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Heat Tolerant Ability of Thai Indigenous, Crossbred Thai Indigenous and Broiler Chickens under Chronic Heat Stress by Using Histopathological Indications

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Abstract: The objective of this experiment was to compare the ability to tolerate heat between Thai Indigenous (TIC), crossbred Thai Indigenous (TICC) and commercial Broiler Chickens (BC). A Split-plot completely randomized design was used. Twenty four TIC, 24 TICC and 24 BC (12 males; 12 females), each 1 kg of weight and infectious disease-free, were obtained from a commercial farm near Mahasarakham University. The 3 breeds were maintained at 26±2°C (continuous temperature) and 38±2°C (cyclic temperature) for 28 days. Their pathological changes were observed. Lesion scores, according to pathological changes were determined under microscopic examination and compared between breeds. The results revealed the following information: generalized edema and hemorrhage in the kidney, especially in the renal pelvis and renal tubular epithelia was observed. A space in the renal tubular increased with water accumulation, which might cause renal edema. The glomeruli were damaged. Most of the liver cells in all chickens showed fatty degeneration by vacuolation with dilation of sinusoids. Lesions in the lung were related to the veins and massive congestion of the veins and arterioles was noted. Massive hemorrhage was mainly observed in the parabronchus, alveolar ducts and the alveolar sac. In the cardiac muscle, massive myofibrillar degeneration with haemorrhage and in some cases generalized and diffuse myocarditis containing organisms, was observed. When broiler chickens were maintained at 38±2°C, the severity of pathologic lesions was higher than in the crossbred Thai ingenuous and the Thai ingenuous chicken ($p<0.05$).

Key words: Pathology, kidney, liver, lung, heart, Thai indigenous chicken, Thai ingenuous chicken crossbreds, broilers, chronic heat stress

INTRODUCTION

The body temperature of domestic chickens is maintained within a relatively narrow range that is usually reflected by the upper and lower limits of a circadian rhythm. In well-fed chickens that are neither dissipating heat to the environment nor gaining heat from the environment, the upper limit of the circadian rhythm is usually about 41.5°C and the lower limit is about 40.5°C (Daghir, 1995). A comfortable temperature range for chickens is between 21-26°C (Ewing *et al*., 1999). After chickens are exposed to high ambient temperatures, their body temperature rises to higher than their normal body temperature. They attempt to reduce their body temperature through radiation, conduction, convection and evaporative cooling (Whittow, 2000). This is the definition of heat stress in birds (Aengwanich and Simaraks, 2002). Heat stress in chickens is similar to heat stroke in mammals. Heat stroke may result from exposure to high ambient temperatures or from the inability to dissipate the metabolically generated heat (Cotran *et al*., 1999).

Heat stress is considered to be one of the most important variables affecting feed intake, body weight gain, mortality rate of broilers (Daghir, 1995) and the reduced profitability of poultry meat production, particularly in hot climates (Deeb and Cahaner, 2001). Aengwanich *et al*. (2003a, b) found that when broilers are under heat stress the kidney, liver, lung and heart become damaged. If chickens cannot reduce their body temperature and it rises to 46-47°C, they will die (Whittow, 2000).

Three breeds of chickens were investigated in this study. The first, Broilers (BC), are considered commercial livestock in Thailand. Broilers in Thailand are under the threat of heat stress because summer ambient temperatures in the country frequently rise to between 36-40°C. The 2nd breed, Thai Indigenous Chickens (TIC)

are wild birds that have been domesticated in rural villages in Thailand over a long period of time. They have become familiar with the high environmental temperatures; however, they have a lower productive performance than broilers. To improve the TIC's production performance, breeders have crossbred them with chickens imported from overseas resulting in the crossbreed called Thai Indigenous Chicken Crossbreed (TICC), the third breed in the study. The TICC is a crossbreed between ½ TIC (cock) and ¼ Rhode Island Red and ¼ Plymouth Rock (hen). Knowledge about pathological changes and heat tolerance between TIC, TICC and Broiler (BC) is limited. As such, the purpose of this experiment was to compare the tolerance to high temperature between TIC, TICC and BC by assessing the damage to internal organs and the lesion scores of these chickens under chronic heat stress. Results from this preliminary study, would provide fundamental knowledge for improving poultry production by identifying a heat tolerant genetic resource for poultry production in tropical regions.

MATERIALS AND METHODS

Twenty four TIC (12 males; 12 females), 84 days old, 24 TICC (12 males; 12 females), 70 days old and 24 commercial BC (12 males; 12 females), 28 days old, 1 kg of weight and infectious disease-free were obtained from a commercial farm near Mahasarakham university. The experiments were begun after a 7 day adaptation period. The chicks were fed a standard ration *ad libitum* with continuous light and water supply. The experimental design was a split-plot design in completely randomized design. The main plot was at two temperatures i.e., 26±2°C (continuous temperature) and 38±2°C (cyclic temperature; 26±2°C-38±2°C-26±2°C; chickens were maintained at 38±2°C for 8 h/day). The ambient temperature of 26+2°C is a comfortable temperature for commercial BC and is commonly used during the raising process. The cyclic temperature represents a normal temperature range during the summer season in Thailand. Sub plot was 2×3 factorial i.e., sex (male and female) and breed of chickens (TIC, TICC and commercial BC). Six TIC, 6 TICC and 6 commercial BC were maintained at each environmental temperature. Relative humidity was controlled at 65%. On day 28 of the experimental period, chickens were killed by cervical dislocation. The lung, liver, kidney and heart of each chicken were collected and fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned at 5 μm and stained with Hematoxylin and Eosin (Luna, 1968). Pathological changes to the internal organs of the TIC, TICC and commercial BC were observed. The stained tissues were scored according to the pathological changes of the lesions by using the guideline of Aengwanich *et al.* (2003b) (Table 1) under microscopic examination. All data were analyzed by using the ANOVA procedure. Means were separated by Duncan's multiple range tests. The level of significance was determined at $p<0.05$.

Table 1: Lesion scores of heat stressed broilers which reported by Aengwanich *et al.* (2003b)

Lesion scores of visceral organs of heat stressed broiler	Scores	Pathological changes
Kidney lesion score	0	Normal finding
	1	Mild hemorrhage between renal tubules
	2	Slightly moderate hemorrhage between renal tubules
	3	Moderate hemorrhage between renal tubules
	4	Severe hemorrhage between renal tubules and in renal papillae
Liver lesion score	0	Normal finding
	1	Mild accumulation of fat globules in liver cells
	2	Slightly moderate accumulation of fat globules in liver cell
	3	Moderate accumulation of fat globules in liver cell
	4	Liver cell degeneration, or pyknotic nucleus and cell death
Lung lesion score	0	Normal finding
	1	Mild hemorrhage in alveolar duct and sac
	2	Slightly moderate hemorrhage in alveolar duct and sac
	3	Moderate hemorrhage in alveolar duct and sac
	4	Severe or generalized hemorrhage in lung
Cardiac lesion score	0	Normal finding
	1	Inflammation of cardiac muscle, mild damage of cardiac muscle fiber and accumulation of leukocyte
	2	Slightly damage of cardiac muscle, hemorrhage and accumulation of leukocyte
	3	Moderate cardiac muscle damage, hemorrhage and leukocyte accumulation
	4	Severe cardiac muscle damage, degeneration of muscle fiber, hemorrhage and accumulation of leukocyte

RESULTS

Pathological changes: There were generalized edema and hemorrhage in the kidney, especially in the renal tubular and renal pelvis. Cell edema could be observed in all of the renal tubular epithelia. The glomeruli were damaged. A space in the renal tubular increased with water accumulation (Fig. 1). Most of the liver cells in all chickens showed fatty degeneration by vacuolation with dilation of sinusoids (Fig. 2). Lesions in the lung were related to veins and massive congestion of veins and arterioles. Hemorrhage was mainly observed in the parabronchus, alveolar ducts and alveolar sacs (Fig. 3). In the cardiac muscle massive myofibrillar degeneration with hemorrhage and in some cases generalized and diffuse myocarditis containing organisms, were observed (Fig. 4).

Comparison of tolerance to heat between TIC, TICC and BC: When chickens were maintained at 38±2°C, pathologic lesions of the visceral organs were significantly greater in number than those of chickens maintained at 26±2°C ($p<0.05$). The kidney lesion scores of the male and female broilers and male Thai indigenous chickens crossbred were significantly higher than the kidney lesion scores of the male and female Thai indigenous chicken and female Thai indigenous chickens crossbred ($p<0.05$) (Table 2). The liver lesion scores of

Table 2: Kidney Lesion Score (KLS), Liver Lesion Score (LLS), Lung Lesion Score (LuLS) and Cardiac Lesion Score (CLS) of TIC, TICC and commercial BC maintained at 26±2°C continuously and 38±2°C (for 8 h/day) for 28 days

	26±2°C						38±2°C						
	TIC		TICC		BC		TIC		TICC		BC		
Parameters	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	SEM
KLS	0.67^{d}	0.84^{d}	0.96^{d}	0.50^{d}	0.84^{d}	0.63^{d}	2.67^{b}	2.17^{c}	3.38^{a}	2.75^{b}	3.29^{a}	3.25^{a}	0.040
LLS	0.50^{d}	0.42^{d}	0.34^{d}	0.42^{d}	0.50^{d}	0.59^{d}	2.04^{c}	2.59^{b}	2.67^{b}	2.71^{b}	3.55^{a}	3.25^{a}	0.046
LuLS	0.42^{c}	0.42^{c}	0.42^{c}	0.25^{c}	0.38^{c}	0.30^{c}	2.83^{c}	2.38^{d}	3.38^{ab}	3.09^{bc}	3.21^{abc}	3.50^{a}	0.037
CLS	0.96^{c}	1.00^{c}	0.92^{c}	0.71^{c}	0.75^{c}	0.84^{c}	2.34^{b}	2.25^{b}	2.33^{b}	2.46^{b}	3.00^{a}	2.96^{a}	0.037

$^{a,b,c \text{ and } d}$within row, mean with different superscript differ significantly ($p<0.05$). SEM = Standard Error of the Mean, TIC=Thai Indigenous Chickens; TICC = Crossbred Thai Indigenous Chickens; BC = Commercial Broilers

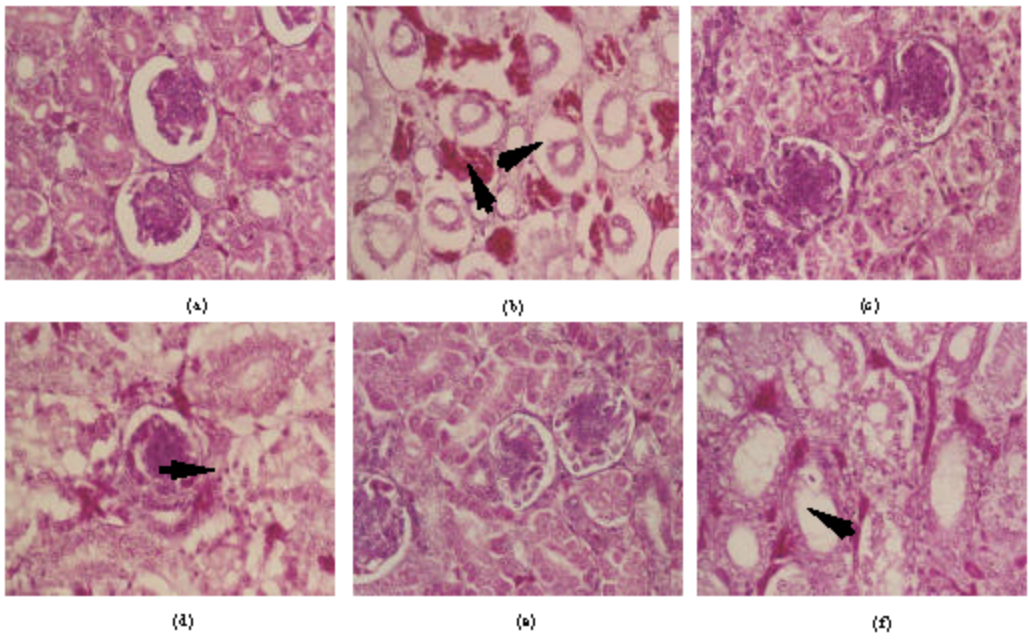

Fig. 1: Pathological changes of kidney in TIC, TICC and commercial BC maintained at 26±2°C and 38±2°C. Hemorrhage, cell edema, cell degeneration and water accumulation in renal tubules in kidney of heat stressed chickens were observed (arrow). (a. Kidney of commercial BC at 26±2°C; b. Kidney of commercial BC at 38±2°C; c. Kidney of TICC at 26±2°C; d. Kidney of TICC at 38±2°C; e. Kidney of TIC at 26±2°C; f. Kidney of TIC at 38±2°C), (H and E staining, X 400)

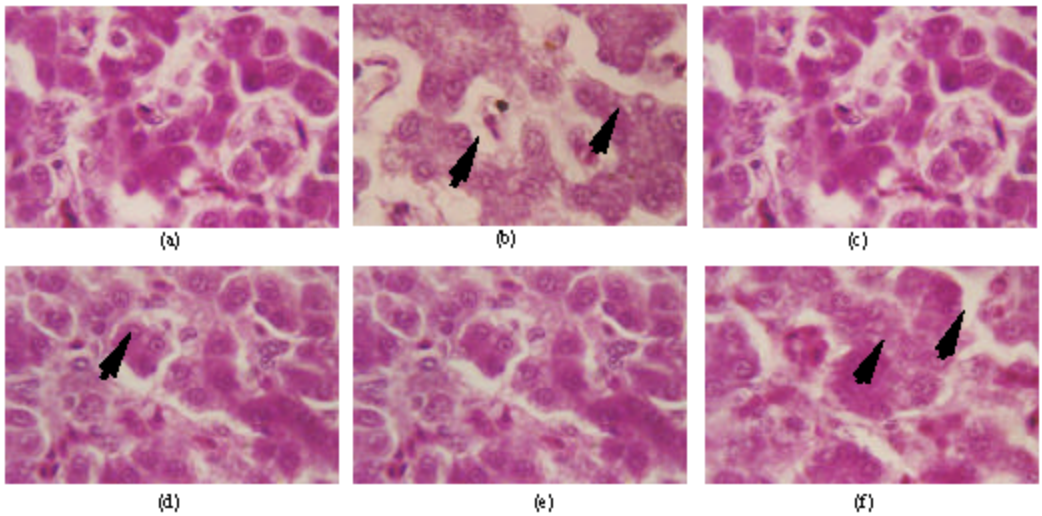

Fig. 2: Pathological changes of liver in TIC, TICC and commercial BC maintained at 26±2°C and 38±2°C. Fatty degeneration by vacuolation with dilation of sinusoids in liver of heat stressed chickens were observed (arrow). (a. Liver of commercial BC at 26±2°C; b. Liver of commercial BC at 38±2°C; c. Liver of TICC at 26±2°C; d. Liver of TICC at 38±2°C; e. Liver of TIC at 26±2°C; f. Liver of TIC at 38±2°C), (H and E staining, X400)

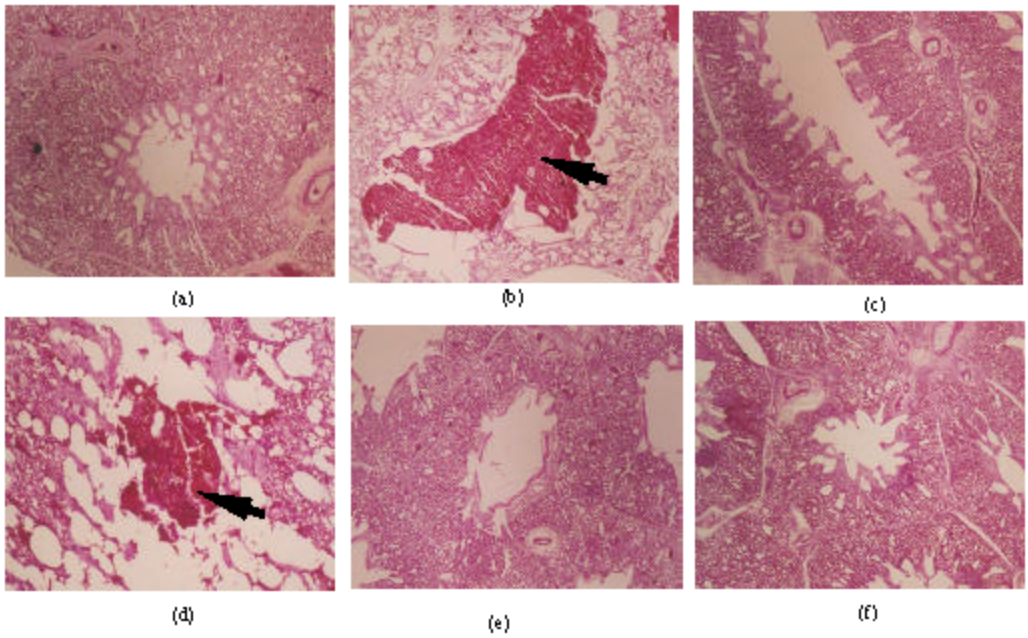

Fig. 3: Pathological changes of lung in TIC, TICC and commercial BC maintained at 26±2°C and 38±2°C. Hemorrhage in the parabronchus, alveolar ducts and alveolar sacs in lung of stressed chickens were observed. (a. Lung of commercial BC at 26±2°C; b. Lung of commercial BC at 38±2°C; c. Lung of TICC at 26±2°C; d. Lung of TICC at 38±2°C; e. Lung of TIC at 26±2°C; f. Lung of TIC at 38±2°C); (H and E staining, X 400)

male and female broilers were significantly higher than the liver lesion scores of the male and female Thai indigenous chicken and Thai indigenous chickens crossbred ($p<0.05$). The lung lesion scores of male and female broilers and male Thai indigenous chickens crossbred were significantly higher than the lung lesion scores of the female Thai indigenous chickens crossbred and male and female Thai indigenous chickens ($p<0.05$). The cardiac lesion scores of male and female broilers were significantly higher than the cardiac lesion scores of the male and female Thai indigenous chickens and Thai indigenous chickens crossbred ($p<0.05$), respectively.

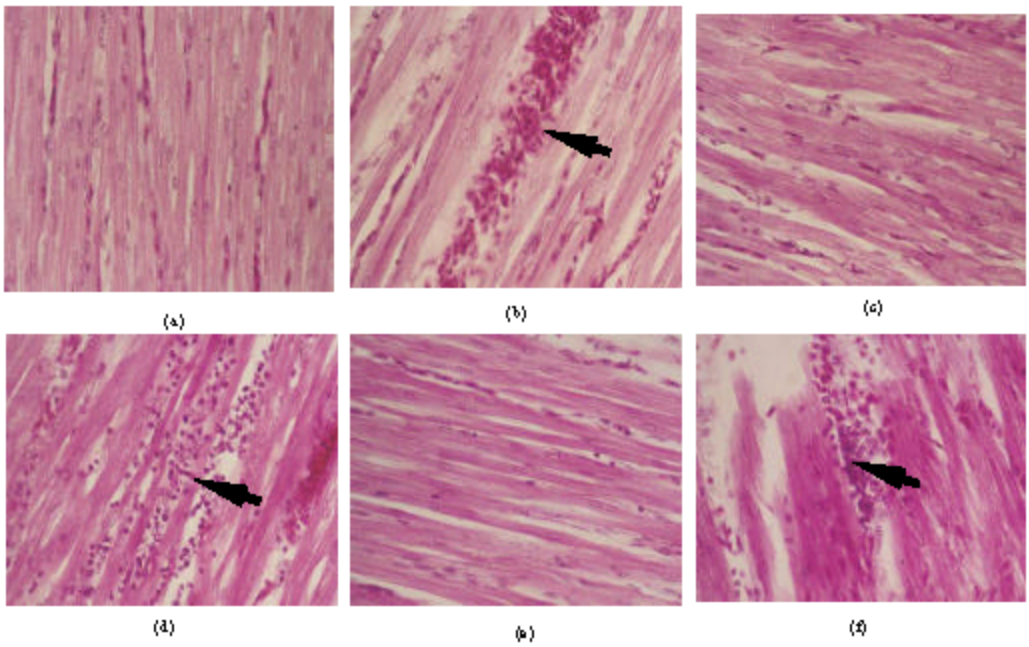

Fig. 4: Pathological changes of heart in TIC, TICC and commercial BC maintained at 26±2°C and 38±2°C. Massive myofibrillar degeneration with hemorrhage and myocarditis containing organisms in cardiac muscle of heat stressed chickens were observed. (a. Heart of commercial BC at 26±2°C; b. Heart of commercial BC at 38±2°C; c. Heart of TICC at 26±2°C; d. Heart of TICC at 38±2°C; e. Heart of TIC at 26±2°C; f. Heart of TIC at 38±2°C); (H and E staining, X400)

These results showed that Thai indigenous chickens and Thai indigenous chickens crossbred were more tolerant to high heat than broilers.

DISCUSSION

Stress challenges the homeostatic state of an organism. Thus, a stress response includes complex reactions to maintain a steady state (Ewing *et al*., 1999). After chickens are exposed to high ambient temperatures, their body temperature rises to a higher than the normal body temperature (Whittow, 2000; Aengwanich *et al*., 2003a; Donkoh, 1989; Cooper and Washburn, 1998; Aengwanich and Chinrasri, 2002) causing an increase in the workload of the physiological system (Ewing *et al*., 1999).

When the body temperature rises above the normal range, the parenchyma of many cells usually begins to become damaged (Guyton, 1966). Moreover, increasing the core body temperature during heat stress causes endothelial cell damage. In addition, high blood pressure might result from an autonomic nervous system response, which may cause circulatory rupture then hemorrhaging in various organs such as the lung, kidney, liver and heart. The hemorrhaging effect is similar to that reported earlier by Aengwanich *et al*. (2003b). They found that heat stress caused hemorrhaging in the visceral organs of commercial BC, while the effects of heat stroke on the chickens showed gross lesions and severe and generalized hyperemia (Aengwanich and Simaraks, 2002; Kumer *et al*., 1999), which was most severe in the respiratory system, especially in the lung (Aengwanich and Simaraks, 2002). This phenomenon was similar to the histological lesions in the kidney, liver, lung and cardiac muscle of the commercial BC, TICC and TIC that were maintained at 38±2°C in this study.

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 (Cheville, 1999). In this study, the hepatocytes of heat stressed chickens were largely damaged and had developed cells with fat globules and/or fatty degeneration like those reported by Aengwanich *et al*. (2003b).

In mammals, heat stroke might cause degenerative changes in the renal tubules. Several days after heat stroke occurs, there is often evidence of renal failure caused by degeneration and necrosis of the renal tubules (Cheville, 1999). These changes are similar to the effects of heat stress on renal tubule epithelia in the chickens in this study. This indicates that chickens living in a prolonged high temperature condition develop nephropathy.

Berrong and Washburn (1998) and Mashaly *et al.* (2004) reported that when chickens were under heat stress their mortality rate increased. After TIC, TICC and commercial BC were under heat stress, tissue damage and abnormalities in the visceral organs were observed. This phenomenon may be the cause of death in chickens under heat stress. Nakamura *et al.* (1999) reported that histological lesions, including myocardial, hepatocytic degeneration and/or necrosis, were the cause of death of chickens (Aengwanich and Chinrasri, 2002).

When chickens were maintained at 38±2°C, pathologic lesions of the visceral organs were greater in number than those of the chickens maintained at 26±2°C. The kidney lesion scores of male and female commercial BC were higher than the kidney lesion scores of the male and female TIC and female TICC. The lung lesion scores of male and female commercial BC were higher than the lung lesion scores of the male and female TIC and the lung lesion score of female commercial BC was higher than the lung lesion score of the female TICC. The liver lesion scores of male and female commercial BC were higher than the liver lesion scores of the male and female TIC and TICC. The cardiac lesion score of male and female commercial BC was higher than the cardiac lesion score of the male and female TIC and TICC. These indicated that the commercial BC was less tolerant to high environmental temperature than TICC and TIC, respectively.

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REFERENCES

Aengwanich, W. and O. Chinrasri, 2002. Effect of heat stress on body temperature and hematological parameters in male layers. Thai. J. Physiol. Sci., 15 (1): 27-33.

Aengwanich, W. and S. Simaraks, 2002. Heat stress in chickens. Songklanakarin. J. Sci. Technol., 24 (1): 159-167.

Aengwanich, W., Y. Phasuk, T. Vongpralab, P. Pakdee, S. Katavetin and S. Simaraks, 2003a. Physiological adaptation and effect of ascorbic acid on adaptation in broilers under heat stress. Thai. J. Agric. Sci., 36 (3): 225-232.

Aengwanich, W., U. Chuachan, Y. Phasuk, T. Vongpralab, P. Pakdee, S. Katavetin and S. Simaraks, 2003b. Effect of ascorbic acid on respiratory rate, body temperature, heterophil: Lymphocyte ratio and microscopic lesion score in lung, liver, kidney, cardiac muscle and spleen in broilers under chronic heat stress. Thai. J. Agric. Sci., 36 (2): 207-218.

Berrong, S.L. and K.W. Washburn, 1998. Effects of genetic variation on total plasma protein, body weight gains and body temperature responses to heat stress. Poult. Sci., 77 (3): 379-385.

Cheville, N.F., 1999. Introduction to Veterinary Pathology. 2nd Edn. Iowa State University Press, Ames, Iowa, pp: 5-28. ISBN: 0-8138-2496 6.

Cooper, M.A. and K.W. Washburn, 1998. The relationships of body temperature to weight gain, feed consumption and feed utilization in broilers under heat stress. Poult. Sci., 77: 237-242. PMID: 949 5487.

Cotran, R.S., V. Kummar and T. Collin, 1999. Robbins: Pathologic Basis of Disease. W.B. Saunders Company, Philadelphia, pp: 434-435. ISBN: 97807216 73356.

Daghir, N.J., 1995. Poultry Production in Hot Climates. The University Press, Cambridge, pp: 32. ISBN: 9780851-989075.

Deeb, N. and A. Cahaner, 2001. Genotype-by-environment interaction with broilers genotype differing in growth rate: 2. The effect of high ambient temperature on dwarf versus normal broilers. Poult. Sci., 80: 541-548. PMID: 11372701.

Donkoh, A., 1989. Ambient temperature: Affecting performance and physiological response of broiler chickens. Int. J. Biometeorol., 33: 259-265. DOI: 10.1007/BF01051087.

Ewing, S.A., D.C. Lay and E.V. Borell, 1999. Farm Animal Well-Being: Stress Physiology, Animal Behavior and Environmental Design Simon and Schuster Company, New Jersey, pp: 50-77. ISBN-13: 9780136602002.

Guyton, A.C., 1966. Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia, pp: 985-1000. ISBN: 0-7216-5944-6.

Kumer, V., Abbas, A.K. and N. Fausto, 1999. Robbins and Cotran: Pathologic Basic of Disease. Elsevier Saunder, Philadelphia, pp: 445. ISBN: 0721601871.

Luna, L.T., 1968. Manual of Histologic Staining Methods of the Armed force Institute of Pathology. McGraw-Hill Book Company, New York, pp: 1-39. http//www.nature.com/nature/journal/v257/n5529/abs/257790a0.html; jds.fass.org/cgi/content/full/87/11/3762.

Mashaly, M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad A.O. Abbas and P.H. Patterson, 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci., 83 (6): 889-894. PMID: 15206614.

Nakamura, K., Y. Ibaraki, Z. Mitarai and T. Shibahara, 1999. Comparative pathology of heart and liver lesion of broiler chickens that died of ascites, heart failure and others. Avian Dis., 43: 526-532. PMID: 10494423.

Whittow, G.C., 2000. Sturky's Avian Physiology. Academic Press, San Diego, pp: 343-390. ISBN-139 78-0-12-747605-6; ISBN-10 0-12-747605-9.