SHORT COMMUNICATION

Pathology of heart, lung, liver and kidney in broilers under chronic heat stress

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Abstract

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The purpose of this experiment was to investigate pathological changes in broilers under chronic heat stress. Fifteen birds (n = 15), twenty-eight days old were kept at 33 ± 1°C environmental temperature for 21 days. While broilers were maintained in environmental temperature at 33 ± 1 °C, body temperature, respiratory rate and urine excretion rose. Macroscopic and microscopic lesions of heart, lung, liver and kidney were examined. Right atrium hypertrophy with excessive blood accumulation, heart enlargement and right ventricular hypertrophy were observed in 12 out of 15 broilers (80%). Congestion, edema and hyperemia in lung were present in all broilers. Yellow and pale livers were observed in 4 out of 15 broilers (26.67%). Kidneys were highly affected i.e. generalized edema and hemorrhage in subrenal capsule. Under microscopic examination of heart, massive myofibrillar degeneration with hemorrhage, general fatty degeneration /or/ vacuolation of myofibers and diffused myocarditis containing organisms were found in some areas. The principal histopathologic lesions in the lung were related to vein and arteriole massive congestion. Massive hemorrhage was largely observed in parabronchus and alveoli of all broilers. Liver cells showed "fatty degeneration" with dilation of sinusoid of all broilers. Besides, necrosis with heterophils and lymphocytes was observed in some parts of the liver, especially in the centritubular region. In the

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kidney, leukocytes such as heterophils accumulated in many inflammatory areas. Fatty degeneration was observed in renal tubular epithelia of all broilers. Glomeruli were damaged. A space between renal papillae increased and accumulated water. Moreover, ureters in 9 out of 15 (60%) birds showed sac-like expansion "ureteral pseudobladder". These sacs were filled with urine and similar to bladder in mammals.

Key words : pathology, heart, lung liver, kidney, chronic heat stress, broiler

บทคัดย่อ

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พยาธิวิทยาของหัวใจ ปอด ตับ และไตในไก่เนื้อเมื่ออยู่ในภาวะเครียดเนื่องจากความร้อนเป็นเวลานาน

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วัตถุประสงค์ของการศึกษานี้คือเพื่อตรวจการเปลี่ยนแปลงทางพยาธิสรีรวิทยา ในไก่เนื้อเมื่ออยู่ในภาวะเครียดเนื่องจากความร้อนเป็นเวลานาน ไก่เนื้ออายุ 28 วัน จำนวน 15 ตัว ถูกเลี้ยงที่อุณหภูมิ 33±1 °C เป็นเวลา 21 วัน ขณะที่ไก่อยู่ที่อุณหภูมิ 33±1 °C อุณหภูมิร่างกาย อัตราการหายใจ และการขับถ่ายปัสสาวะของไก่เพิ่มขึ้น เมื่อทำการตรวจพยาธิสภาพของอวัยวะภายในทั้งระดับที่มองเห็นด้วยตาเปล่าและตรวจภายใต้กล้องจุลทรรศน์พบว่าไก่เนื้อจำนวน 12 ตัวจาก 15 ตัว (80%) มีหัวใจห้องบนขวาขยายใหญ่พร้อมกับมีเลือดคั่งจำนวนมากและหัวใจห้องล่างขวาขยายใหญ่ ปอดของไก่ทุกตัวมีสีแดงเนื่องจากเกิดเลือดคั่งและบวมน้ำ ไก่ 4 ตัวจาก 15 ตัว (26.67%) ตับมีสีเหลืองและซีด ไตของไก่เป็นอวัยวะที่ได้รับผลกระทบสูงมาก โดยพบว่าไตของไก่มีการบวมน้ำโดยทั่วไป มีเลือดออกในชั้นใต้เยื่อหุ้มไต เมื่อตรวจพยาธิสภาพของเนื้อเยื่อภายใต้กล้องจุลทรรศน์พบว่า เซลล์กล้ามเนื้อหัวใจเกิดการเสื่อมอย่างรุนแรงพร้อมกับมีเลือดออกและมีการเสื่อมแบบสะสมไขมันหรือมี vacuole ภายในเส้นไยของกล้ามเนื้อ กล้ามเนื้อหัวใจมีการอักเสบพร้อมกับมีเลือดออกและพบเม็ดเลือดขาวบางบริเวณที่ปอดจะพบเส้นเลือดแดงและดำมีการคั่งเลือดอย่างรุนแรง มีเลือดออกปริมาณมากทั้งใน parabronchus และ alveoli ตับของไก่จะพบ fatty degeneration และช่องว่างระหว่างเซลล์ของตับ (sinusoid) มีช่องว่างเพิ่มมากขึ้นในไก่ทุกตัว นอกจากนี้ยังพบกลุ่มเนื้อตายพร้อมกับมีเม็ดเลือดขาวชนิดเฮทเทอโรฟิลและลิมโฟไซต์มาสะสมบางส่วนในตับโดยเฉพาะบริเวณ centrilobular region เนื้อเยื่อไตจะพบการอักเสบและมีเม็ดเลือดขาวชนิดเฮทเทอโรฟิลมาสะสมหลายบริเวณ เซลล์เยื่อบุท่อไตมีการเสื่อมแบบมีการสะสมไขมัน glomeruli ของไตมีความเสียหาย ช่องว่างบริเวณ renal papillae มีการขยายเพิ่มขึ้นและมีน้ำมาสะสม และไก่เนื้อ 9 ตัวจาก 15 (60%) ตัวมีถุงขยายใหญ่ที่เรียกว่า uretheral pseudobladder ซึ่งถุงนี้จะมีน้ำปัสสาวะอยู่เต็มคล้ายกับกระเพาะปัสสาวะในสัตว์เลี้ยงลูกด้วยน้ำนม

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Like all other warm-blooded animals, poultry must regulate their body temperature within a fine limit. Normally, the body temperature of an adult chicken is 41 - 42 °C. There is a diurnal temperature variation of about 1.5 °C depending on the activity of birds (Reddy, 2000). Optimal temperature for efficient production ranges from 18 – 21 °C. When ambient temperature rises, the thermal equation (heat produced – heat loss) shifts to the left side and body temperature tends to rise. Under heat stress condition, the heat loss from the body is less than heat gained, the body temperature tends to rise (Mehta and Shingari, 1999). During heat stress, catecholamines are released from the

adrenal medulla (Gregory, 1998) due to the effect of accelerated heart rate (Ewing *et al*., 1999); moreover this condition stimulated parabrachial nucleus causing increase respiration (Gregory, 1998). Packed cell volumes (PCV) and hemoglobin concentration (Hb) decreased in broilers under chronic heat stress (Teeter *et al*., 1992, Deaton *et al*., 1996, Yahav *et al*., 1997, Zhou *et al*., 1998, Borges *et al*., 1999, Furlan *et al*., 1999, Altan *et al*., 2000) and might cause hypoxia.

Generally, oxygen deficiency is one of the most common causes of tissue injury when combined with increased body temperature and hypoxia becomes a potent cause of death. Normally, the function of cardiac muscle is to extract oxygen from blood efficiently, the failure of which causes the lack of oxygen in the heart muscle and results in hypoxic damage and finally heart failure. Mammalian skeletal muscle uses about 40% of oxygen in the blood that circulates through the muscle. In contrast, cardiac muscle uses nearly 100% of the oxygen from the blood circulating through the myocardial capillaries. This high oxygen demand makes myocardium susceptible to systemic hypoxia. Besides, necrosis from oxygen deficit develops in centrolobular areas of the hypoxic liver. In mammal, ischemic hepatic necrosis occurs in cardiac failure, severe anemia, and shock with prolonged low circulatory rates. Moreover, the renal cortex is highly sensitive to hypoxia, especially the proximal convoluted tubules, which are often affected in ischemic injury (Cheville, 1999).

At present, information about pathology of broilers in response to heat stress has not been reported. Therefore, the objective of this experiment was to study the effect of chronic heat stress on pathogenic lesions of heart, lung, liver and kidney of broilers. This pathogenesis may explain the phenomenon of body changes and cause of death under this condition.

Materials and Methods

Thirty-six infectious disease-free broilers were obtained from a commercial hatchery. They were brooded for 21 days before being placed in layer cages. Experiments began 7 days after adaptation. Chicks were fed on standard broiler starter in continuous light and water supply.

At 28 days of age (7 days after adaptation), they were transferred into an environmentally controlled house under heat stress (33 $\pm$ 1 ^{o}C) and kept (two birds/ cage) in layer cages with wire floor. All broilers were subjected to a 5-h episode of heat stress at 33$\pm$1^{o}C environmental temperature each day. Relative humidity was 60-70%. The total mixed diet (Table 1) was fed *ad libitum* with continuous light and water supply. Body temperature and respiratory rate and behavior were investigated and observed. On day 49 of the experimental period (21 days after heat exposure), fifteen birds were killed by cervical dislocation. Gross lesions of visceral organs of broilers were examined. Lung, liver, kidney and heart of each

Table 1. Total mixed of feed ration for growing broilers in experiment.

Ingredients	Percentages of mixed
1. Corn#2	62.00
2. Fish meal 58%	10.00
3. Soy bean meal china 44%	23.00
4. Rice bran oil	2.96
5. Premix*	0.5
6. Alimethionine	0.25
7. L – Lysine	0.14
8. Limestone	0.50
9. Salt	0.20
10. DCP (Rock 16%)	0.30
11. DCP (Rock 18%)	0.15
Total	**100.00**

*** Vitamin AD$_3$E 500/1000 (400mg); Vitamin E $_{50}$(2,000 mg); Vitamin B$_1$ (180 mg); VitaminB$_2$ (100 mg); VitaminB$_6$ (310 mg); VitaminB$_{12}$ 1% (120 mg); Vitamin K$_3$ 51% (100 mg); Niacin, B$_5$ (2, 700 mg); D – calciumpentothinate (1,000 mg); Folic acid (50 mg); Biotin, 2%(750 mg); Chlorine chloride 50%(20, 000 mg); Magnesium sulphate (19,600 mg); Potassium iodide, KI (44 mg); Cobalt chloride (35 mg); Zinc Oxide, ZnO (1,980 mg); Copper sulphate, Cu $^{2+}$. 5 H$_2$O (210 mg); ferrous sulphate, Fe. 7H$_2$O (40,600 mg); Selenium (150 mg); Dicalcium phosphate (406.37g).**

bird were taken from necropsied birds, fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination (Luna, 1968).

Results and Discussion

When broilers were maintained in an environmental temperature at 33 ± 1 °C, body temperature, respiratory rate and urine excretion of broilers rose. The following changes were observed in the broilers exposed to 21 days heat stress (33 ± 1 °C).

Heart: Right atrium hypertrophy with large blood accumulation (Figure 1A.), heart enlargement and right ventricular hypertrophy (Figure 1B.) were observed in 12 out of 15 broilers (80%). Besides, under light microscope examination; massive myofibrillar degeneration with hemorrhage (Figure 1C.), fatty degeneration and/or vacuolation of myofibers (Figure 1D.), and in some cases generalized and diffuse myocarditis containing organisms were observed.

Lung: Congestion, edema and hyperemia of lung were observed in all broilers. (Figure 2A.). The principal histopathologic lesions in the lung were related to veins and massive congestion of veins and arterioles (Figure 2B.). Massive hemorrhage was mainly observed in parabronchus and alveoli (Figure 2B.).

Liver: Yellow and pale livers in 4 out of 15 broilers (26.67%) were observed (Figure 3A.). Examination under light microscope revealed most liver cells of all broilers showing fatty degeneration by vacuolation with dilation of sinusoids (Figure 3B.). Besides, necrosis with leukocyte granulation tissue was observed in some parts of the liver, especially in the centritubular region (Figure 3C.).

Kidney: There were generalized edema and hemorrhage in the kidney, especially in renal papillae, renal tubular and subrenal capsule (Figure 4B and 4D). Heterophils accumulated in many inflammatory areas in the kidney (Figure 4E). Fatty degeneration could be observed in all of the renal tubular epithelia. Glomeruli were damaged (Figure 4F). A space in renal tubular increased with water accumulation, which might cause renal edema (Figure 4C). Moreover, ureters of 9 in 15 broilers (60%) showed like sac expansion or "ureteral pseudobladder". These sacs were filled with urine and similar to the bladder of mammals.

Stress challenges the homeostatic state of the organism. Thus the stress response includes complex responses to maintain a steady state. Prime examples of such response were increased heart rate and increased blood flow to muscle, brain, and heart (Ewing *et al*., 1999). This might cause congestion in kidney, liver and lung. Effects of heat stroke on the gross lesions were dominated by severe and generalized hyperemia, which was most severe in the respiratory tract, especially in the lung, tracheal and bronchial mucosae. Lungs may also be edematous and occasionally contain focal consolidations of bronchopneumonia. Other organs, such as heart, kidney, may also be severely congested. The microscopic lesions are compatible with those seen grossly. The vasculatures of the lung are severely engorged and edema are evident in alveoli. Centritubular necrosis, dissociation of hepatocytes and congestion are often found in the liver. Subendocardium and subepicardial hemorrhages are found in the heart. Besides, capillary congestion is evident in kidney and other structures (Smith *et al*., 1972). When body temperature rose above normal range, the parenchyma of many cells usually begins to be damaged (Guyton, 1966). So, increasing core body temperature during heat stress caused endothelial cell damage. Besides, high blood pressure might result from an autonomic nervous system response, which may cause circulatory rupture then hemorrhage in various organs such as lung, kidney, liver and heart. Fatty degeneration is the accumulation of neutral lipids in the cytoplasm. This is a diagnostic clue for liver injury. Excess lipids in hepatocytes indicate that sublethal injury has occurred. However, the swollen, yellow, greasy appearance of fatty degeneration is characteristic of liver and less common in kidney and heart. On microscopic examination, lipid accumulation causes cells to be enlarged, pale and lacy, especially in centrolobular zones – areas

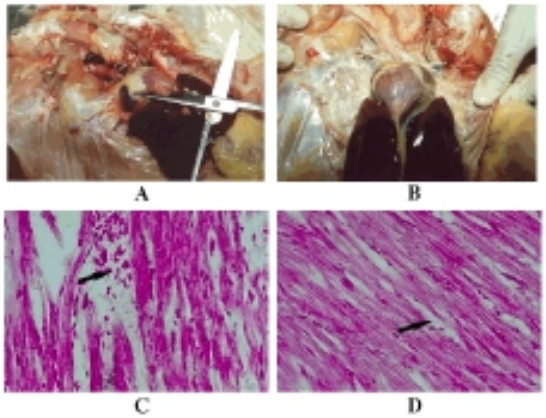

Figure 1. Gross and microscopic lesions of heart in broilers under chronic heat stress.

A. right atrium enlargement and blood accumulation, B. heart enlargement and right ventricular hypertrophy, C. cardiac myofibrilla degeneration with hemorrhage, D. fatty degeneration in cardiac muscle cells. (H&E staining, 400X)

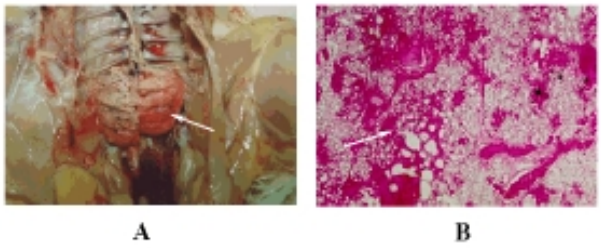

Figure 2. Gross and microscopic lesions of lung in broilers under chronic heat stress.

A. hyperemia, edema and hemorrhage of lung, B. congestion and hemorrhage of parabronchus and alveoli. (H&E staining, 200X)

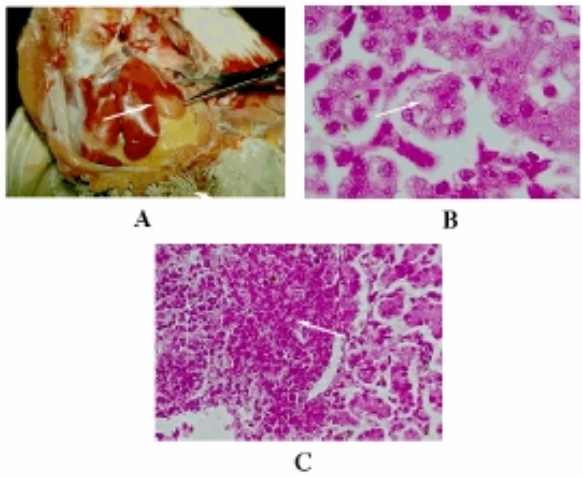

Figure 3. Gross and microscopic lesions of liver in broilers under chronic heat stress.

A. partial yellow and pale liver. B. fatty degeneration of liver cells. C. necrosis of liver with leukocyte granulation. (H&E staining, 400X)

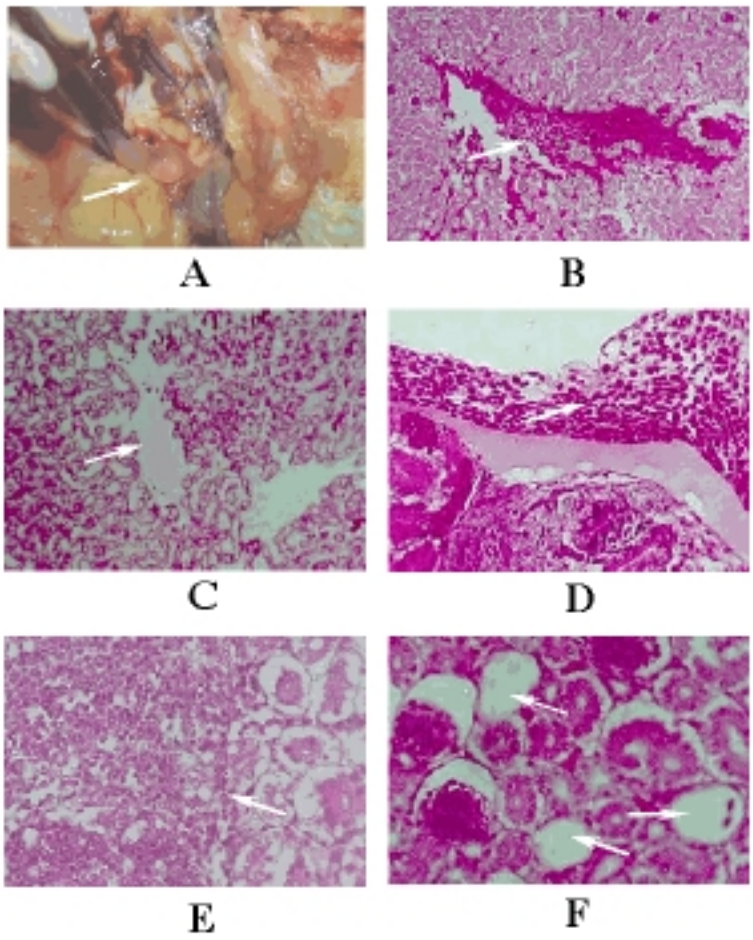

Figure 4. Gross and microscopic lesions of kidney in broilers under chronic heat stress.

A. ureter extension with water accumulation. B. hemorrhage in renal papilla. C. water accumulation in renal papilla, renal edema. D. subcapsular hemorrhage of kidney. E. fatty degeneration of renal epithelial cells and renal necrosis with leukocyte granulation tissue. F. gromerular damage. (H&E staining, 400X)

of liver where hepatocytes are most susceptible to oxygen deficiency (Cheville, 1999), whereas, in this study, fatty degeneration could be mainly found in both cardiac muscle and renal tubular epithelial cell of the broilers. This might be caused by body temperature of the broilers after exposal to high environmental temperature. So many cells of these organs were largely damaged and developed cells with fat globules and/or fatty degeneration.

In mammals, heat stroke might be associated with degenerative changes in the renal tubules. The early signs are hyperpnea (abnormal increase in rate and depth of respiration), tachycardia (rapid heart action). Several days after heat stroke occurs, there is often evidence of renal failure caused by degeneration and necrosis of renal tubules (Cheville, 1999). These changes are similar to the effects of heat stress on renal tubule in broilers in this study. Especially, fatty degeneraration could be found in every cell of the renal tubules. Besides leukocyte cells accumulated in many inflammatory areas and glomeruli were destroyed. This indicates that broilers living in a prolonged high temperature condition develop nephropathy.

Predisposing cause of ascites in chickens were breeds, feed efficiency, cool, heat, diet and

especially oxygen requirement (Richard, 1998). In birds under heat stress, hydropericardium and dilation of right atrium and ventricle were observed. The right side of the heart and vena cava were markedly distended because of filling with blood. The left side of the heart also showed some degree of dilation in cases of markedly dilated right side of the heart. Cardiac histologic lesions included myocardial degeneration. In the chickens with ascites, lesions of both hepatocytic degeneration and/or necrosis were observed and the cause of death of chickens in this condition was heart failure (Nakamura *et al*., 1999). These showed that gross and microscopic lesions of broilers under hypoxia and broilers that have been maintained at high environmental temperature were similar, therefore heat stress might cause ascites in chickens. The increasing respiratory rate caused by stimulation of parabrachial nuclei (Gregory, 1998) may cause lung edema and hemorrhage, because the broiler attempted to accelerate evaporative heat loss by vapor convection. When blood was largely transferred to the lung by increased heart rate, the lung was saturated by this fluid, followed by the increasing backpressure from the pulmonary arteries and right ventricle chamber, and caused right ventricular hypertrophy, right atrium enlargement and other tissue hypoxia.

At 35 ^{o}C, the broilers panted with high respiratory frequency. The ratio of water to feed intake increased up to 9: 1. The excreta had the consistency of soup, and the dry matter content dropped as low as 12%. Besides, the increased water content of the excreta during heat stress was due to an increased flow of urine. Not only did the broilers drink more to supply the respiratory tract with water for panting, but they also produced much more urine. During heat stress, urine entering the cloaca is not absorbed, as happens at normal ambient temperature (Hoppe, 1999). When studying the gross lesions in broilers under chronic heat stress, their ureter showed a sac- like extension filled with urine, - a "ureter pseudobladder". The extension of ureter may assist the broilers to reduce heat from the body by using water convection, because, after they were exposed to high ambient temperature, the urine was frequently voided. These findings have not been reported before.

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