44:13–18.

Shim MY, Karnuah AB, Anthony NB, Pesti GM, Aggrey SE. The effects of broiler chicken growthrate on valgus, varus, and tibial dyschondroplasia. Poultry Science 2012;91:62–65.

Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS Hypoxia in cartilage:HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Development 2001;15:2865-2876.

Thompson HS, Scordilis, SP, Clarkson, PM, Lohrer WA. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. Acta Physiologica 2001;171:187-193.

Tian WX, Li JK, Qin P, Wang R, Ning GB, Qiao JG, *et al.* Screening of differentially expressed genes in the growth plate of broiler chickens with tibial dyschondroplasia by microarray analysis. BMC Genomics 2013;14:276.

Tian WX, Zhang WP, Li JK, Bi DR, Shahzad M, Gao J, *et al.* Expression of genes encoding matrilin-3 and cyclin-I during the impairment and recovery of chicken growth plate in tibial dyschondroplasia. Avian Diseases 2014;58:468–473.

Velada I, Capela-Silva F, Reis F, Pires E, Egas C, Rodrigues-Santos P, *et al.* Expression of genes encoding extracellular matrix macromolecules and metalloproteinases in avian tibial dyschondroplasia. Journal of Comparative Pathology 2011;145:174–186.

Zhang JP, Deng YF, Zhou ZL, Hou JF. Expression and identification of recombinant chicken vascular endothelial growth factor in Pichia pastoris and its role in the pathogenesis of tibial dyschondroplasia. Poultry Science 2013;92:3214.

Zhang H, Mehmood K, Li K, Rehman Mu, Jiang X, Huang S, et. al. Icariin ameliorate thiraminduced tibial dyschondroplasia via regulation of WNT4 and VEGF Expression in broiler chickens. Frontiers in Pharmacology 2018;29:123.