ORIGINAL ARTICLE

Effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress

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Abstract

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Effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress

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The purpose of this study was to conduct an experiment related to the effects of chronic heat stress on total white blood cell changes, pathophysiology of leukocyte and effects of ascorbic acid on lymphocytes, lympholytic cells and humoral immunity of New-castle disease of broilers under chronic heat stress. Randomized complete block was the design. One hundred-forty-four chickens were maintained at 33±1 °C environmental temperature and on four levels of added ascorbic acid i.e. 0 (control group), 200, 400 and 800 mg/kg in diets for 21 days. On days 1, 3, 7, 14 and 21 of the experimental period, total white blood cells count, lympholytic

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cell and HI titer for Newcastle disease were determined. On day 21, histopathology of lung, liver, kidney, heart and bursa of fabricius of randomly selected broilers (n=36; 3 birds per experimental unit) were studied. Total white blood cells (TWBC) of the birds were significantly increased on day 3 (P<0.05) and were highest on days 7 and 14 then significantly decreased on days 21 (P<0.05). Monocytes were significantly increased on day 3 (P<0.05). Lymphocytes were significantly increased on day 7, and were highest on day 14 (P<0.05). On day 21, the value of lymphocyte was significantly lower than on days 7 and 14 (P<0.05), respectively. Lympholytic cells were significantly increased on day 3 and 7 (P<0.05), respectively, but on day 21, lympholytic cells were significantly decreased to lower value than on day 7 (P<0.05). Heterophils were significantly increased on day 3 and 7 and then decreased on day 14 (P<0.05). Tissue injury and hemorrhage in broilers under chronic heat stress caused leukocytosis, heterophilia, lympholysis and monocytosis. The size of lobules within the bursa of fabricius in broilers receiving ascorbic acid at 800 mg/kg in the diet were larger than in birds that received added ascorbic acid at 400, 200 and 0 mg/kg in their diets, respectively. Lymphocytes and lympholytic cells were not significantly different among the ascorbic acid treatment groups. Besides, HI titers of Newcastle disease at 800 mg/kg in the diet were significantly higher than the others (P<0.05). Apparently, adding ascorbic acid at 800 mg/kg in the diet could improve humoral immunity in broilers under heat stress.

Key words : ascorbic acid, immune response, white blood cell, broilers, heat stress

บทคัดย่อ

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ผลของวิตามินซีต่อการตอบสนองภูมิคุ้มกันแบบพึ่งเซลล์ ไม่พึ่งเซลล์และพยาธิสรีรวิทยาของเม็ดเลือดขาวในไก่กระทงเมื่ออยู่ในภาวะเครียดเนื่องจากความร้อน

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วัตถุประสงค์ของการศึกษานี้เพื่อศึกษาผลของภาวะเครียดเนื่องจากความร้อนเป็นเวลานาน ต่อการเปลี่ยนแปลงจำนวนเม็ดเลือดขาวทั้งหมด พยาธิสรีรวิทยาของเม็ดเลือดขาว และผลของวิตามินซีต่อจำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ปกติ และชนิดที่เกิดการแตกเสียหาย และการตอบสนองของระบบภูมิคุ้มกันแบบไม่พึ่งเซลล์ของโรคนิวคาสเซิลในไก่กระทง วางแผนการทดลองแบบบล็อคสมบูรณ์ เลี้ยงไก่กระทงที่อุณหภูมิ 33±1 °C จำนวน 144 ตัว และเสริมวิตามินซี 4 ระดับ ได้แก่ 0 200 400 และ 800 มก./กก. ของอาหาร เป็นเวลา 21 วัน ในวันที่ 1 3 7 14 และ 21 ของการทดลอง ทำการวัดค่าจำนวนเม็ดเลือดขาวทั้งหมด จำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ที่เกิดการแตกเสียหายและภูมิคุ้มกันแบบไม่พึ่งเซลล์ของโรคนิวคาสเซิล ในวันที่ 21 ของการทดลอง สุ่มเลือกไก่กระทง จำนวน 36 ตัว (หน่วยทดลองละ 3 ตัว) แล้วเก็บตัวอย่างอวัยวะของไก่ได้แก่ ปอด ตับ ไต หัวใจและต่อมเบอร์ซ่า มาเพื่อศึกษาพยาธิสรีรวิทยา ผลการศึกษาพบว่าจำนวนเม็ดเลือดขาวทั้งหมดของไก่สูงขึ้นในวันที่ 3 (P<0.05) และสูงที่สุดในวันที่ 7 และ 14 (P<0.05) จากนั้นจึงลดต่ำลงในวันที่ 21 (P<0.05) ของการทดลอง จำนวนเม็ดเลือดขาวชนิดโมโนไซต์เพิ่มสูงขึ้นในวันที่ 3 (P<0.05) ของการทดลอง จำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์เพิ่มสูงขึ้นในวันที่ 7 (P<0.05) และเพิ่มสูงสุดในวันที่ 14 (P<0.05) ของการทดลอง และวันที่ 21 ของการทดลองจำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ต่ำกว่าวันที่ 7 และ 14 (P<0.05) ตามลำดับ จำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ที่เกิดการแตกเสียหายเพิ่มสูงขึ้นในวันที่ 3 และ 7 (P<0.05) ตามลำดับ แต่ในวันที่ 21 ของการทดลอง จำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ที่เกิดการแตกเสียหายต่ำกว่าวันที่ 7 (P<0.05) จำนวนเม็ดเลือดขาวชนิดเฮทเทอโรฟิลเพิ่มสูงขึ้นในวันที่ 3 และ 7 (P<0.05) และลดลงในวันที่ 14 (P<0.05) ของการทดลอง การเกิดความเสียหายของเนื้อเยื่อและมีเลือดออกในอวัยวะต่าง ๆ ของไก่เมื่ออยู่ในภาวะเครียดเนื่องจากความร้อน มีผลทำให้จำนวนเม็ดเลือดขาวทั้งหมด เม็ดเลือดขาวชนิดลิมโฟไซต์ เฮทเทอโรฟิล และโมโนไซต์

เพิ่มขึ้น เมื่อทำการเสริมวิตามินซีทั้ง 4 ระดับพบว่าขนาดของ lobule ภายในต่อมเบอร์ซ่าของไก่กลุ่มที่ได้รับวิตามินซีที่ระดับ 800 มก./กก. ของอาหาร มีขนาดใหญ่กว่าไก่กลุ่มที่ได้รับวิตามินซีที่ระดับ 400 200 และ 0 มก./กก. ของอาหาร ตามลำดับ และวิตามินซีทั้ง 4 ระดับไม่มีผลต่อจำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ และจำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ที่เกิดการแตกเสียหาย (P>0.05) และระดับภูมิคุ้มกันแบบไม่พึ่งเซลล์ของโรคนิวคาสเซิลในไก่เนื้อเมื่ออยู่ในภาวะเครียดเนื่องจากความร้อนได้รับวิตามินซีที่ระดับ 800 มก./กก. ของอาหาร สูงกว่ากลุ่มควบคุมและกลุ่มที่ได้รับวิตามินซีระดับอื่นทางสถิติ (P<0.05) แสดงให้เห็นว่าวิตามินซีที่ระดับ 800 มก./กก.ของอาหาร มีผลช่วยให้ระดับภูมิคุ้มกันของไก่แบบไม่พึ่งเซลล์เพิ่มขึ้น

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When broilers were exposed to acute heat stress, the percentage of monocyte and lymphocyte decreased while the percentage of the heterophil increased (Altan *et al*., 2000). Moreover, Borges *et al*. (1999) reported that heat stress increased the percentage of heterophils and decreased the percentage of lymphocytes. Total white blood cell of broilers under stress increased (Puvadolpirod and Thaxton, 2000).

Corticosteroid administration results in a lymphopenia and an increase in circulating heterophils in chicken. Therefore, it appears that chickens have a "stress leukogram" similar to that of mammals (Harmon, 1998). Jain (1993) reported that corticosteroid caused lympholysis in blood and lymphoid tissue, increased shift of lymphocytes from blood to other body compartments, or both in mammals. Moreover, T cell in blood and tissues are most sensitive to the lympholytic effect. After broilers were exposed to high ambient temperature, their body temperature increased more than the normal body temperature (Reddy, 2000), corticosterone stored in adrenal cortex was released into the blood circulation to help broilers increase metabolism (Richard, 1998). This hormone might cause cell mediated and humoral immunity failure because changes in the plasma concentrations of corticosteroides and ACTH affected the lymphoid tissues; for example, a diminution in the mass of the spleen, thymus and bursa of fabricius (Daghir, 1995).

Ascorbic acid has been widely used to reduce the stress in chickens, because this vitamin could decrease corticosterone level in the blood circulation (Nockel *et al*., 1973; Sheila and Cheryl, 1978). However, information concerning the effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress has not been reported. Therefore, the purpose of this study was to conduct an experiment related to the effects of ascorbic acid on lymphocyte, lympholytic cell, humoral immunity, white blood cell changes and pathophysiology of leukocyte in broilers under heat stress.

Materials and Methods

One hundred and forty four, symptomatically disease - free day - old broiler chicks were obtained from a commercial hatchery. They were incubated for 21 days before being placed in layer cages. Live Newcastle disease virus (Lasota strain) was administerated by occulonasal instillation of 0.1 ml. on day 14 of age. Experiments began after 7 days adaptation period in the cages at 26-28 $^{\circ}$C environmental temperatures. The chicks were fed on standard broiler starter (commercial feed) with continuous light and water supply.

The experiment was designed as a Randomized complete block design (RCBD) with four treatments i.e. supplementation of diets with ascorbic acid at 0, 200, 400 and 800 mg/kg. On day 1 of the experimental period (28 days of age), broilers were transferred into an environmentally controlled housing and kept in wire - floored layer

cages. All broilers were subjected to 5-hour episode of heat stress at 33±1 °C each day. Relative humidity was 60-70%. The total mixed diet (Table 1) with the four levels of ascorbic acid was fed *ad libitum*. On day 1, 3, 7, 14 and 21 of the experimental period, blood samples (via wing vein: 3.75 ml.) from randomly selected five birds per the experimental unit were collected and transferred to vial tubes containing EDTA as anticoagulant and without anticoagulant for white blood cell parameters and NDV-HI titers determination, respectively. Air-dried blood films were stained with Giemsa-Wright's stain. Differential WBC counts and lympholytic cell were performed by using standard avian guidelines introduced by Ritchie, *et al*. (1994). Total white blood cells were determined by the Unopett method (Campbell, 1995). NDV-HI titers were investigated using the method described by Wongwatcharadumrong (1990).

All data were analysed by using repeated measurement of the ANOVA procedure of Statistical Analysis System (SAS, 1990). Influence of time on parameter changes when broilers were maintained under prolonged heat stress and treatments were considered. Means were separated by Duncan's multiple range tests (Duncan, 1955). The level of significance was determined at $P < 0.05$.

On day 21 of the experimental period (49 days of age), three randomly selected broilers per experimental unit were killed by cervical dislocation. Lung, liver, kidney, heart and bursa of fabricius of each bird were collected. These organs were fixed in 10% buffered formalin, then sectioned, and stained with Hematoxylin and Eosin (H&E) for microscopic examination (Luna, 1968).

Table 1. Total mixed feed ration for growing broilers.

Ingredients	Percentage of mix
1. Corn#2	62.60
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. D.C.P. (Rock 16%)	0.30
11. D.C.P. (Rock 18%)	0.15
Total	**100.00**

Each kg contains 50 Vitamin AD_3E 500/1000 (400mg); Vitamin E_{50}(2,000 mg); Vitamin B_1 (180 mg); Vitamin B_2 (100 mg); Vitamin B_6 (310 mg); Vitamin B_{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_2O (210 mg); ferrous sulphate, Fe. 7H_2O (40,600 mg); Selenium (150 mg); Dicalcium phosphate (406.37g).

Results

Effects on white blood cell and differential counts

When the broilers were subjected to 33± 1 °C temperature episode during 1 - 21 days of experimental period, total white blood cells (TWBC) of the birds were significantly increased on day 3 ($P<0.05$) and were highest on days 7 and 14, then significantly decreased on day 21 ($P<0.05$). Monocytes were significantly increased on day 7 ($P<0.05$). Lymphocytes were significantly increased on day 7, and were highest on day 14 ($P<0.05$). On day 21, the value of lymphocyte was significantly lower than on days 7 and 14 ($P<0.05$), respectively. Lympholytic cells were significantly increased on day 3 and 7 ($P<0.05$), respectively, but on day 21, they significantly decreased to a lower value than on day 7 ($P<0.05$). Heterophils were significantly increased on days 3 and 7 and then decreased on day 14 ($P<0.05$) (Table2). The patterns of white blood cell parameters are presented in Figure 1. Level of added ascorbic acid produced no effect on white blood cells and differential counts.

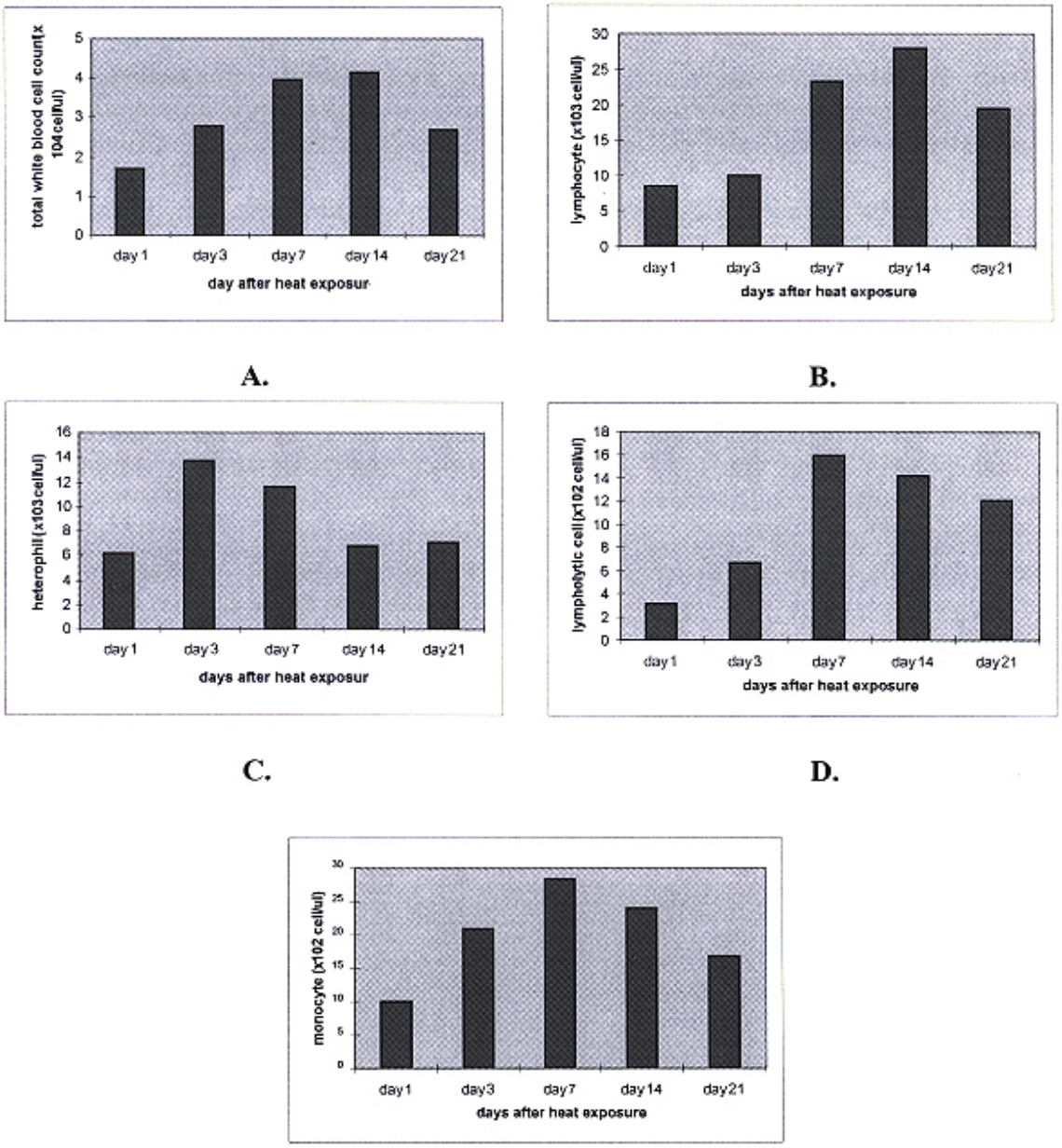

Figure 1. White blood cell parameters pattern of broiler response to heat stress for 21 days (A: total white blood cell; B: lymphocyte; C: heterophil; D: lympholytic cell; E: monocyte)

Microscopic changes

In all birds on day 21 of the experimental period, generalized edema and hemorrhage were observed in the kidney especially in renal papillae and renal tubulae. In addition, leukocytes accumulated in many inflammatory areas (Figure2A). Fatty degeneration was mostly found in renal tubular epithelial cells. Most liver cells accumu-

Table 2. White blood cell parameters changes in broiler chickens under chronic heat stress.

Parameter	Days after heat exposure					SEM[1]
	Day1	Day3	Day7	Day14	Day21	
Total WBC (10^4cell/μl)	1.68[c]	2.79[b]	3.96[a]	4.16[a]	2.67[b]	0.46
Monocyte (10^2cell/μl)	10.05[c]	21.00[abc]	28.39[a]	23.97[ab]	16.76[bc]	3.57
Lymphocyte (10^3cell/μl)	8.50[d]	10.05[d]	23.34[b]	27.98[a]	19.48[c]	2.60
Lympholytic cell (10^2cell/μl)	3.16[d]	6.67[c]	15.98[a]	14.23[ab]	12.09[b]	1.84
Heterophil (10^3cell/μl)	6.13[b]	13.69[a]	11.57[a]	6.78[b]	7.11[b]	2.18

[a, b, c and d] **within row, mean with no common superscript differ significantly (P<0.05).**
[1] **Standard error of the mean**

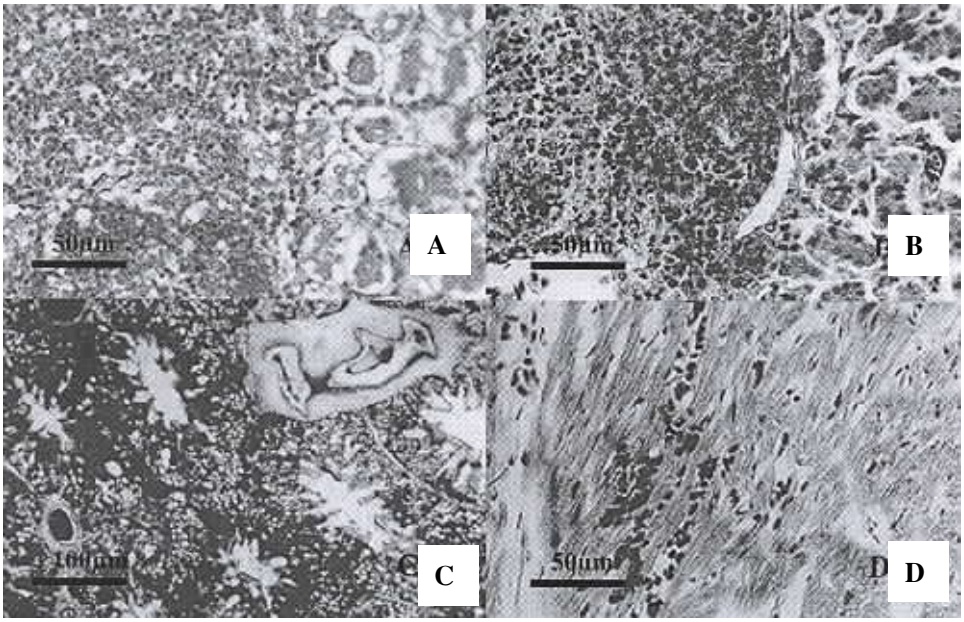

Figure 2. Microscopic examination of kidney, liver, lung and cardiac muscle in broilers under heat stress.(A= kidney, B=liver, C=lung and D=cardiac muscle)

lated fat by vacuolation of fat with dilation of sinusoid. Necrosis with leukocyte granulation tissue was seen in some parts of the liver (Figure 2B). Lung tissue with massive congestion and hemorrhage was largely observed in alveolar duct and alveolar sac (Figure2C). Massive myofibrillar degeneration with hemorrhage, vacuolation of myofibers and diffuse myocarditis containing white blood cells was found in some areas (Figure 2D). The lobules within the bursa of fabricius of broilers were atrophied when exposed to heat stress (Figure3A and 3B). Whereas, only the size of lobules in broilers receiving ascorbic acid at 800 mg/kg in the diet were larger and active than this birds that received ascorbic acid at 400, 200 and 0 mg/kg in their diets, respectively (Figure 3A, 3B, 3C and 3D).

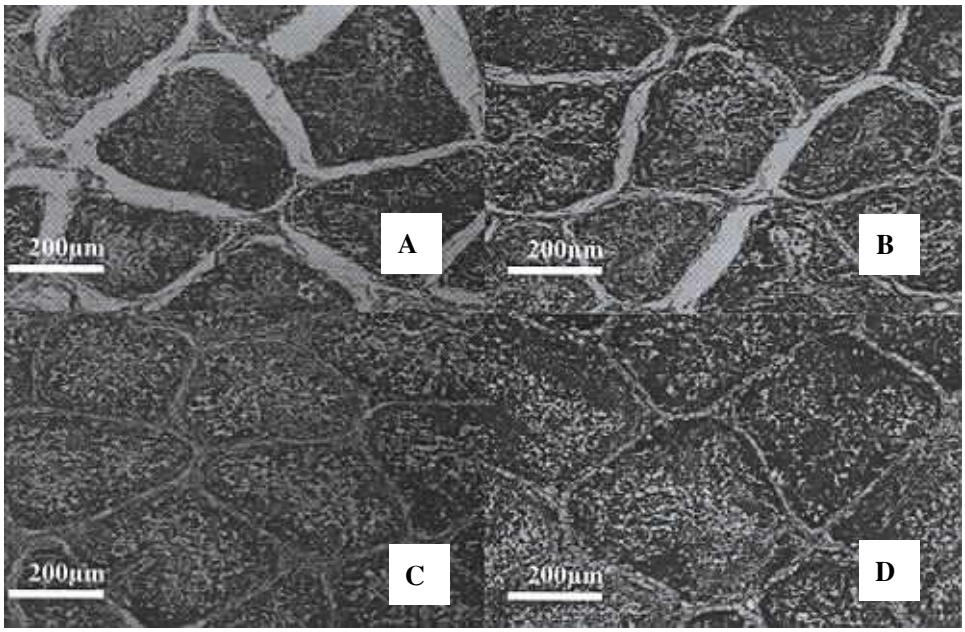

Figure 3. Microscopic examination of lobules within bursa of fabricius in broilers with added ascorbic acid in their diets after exposed to heat stress for 21 days (A = 0 mg/kg; B=200 mg/kg; C= 400 mg/kg; D=800 mg/kg).

Table 3. Effects of ascorbic acid on lymphocyte, lympholytic cell and NDV-HI titers in broilers chickens under chronic heat stress.

Parameter	Levels of ascorbic acid				SEM[1]
	0mg/kg	200mg/kg	400mg/kg	800mg/kg	
Lymphocyte ($\times 10^3$cell/µl)	17.10	18.60	18.52	17.27	2.60
Lympholytic cell ($\times 10^2$cell/µl)	10.38	10.62	10.60	10.10	1.84
NDV - HI titer	1.30[b]	1.52[b]	1.07[b]	2.54[a]	2.18

[a and b] within row, mean with no common superscript differ significantly ($P<0.05$).
[1] Standard error of the mean

Effects of ascorbic acid on lymphocytes, lympholytic cells and HI titer of ND

Birds were fed with four levels of added ascorbic acid i.e. 0, 200, 400 and 800 mg/kg diets and subjected to 33±1 °C temperature episode to determine lymphocytes, lympholytic cells and HI titer of Newcastle disease on day 1, 7, 14 and 21 of experimental period. Lymphocytes and lympholytic cells showed no significant difference among the treatments. However, HI titer of Newcastle disease at 800 mg/kg diets were significantly higher than others. Apparently, adding ascorbic acid at 800 mg/kg in the diet could induce humoral immunity in broilers under heat stress (Table 3).

Discussion

Generally, normal total leukocyte counts in chickens (*Gallus gallus* domesticus) were 1.2-3.0 $\times 10^4$cell/µl (average 1.2) (Jain, 1993). A count that is greater than normal range is considered suggestive leukocytosis. General causes of leukocytosis include infection, trauma, toxicities, hemorrhage into a body cavity, rapidly growing neoplasm and leukemias. The leukocyte count aids in the assessment of the leukocytosis, because a heterophilia is usually present in leukocytosis caused by inflammation, (Ritchie *et al*., 1994). Typically, heterophil infiltration predominates in the first 6 to 12 hrs of the inflammatory response, but macrophages, lymphocytes, and event giant cells are present at 48 h and there are numerous giant cells at 72 h (Harmon, 1998).

In this study, heterophils started increasing on day 3 and returned to the normal level on day 14 of the experimental period, while total lymphocytes increased on day 7 and day 14, but subsequently decreased. The magnitude of the heterophilia usually indicates severity of the initial inflammatory process. Ritchie *et al*. (1994) reported that birds that normally had high numbers of circulating lymphocytes and may develop leukopenia and lymphopenia in the initial stress response, but 12 hrs later showed leukocytosis and heterophilia. Similarly Jain (1993) reported that lymphocytosis might be physiologic, reactive, or proliferative. A transient physiologic lymphocytosis is observed with a marked release of epinephrine because of physical or emotional stress. It usually occurs in association with neutrophilia in mammals. The monocytes in this study increased on day 3, but significantly increased on days 7 and 14. Jain (1993) reported that monocytosis characteristic in mammals in subacute and chronic inflammatory condition. Monocytes accumulated in areas of inflammation and tissue destruction in response to appropriate chemotactic factors. Under appropriates circumstances, monocytes in tissue transform to macrophage, epithelioid cells, and giant cells, all of which are important cellular components of chronic inflammation. Based on histopathology of visceral organs in broilers under chronic heat stress such as liver, heart, kidney and lung tissues injuries and hemorrhage were observed. These might explain leukocytosis, heterophilia, lymphocytosis and monocytosis in this study.

After the broilers were exposed to high ambient temperature, there was an increased release of corticosterone, stored in the adrenal cortex, into the blood circulation, (Richard, 1998). Furthermore, Jain (1993) reported that corticosteroid induced lymphopenia attributed to lympholysis in blood and lymphoid tissue, increased shift of lymphocytes from blood to other body compartments, or both. T-cells in blood and tissues are most sensitive to the lympholytic effect. Lymphocytes have high affinity receptors for corticosteroids in their cytoplasm. After ligand receptor interaction in the cytoplasm, the ligand receptor complexes bind to specific DNA sequences and induce the synthesis of mRNA, which in turn triggers the synthesis of protein that inhibits intracellular glucose transport and lipid synthesis. In addition, an endonucleas may become activated, causing DNA fragmentation. Glucocorticoids also markedly inhibit the synthesis of IL-1 by macrophages and IL-2 by activated T cell, thereby thwarting an immune response (an immunosuppressive effect), while ascorbic acid could decrease corticosterone level in the circulation (Nockel *et al*., 1973; Sheila and Cheryl, 1978). However, in this study, lympholytic cells started increasing on day 3, then significantly increased on days 7, 14 and 21 of the experimental period. Four levels of ascorbic acid could not reduce lympholytic cells, but could improve HI titer of Newcastle disease. Similarly Gross (1992) reported that ascorbic acid could improve immune response in birds under stress and disease condition. But the pathogenesis of heat stress in broilers in this study caused different physiological changes from stress under infection. Besides, the NDV-HI titer of broilers that received ascorbic acid at 800 mg/kg in the diet was related with the largest size of the lobules within their bursa of fabricius. This illustrates that tissue injury and hemorrhage in broilers under chronic heat stress

cause leukocytosis, heterophilia, lymphocytosis and monocytosis. Lympholytic cells were also increased. Adding ascorbic acid at 0, 200, 400 and 800 mg/kg in diets had no effect on lympholytic cells and lymphocyte numbers. However, adding ascorbic acid at 800 mg/kg in the diet could induce the highest humoral immunity in broilers under chronic heat stress because ascorbic acid at this level could protect the bursa of fabricius from the effect of glucocorticoid that were released during broilers under heat stress.

References

Altan, O., Altan, A., Cabuk, M. and Bayraktar, H. 2000. Effect of heat stress on some blood parameter in broilers. Turk Veternerlik Ve Hayvancilik Dergisi. 24: 2, 145 - 148. (Abstract)

Borges, S.A., Ariki, J., Martins, C.L. and Moraes, V.M.B.de. 1999. Potassium chloride supplementation in heat stress broilers. Revista Brasileira de Zootecna, 28: 2, 313 - 319. (Abstract)

Campbell, T.W. 1995. Avian Hematology and Cytology, Iowa State University Press, Ames, Iowa. pp. 3-19.

Daghir, N. J. 1995. Poultry production in hot climates. The university Press, Cambridge.

Duncan, D.B. 1955. Multiple ranges and multiple F test. Biometric 11:1 - 42.

Gross, W.B. 1992. Effect of ascorbic acid on stress and disease in chicken. Avian Disease. 36: 688- 692.

Harmon, B.G. 1998. Avian heterophil in inflammation and disease resistance. Poultry Sci. 77: 972-977.

Jain, N.C. 1993. Essential of Veterinary Hematology. Lea& Febiger, Philadelphia, pp. 266-277.

Luna, L.T. 1968. Manual of Histologic Staining Methods of the Armedforce Institute of Pathology. McGraw-Hill Book Company, NY. pp. 1-39.

Nockels, C.F., Lopez, G.A. and Phillips, R.W. 1973. Influence of vitamin A and C on corticosterone and carbohydrate metabolism in chickens. Poultry Sci. 52: 1261-1269.

Puvadolpirod, S. and Thaxton, J.P. 2000. Physiology stress in chickens 1. Response parameters. Poultry Sci. 76: 363-369.

Reddy, C.V. 2000. Maintaining growth and production. Poultry International: Asia Pacific Edition. 31(1): 36-40.

Richard, J.J. 1998. Physiological management and environmental triggers of the ascites syndrome. Poultry International: Asia Pacific Edition. 37(8): 28-33.

Ritchie, B.W., Harrison, J. G. and Harrison, R. L. 1994. Avian Medicine. Winger's Publishing, Inc, Florida, pp. 176-198.

SAS institute. 1990. SAS/STAT User's Guide: statistics, Release 6.04 Edition. SAS institute, Inc., Cary, NC.

Sheila, K.S. and Cheryl, F.N. 1978. Effect of age, sex, and ascorbic acid ingestion on chicken plasma corticosterone levels. Poultry Sci. 57: 527-533.

Wongwatcharadumrong, R. 1990. Laboratory for Veterinary Virology. Department of Pathology, Faculty of Veterinary Medicine, Chulalongkorn University. (in Thai)