



The Effects of Stress Exposure on One Day Aged Broiler Chicken: A pathological Study



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Abstract

ENVIRONMENTAL stresses such as (transportation, stocking density) increase the risk of animals being exposed to infection and injury, affect the efficiency of the immune system, the health status of the animal, and the ability of animals to adapt to these changes. The current study aimed to detect TGF-B and IL-B1 expression associated with microscopic lesions in one day aged broiler chicken. Twenty-eight birds were collected from the Bashiqa district in Mosul city. Results of the current work showed the presences of macro and micro changes, the macro lesions represented by swelling, congestion and hemorrhage spots in different part of the body. In contrast the microscopic exam of the brain, liver and proventriculus were scored into mild, moderate and severe lesions which represented by disturbances of growth including (Atrophy of glandular stomach and Sloughing of proventriculus folds), disturbance of circulation represented by (edema and congestion), in addition to degeneration and necrosis cases, immunohistochemistry marker represented by TGF-B and IL-B1 were detected in birds as a biomarker proteins for pathological samples. These findings suggest that these proteins are the significant proliferative markers in broilers under stress condition and could be used as a prophylaxis for diagnosis of different disease.

Keyword: IL-B1, TGF-B, Histopath lesion, Transport.

Introduction

Consumers prefer raised chickens meats over other type of meats due to its characteristics while, developing of poultry production blindly will produce more recurrent disability like loss of weight, reduction of welfare and metamorphosis of meat [1], it's necessary to maximize broilers production to meet the rising request for proteins of animals due to raising human population in global [2], the capacity of broiler chickens to deal with environmental stress difficulties is one of the major factors in poultry production. Stress is the outcome of biological reaction to an inner or exterior stimuli that endangers an organisms normal physiological homeostasis commercial production of poultry is faced with a various of stress factor such as environmental condition, nutritional factors and inner stress factor which reduce reproduction activity and production and affects the healthy condition of birds [3 and 4]. Birds suffering from different environmental stress factor like thermal stress [5] cool stress [6], limited of feed [7], density of stocking and multiple others.

In spite of improvements in housing and management techniques to counteract the impacts of environmental stress, stressors still vigorously affects poultry output (Abo Ghanima *et al.*). Body performance, health condition and welfare of birds can all be impacted by Thermal stress, (TS), a well-known physical ecological stressor [5]. TS develops when the body's ability to dissipate heat is exceeded by the amount of heat produced by the body [8]. According to [9], HS is a significant environmental component that lowers hens growth performances. Poultry exposed to TS have a variety of physiological disruption like systemic dysregulation of immunological, endocrine problems, alkalosis of respiratory system and electrolyte imbalance [8,10] and these substantially decreased performance of growth and barrier of the digestive system [9]. by decreasing thermoregulation, heat stress can increase poultry mortality rates, another stress. Cold stress (CS) is another significant environmental stressor. When the ambient temperature falls below 18C, cold stress develops [11]. In this cases, the animals may try to warm itself, leading to tissue damage, sever

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cold-related illness, and ultimately death [12] adult birds have the ability to regulate their body temperature and generate heat through metabolism[12]. however, neonatal birds are sensitive to cold temperature and are unable to endure the extreme condition until they develop ripe organs for thermogenesis[13]. restriction of feed has been classify as one of the physiological stress in poultry[14], particularly in young birds due to their rapid growth and metabolic rate[15] feed restriction negative effects in chickens may depended on time, intensity and extent [16]. There is inclusive evidence that limitation of feeding can result in stressful of physiological reaction, red blood counts, haemoglobin level, and concentration of mean cell haemoglobin were all lower in broiler fed on a limited diet after 3 days [17]. Stocking density (SD) is a crucial environmental factor that has been linked to physiological stress in certain kinds of chicken, which has become a major reason for worry [18], SD effects on health condition, performance activity, welfare, trait of carcass and guilty of meat [19]. Immunity is a main aspect of broiler industry due to its relation with production and growth performance .pervious research have recorded that immunity activity is suppressed under thermal stress [20]. Chemokine and cytokine are important immunity marker. These marker proteins imply the regulation of immunity through hematopoietic and facilitated the homeostasis and host defenses mechanism [21]. primarily they comprise TNF, TGF and IL-S, thermal stress-leading to suppression of the poultry immunity which take place through regulation of gene, thermal stress

increases the expression of TLR1, TLR5, TN- α and IL-4[22]. The aims of this study is to punctuate the pathological lesions that affected one day aged broiler chicken

Material and Methods

Ethical approve

The supervision and ethical approval were received from the Ethics Committee for the experimental animal using under the code number UM.VET.2022.075, college of Veterinary Medicine, University of Mosul.

Sample collection

Twenty-eight Cobb birds with one-day aged were randomly collected from the poultry fields of Bashiqa district in Mosul city.

Histopathological analysis

The present study focused on clinical symptoms, posthumous and Histopathological lesion. Twenty-eight of dead birds with one-day-old aged were collected randomly and examined for any abnormal changes. Brain, liver and periventricular of all affected samples were grasped from necropsied chicks, fixed in neutral buffered formalin with 10%con, severed, and stained with routine stain (H&E) for examination under light microscope [23]. After conducting the anatomical and histological characteristics of the affected samples, the pathological lesions were classified according [24], represented by their categories and characteristic of lesion Table (1).

TABLE 1. Categories and characteristic of lesion

Categories	Lesion characteristic
Disturbances of growth	Hyperplasia, Atrophy in neuron cell & Sloughing of Proventriculus folds
Disturbances of circulation	Edema & congestion
Inflammation	Encephalitis & infiltration of inflammatory cell in brain and Proventriculus
Degeneration	Vacuolar degeneration
Necrosis	Premature death of cell and Loss of hepatocytes architectures

Immunocytochemistry

Tissue section were fixed in a clearing agent (xylene), in a chain of ethanol they were rehydrated and then washed in phosphate buffered saline (BPS).After that, H₂O₂ with 3% concentration was added to repress the activity of endogenous peroxidase for 30 minutes .For 60 minutes at 25 Celsius the section were blocked and incubated overnight at 4 Celsius with the primary antibody .The primary antibodies reaction used were transforming growth factor-beta (TGF-B) rabbit polyclonal (dilution 1:200, Minneapolis, MN,

China)and Interleukin 1 beta(IL-B) mouse monoclonal(dilution 1:50, Clone sc-7966). After washing of the section it incubated for 1 hours at 37 Celsius with poly-HRP Goat Anti-rabbit IgG (dilution 1:50, China) then the section detected with a complex of an avidin-biotin ,stained with H&E for 60 second and by a distal water they washed, dehydrated and covering by slide slipped. The histological sections were seen by electron microscope and photographed with a digital camera[25].

Result

Histopathological finding was observed in brain represented by inflammation, gliosis of cell, oedema and degenerative and necrosis changes (Figure 1 a, b, c), vacuolation and necrosis of hepatocytes, loss of architectures in addition to

congestion and Binucleated of hepatic cell were observed in (Figure 2 d, e, f). There were also atrophy of proventriculus glands, hyperplasia in addition to sloughing of the fold were observed in (Fig. 3 g, h, i). as well the stage and score of Histopathological lesions are showed in Table (2).

TABLE 2. Grade and score of Histopathological lesions

Grade and score of Histopathological findings					
	Growth disturbances	Circulation disturbances	Inflammation	Degeneration	Necrosis
Grade	Moderates & Sever	Mild	Mild	Moderates	Sever
Score	2&3	1	1	2	3

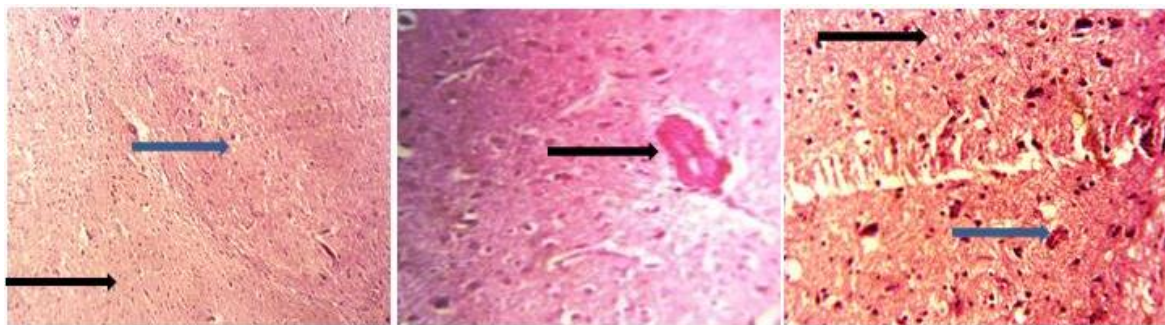


Fig. 1. Micrograph of broiler brain showed necrosis (black arrow) with inflammation (blue arrow),10X A, congestion (black arrow),40X B, hyper atrophy of neuron cell (blue arrow) and vacuolation (black arrow), 40X C .H&E.

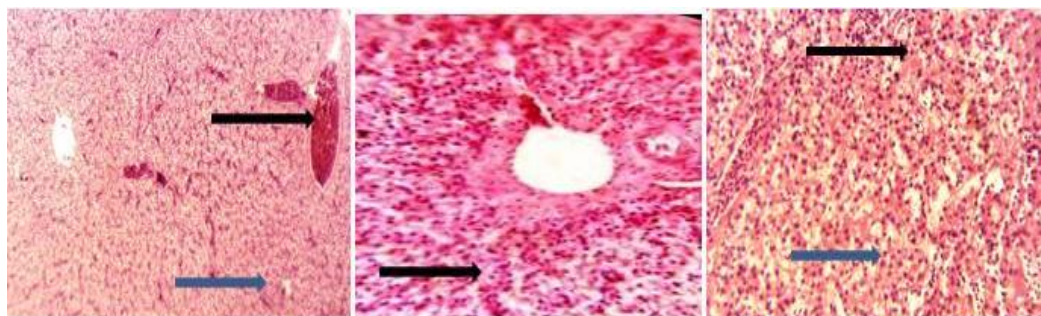


Fig. 2. Micrograph of broiler liver showed Loss of hepatocytes architectures (blue arrow) and congestion (black arrow) 10X A, infiltration of inflammatory cell (black arrow),10X B, Binucleated hepatocytes (black arrow) in addition to vacuolation (blue arrow) ,40X C .H&E.

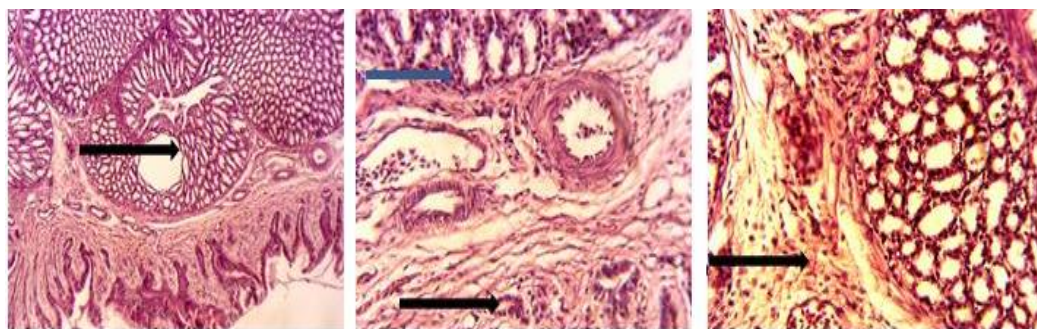


Fig. 3. Micrograph of broiler Proventriculus showed atrophy of gland (black arrow)10X A, atrophy of glands (black arrow) as well infiltration of inflammatory cell (blue arrow), 40X , C , infiltration of giant cell 40 X C .H&E.

IHC Results

Immunocytochemistry scoring analysis of TGF-B and IL-B1 proteins in affected section showed positive stained in the cytoplasm and extracellular.

For the both protein markers figure 4(g,h,i) showed sever expression while figure 5 (k,l,m) showed moderate expression for TGF-B and ILB1.

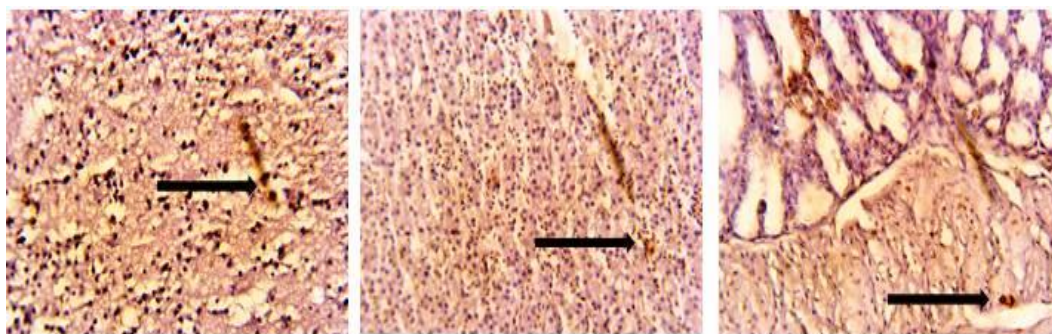


Fig. 4. Cytoplasmic expression of TGF-B in brain (g), (h)liver and Proventriculus (i), IHC,400µm.

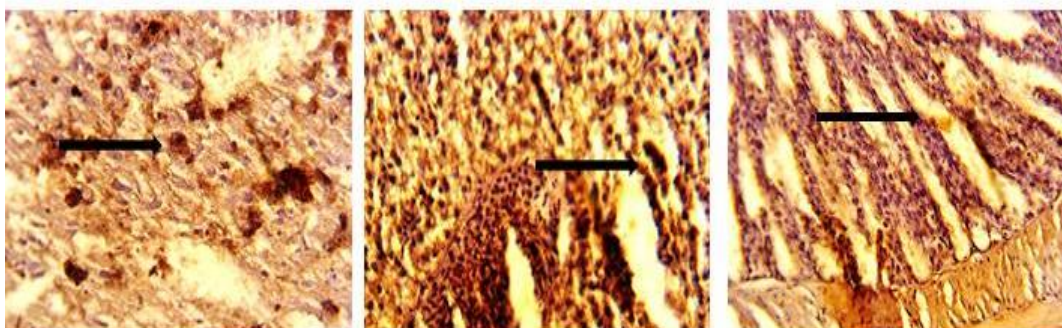


Fig. 5. Extracellular expression of IL-B1 in brain (k), liver (l) and proventriculus (m), IHC,400µm.

Discussion

Stress factors challenge the homeostatic condition of the living organisms. The response to stress includes intricate reaction to maintain invariable state. The Histopathological section of brain, liver and proventriculus showed the presence of degeneration and liquefactive necrosis of cells. The earliest reaction that can be seen before the occurrence of necrosis at the organs and tissue is a cell degeneration, degeneration and necrosis occurs due to insufficiency of oxygen (O_2) caused the failure of adenosine triphosphate (ATP) synthesis leading to sodium (Na^+) and potassium (K^+) pumps failure and loss control of cell volume [26]. Degeneration of cell is also known as vacuolar or hydropic degeneration and also called swelling of cell. Due to overabundance of water, the volume and size of cell increased leading to failure of it to maintain proper homeostasis. Usually degeneration of cell involve cellular membranes damage, deficiency in the production of cellular energy or enzymes injury regulation of ion channels membranes [27]. According to [28], when stress factors such as heat, overcrowding and transport occurs on chicken it will finally reduce the intake of oxygen and these associated with hypothalamic

hypoxia, pathways of oxygen and abnormalities in the tissue of lung. Environmental pollutants like ammonia (NH_3), copper (Cu), hydrogen sulfide (H_2S) and Arsenic (As) can cause oxidative stress when gulped by birds [29]. These pollutants material causing inhibition in the activity of antioxidant enzyme like, SOD, GPX and CAT, inducing oxidative stress through increasing levels of MDA and H_2O_2 which results in inflammatory reaction injury and infiltration of inflammatory cell mediated by signalling pathway [30] enhancing the production of reactive oxygen species (ROS) that impairing and damage the respiratory and immune system in broiler [31 & 32]. There were generalized oedema and congestion in the liver and brain. Environmental stress factors challenge the live organism homeostatic state, Binucleated of hepatocytes occurs as results of cell injury. After that regeneration. Therefore, in order to maintain a steady state, the stress response involves. increase rate of heart and increased blood flow to liver and brain [34] brain and Proventriculus Histopathological features showed the presence of atrophy, hyperplasia and sloughing of epithelial cell and these belong to present of irritation causes, inflammatory factor, bacterial, parasitic and even fungal infection [26]. The more powerful

fibrogenic cytokine involved in the causes of fibrotic diseases is TGF- β , these markers transmit signals via its trans membrane receptors of serine, threonine (Type 1 and 2 receptors). These receptors –regulated Smad2 and Smad3 are then phosphorylated and translocate into the nucleus where they bind to DNA and control the transcription of inflammation gene [34]. Heat shock protein are a type of proteins that produced from a cell in responses to different type of stress factor, TGF- β and IL-1 can regulate HSP70 and HSP 90 expression these refers to the role of these cytokines in regulatory of body immunity. Heat shock proteins is later known to stimulate the production of other types of inflammatory cytokines or pro-inflammatory cytokine [35].

Conclusion

Ecological stress like transport and density has a negative impacts health broilers performance and these can causes increasing in secretion of stress hormone which adversely affects performance growth and leading to mortality effective conducted techniques are key to increasing healthy condition and profit boosting to enhance chickens adaptive under stress, proteins marker expression (IL-1 and TGF- β) increasing during stress circumstances.

Authors contributions

All authors participated equally in preparing this work.

Interest Conflict

No conflict of interest about this work

Acknowledgment

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تأثير التعرض للاجهاد على دجاج التسمين بعمر يوم واحد: دراسة مرضية

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الخلاصة

الضغوط البيئية مثل (النقل والكثافة) تزيد من خطر تعرض الحيوانات للعدوى والاصابة ,وتؤثر على كفاءة الجهاز المناعي ,الحالة الصحية للحيوان وعلى قدرة الحيوانات للتكيف مع هذه التغيرات .تهدف الدراسة الحالية الى تحديد التعبير البروتيني لل TGF-B و IL-B1 وعلاقتها بالآفات النسيجية المرضية بدجاج التسمين بعمر يوم واحد ,تم جمع ثمانية وعشرين حالة مصابة من منطقة بعشيق بمدينة الموصل ,واظهرت النتائج وجود التغيرات العينية والمجهرية تمثلت التغيرات العينية بالانتفاخ والاحتقان مع وجود بقع دموية في اماكن مختلفة من الجسم وبالمقابل كانت الآفات النسيجية للدماغ ,الكبد والمعدة الحقيقية مصنفة الى خفيفة ,متوسطة وشديدة للتعبير البروتيني وتمثلت الآفات النسيجية باضطرابات النمو(ضمور المعدة الغدية وانسلاخات المعدة الحقيقية) واضطرابات الدوران (الوذمة والاحتقان)بالإضافة الى التنكس والنخر بروتينات الكيمياء المناعية النسيجية لل TGF-B و IL-B1 اعطت اشارات موجبة للآفات المرضية لعينات الطيور .تشير هذه النتائج الى ان هذه البروتينات هي علامات تكاثيرية مهمة في دجاج التسمين تحت ظروف الاجهاد ويمكن استخدامها كالمنبئ لتشخيص امراض مختلفة .

الكلمات المفتاحية: TGF-B , IL-B1 , التغيرات المرضية ، النقل.