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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Effects of Polyphenols Extracted from Tamarind (*Tamarindus indica* L.) Seed Coat on Body Weight, White Blood Cells, Bursa of Fabricius and NDV-HI Titer of Broilers under Chronic Heat Stress

T. Srikhun<sup>1</sup>, W. Aengwanich<sup>1</sup> and W. Kongbuntad<sup>2</sup>

<sup>1</sup>Stress and Oxidative Stress in Animals Research Unit,  
Faculty of Veterinary Medicine and Animal Science, Mahasarakham University,  
Maha Sarakham 44000, Thailand

<sup>2</sup>Faculty of Veterinary Medicine and Animal Science, Mahasarakham University,  
Maha Sarakham 44000, Thailand

**Abstract:** The purpose of this experiment was to investigate the effects of polyphenols extracted from tamarind (*Tamarindus indica* L.) seed coat on total body weight, white blood cells, bursa of Fabricius and NDV-HI titer of broilers maintained in high environmental temperature. The broilers under study were divided into two main groups. Those in the first group were maintained at  $26\pm 2^{\circ}\text{C}$  and the second group at  $38\pm 2^{\circ}\text{C}$ . The broilers in the second group were subdivided into six sub-groups, each of which received polyphenols at 0, 100, 200, 300, 400 and 500 mg/kg in their diets. The results revealed that the body weights of the broilers in all groups were not different. The total white blood cell counts and lymphocyte counts of the broilers in all sub-groups of the second group were higher than that of the broilers in the first group ( $p<0.05$ ). On day 21 of the experimental period, the total number of white blood cells and lymphocytes of the broilers in the first group was not different from those of the broilers in the first, second, fifth and sixth sub-groups of the second group ( $p>0.05$ ). The relative bursa of Fabricius weight of the broilers in the first group and the third sub-group of the second group were not different ( $p>0.05$ ). Furthermore, lesion scores of the bursa of Fabricius of the first group and the third and fourth sub-groups of the second group were not different ( $p>0.05$ ). Moreover, NDV-HI titer of the broilers in the first group and all sub-groups of the second group were not also different ( $p>0.05$ ). These phenomena indicated that polyphenols could increase the number of lymphocytes and relative bursa of Fabricius weight. On the other hand, the extract could decrease lesion scores of bursa of Fabricius in heat stressed broilers.

**Key words:** Polyphenols, tamarind (*Tamarindus indica* L.), broiler, immunity, heat stress

### INTRODUCTION

The main environmental factor affecting the immune system of broiler chickens is ambient temperature. Exposure of chickens to heat stress causes significant physiological response resulting immunosuppression and high mortality rate (Mujahid *et al.*, 2005). Generally, when the environmental temperature is over  $32^{\circ}\text{C}$ , broilers are induced to heat stress (Cooper and Washburn, 1998). Leukocyte responses have been used as an indicator of heat stress in poultry (Altan *et al.*, 2000). White blood cell parameters i.e. total white blood cells (Aengwanich *et al.*, 2003) and percentage of lymphocytes (Borges *et al.*, 2004; Yalcin *et al.*, 2004; Lien *et al.*, 2007) are also used as indicators of heat stress in chicken. Altan *et al.* (2000) reported that when broilers were exposed to high environmental temperature their lymphocytes decreased. On the other hand, Aengwanich *et al.* (2003) found that total white

blood cells of heat stressed broilers increased. Moreover, Naseem *et al.* (2005) and Aengwanich (2008) reported that heat stress decreased the immune response and bursa of Fabricius weight of broilers. This is in accordance with the report of Al-Ghamdi (2008) who found that heat stress caused the decrease of antibodies' levels of broilers.

Tamarind (*Tamarindus indica* L.) is a tree-type of plant which belongs to the Leguminosae family. It is indigenous to tropical Africa but has become naturalized in North and South America. It is also cultivated in China, India, Pakistan, the Philippines, Indonesia and Spain (Komutarin *et al.*, 2004). Gu *et al.* (2003) found that the seeds of tamarind contained 29.32 procyanidin oligomers and 101.89 g/kg high molecular weight tannins. Moreover, Sudjaroen *et al.* (2005) reported that the content of tamarind seeds comprised only procyanidins such as oligomeric procyanidin tetramer,

procyanidin hexamer, procyanidin trimer, procyanidin pentamer, lower amounts of procyanidin B<sub>2</sub> and (-)-epicatechin. In the previous studies, Aengwanich *et al.* (2009a) found that when broilers were under heat stress and received polyphenols extracted from tamarind seed coat, the percentage of lymphocytes of heat stressed broilers increased. They concluded that polyphenolic compound in the extracts could reduce heat stress in broiler chickens. Since knowledge about the effect of polyphenols extracted from tamarind seed coat on cell mediated, humoral immunity and bursa of fabricius in heat stressed broilers is limited, this experiment aims to examine the effects of polyphenols extracted from tamarind seed coat on final body weight, total white blood cells, number of lymphocytes, relative weight and scores of bursa of Fabricius and NDV-HI titer in broilers maintained in the high environmental temperature. Results from this study would provide fundamental knowledge for using polyphenols from natural products as feed additives in poultry production industry.

## MATERIALS AND METHODS

**Chemicals:** Tamarind seeds were obtained from a local market in Maha Sarakham Province, Thailand. The seeds were heated in a hot air oven at 140°C for 45 min, cooled and cracked to separate their outside brown layer coat from the seeds. Only brown-red seed coats were collected and these were then grounded into fine powder (Komutarin *et al.*, 2004). The polyphenols in the tamarind seed coat powder was extracted following the method of Aengwanich (2009). Total phenolic compounds in each gram of tamarind seed coat extract powder were analyzed by using the Folin-Ciocalteu method (Kahkonen *et al.*, 1999).

**Animals and experimental design:** This experiment was performed under the care and use of the experimental animals committee of Mahasarakham University. 147 male one-day-old Arbor Acres chicks were obtained from a local commercial farm near the laboratory of the Faculty of Veterinary Medicine and Animal Science, Mahasarakham University. The chicks were brooded and fed with the diet with proximate composition of 22.6% CP and 13.4 MJ/kg ME for 14 days. Then, the broilers were fed with the proximate composition of 21.4% CP and 13.0 MJ/kg ME until the experiment period. A completely randomized design (3 replications/treatment and 7 broilers/experimental unit) was used. When the broilers were 18 days old, they were divided into two main groups. In the first group, the broilers were maintained in the environmental temperature at 26±2°C throughout the experimental period, while in the second group, the broilers were maintained in the controlled

room temperature at 38±2°C for 6 h per day and then rested at 26±2°C for 18 h per day (cyclic temperature). The second-group broilers were subdivided into six sub-groups according to their feeds. The sub-groups received polyphenols extracted from tamarind seed coat at 0, 100, 200, 300, 400 and 500 mg/kg in their diets. Throughout the experimental period, the broilers were treated *ad libitum* with continuous light and water supplies. The experiment was performed for 28 days.

**Hematological techniques:** On days 1, 7, 14 and 21 of the experimental period, the blood samples were collected after the chickens were subjected to 38±2°C for two hrs. The chickens were restrained manually and 3 ml of blood sample were collected from the cervical vein using a 3 ml syringe and a 23-gauge needle with 1.5 inches in length. The collected blood samples were divided in to two parts. In Part 1, 1.5 ml of blood was placed into microtube with EDTA. In Part 2, 1.5 ml of blood was placed into a tube without EDTA for collecting serum. The samples were cooled to approximately 4°C using icepacks and were transferred to the laboratory within two hours after blood collection.

**Total white blood cells and lymphocytes:** Blood films were prepared, fixed in 95% methyl alcohol for 5 min and then stained with Wright-Giemsa solution. Differential white blood cell counts were performed using the standard avian guidelines of Ritchie *et al.* (1994). Total white blood cell count was investigated following the method described by Campbell (1995). Total lymphocytes were calculated by using the following equation: total white blood cells X percentage of lymphocytes. Total white blood cell and lymphocyte counts were determined on days 1, 7, 14 and 21 of the experimental period.

**ND-HI titer:** Newcastle Disease (ND) HI titer was determined by the following procedure: A 2-fold serial dilution of serum was made in a 96-well microlitre plate with V-shaped bottom, containing 25 µl of buffer with 7.2-7.4 pH and 25 µl of serum in all wells. 25 µl of ND virus antigen were added to all wells except those in the last row (the controls). Serum dilutions ranged from 1:2 to 1:2,048. The antigen serum mixture was incubated for 10 min at 37°C. 50 µl of 0.5% erythrocyte suspension were then added to each well and the wells were re-incubated for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination was considered at the end point. The geometric mean titer was expressed as reciprocal log<sub>2</sub> values of the highest dilution that displayed anti-ND-HI.

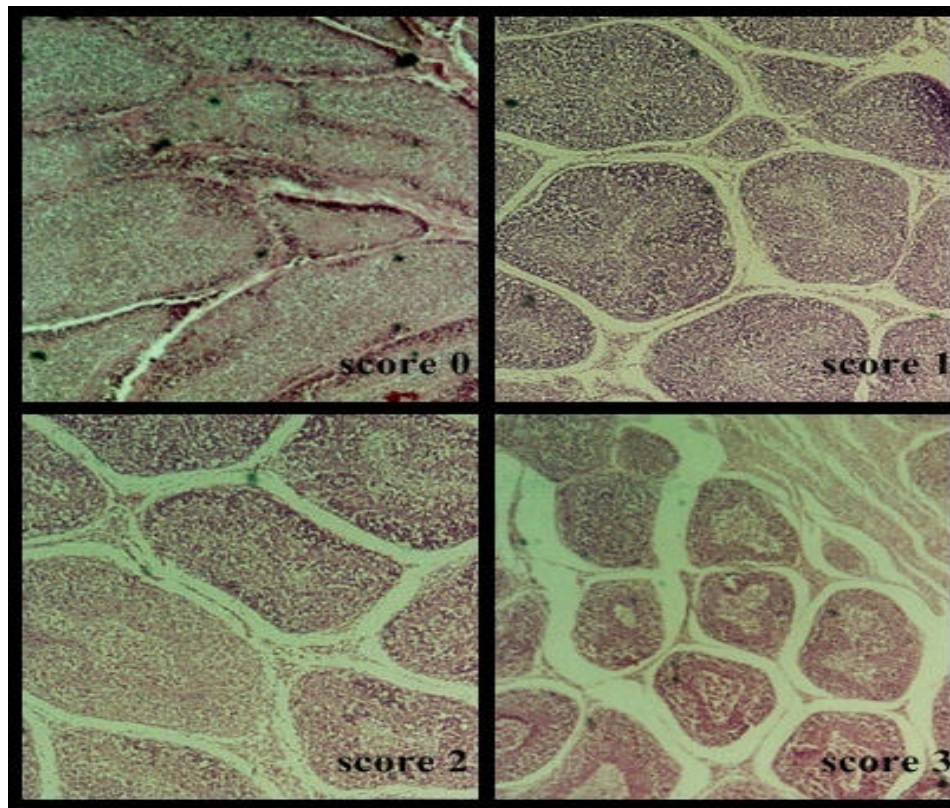


Fig. 1: Lesion scores of bursa of Fabricius in broilers subjected to cyclic temperature for 28 days (H and E, 5x)

**Bursa weight and lesion scores of Bursa of Farbricius:**

On day 28 of the experimental period, the body weight of broilers in both groups was investigated and then they were slaughtered. The bursa of each broiler was collected and weighted. Relative bursa of Fabricius weight was calculated. Lesion scores of the bursa of Fabricius were performed. Briefly, the bursa was fixed in 10% buffered formalin, then sectioned and stained with Hematoxylin and Eosin (H and E) for microscopic examination (Luna, 1968). The lesion scores were judged as follows: Score 0 (100%): normal finding; Score 1 (80%): some follicles in the bursa shrunk and a space between the follicle septum was found; Score 2 (60%): each lobule in the bursa shrunk more than that in Score 1, each follicle was separated from the others and the space between follicles within the bursa was larger than that in Score 1; Score 3 (40%): each follicle in the bursa shrunk more than that in Score 2 and each follicle in the bursa was completely separated from the others and the space between follicles within the bursa was larger than that in Score 2 (Fig. 1).

**Statistical analysis:** Data were analyzed by using the ANOVA procedure. Means were separated by Duncan's

multiple range tests. The level of significance was determined at  $p < 0.05$ .

**RESULTS**

**Total white blood cell count:** On days 1, 7 and 14 of the experimental period, the total white blood cell counts of the broilers in all six sub-groups of the second group (those maintained at  $38 \pm 2^\circ\text{C}$  for 6 h per day and fed with polyphenols at 0, 100, 200, 300, 400 and 500 mg/kg) were higher ( $p < 0.05$ ) than that of the first-group broilers (those maintained at  $26 \pm 2^\circ\text{C}$  and fed with normal diet). On day 7, the total white blood cell count of the broilers fed with polyphenols at 200 mg/kg, was higher than that of the broilers receiving polyphenols at 400 mg/kg ( $p < 0.05$ ). On day 14, the total white blood cell count of the broilers fed with polyphenols at 500 mg/kg was higher than that of the broilers fed with polyphenols at 0 mg/kg ( $p < 0.05$ ). On day 21, the total white blood cell count of the broilers fed with polyphenols at 0 mg/kg was not different from that of the broilers maintained at  $26 \pm 2^\circ\text{C}$  ( $p > 0.05$ ). The total white blood cell count of the broilers fed with polyphenols at 200 mg/kg was higher than those of the broilers with the other feeds ( $p < 0.05$ ) (Table 1).

Table 1: Total White Blood Cell (TWBC) counts of the broilers in the first group and all six sub-groups of the second group

Environmental temperature									
38±2°C									
Parameter	Days	26±2°C	0 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	500 mg/kg	SEM
TWBC (cell/ml)	1	12089 <sup>b</sup>	20876 <sup>a</sup>	21791 <sup>a</sup>	21338 <sup>a</sup>	25905 <sup>a</sup>	26971 <sup>a</sup>	23619 <sup>a</sup>	2415.72
	7	17813 <sup>c</sup>	35810 <sup>ab</sup>	42870 <sup>ab</sup>	44190 <sup>a</sup>	43124 <sup>ab</sup>	33478 <sup>b</sup>	41752 <sup>ab</sup>	3143.76
	14	23467 <sup>c</sup>	40990 <sup>b</sup>	43733 <sup>ab</sup>	45105 <sup>ab</sup>	49067 <sup>ab</sup>	43378 <sup>ab</sup>	57448 <sup>a</sup>	4288.78
	21	26453 <sup>c</sup>	26209 <sup>c</sup>	28160 <sup>bc</sup>	39543 <sup>a</sup>	37008 <sup>ab</sup>	28178 <sup>bc</sup>	29714 <sup>bc</sup>	2811.80
	Mean	19955.5	30971.3	34138.5	37544	38776	33001.3	25207.3	3165.02

<sup>a,b</sup> and <sup>c</sup> within row, mean with no common superscript differ significantly ( $p < 0.05$ ); SEM = Standard Error of the Mean

Table 2: Total lymphocytes of the broilers in the first group and all six sub-groups of the second group

Environmental temperature									
38±2°C									
Parameter	Days	26±2°C	0 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	500 mg/kg	SEM
Lymphocytes (cell/ml)	1	8988 <sup>c</sup>	13495 <sup>bc</sup>	16247 <sup>ab</sup>	15960 <sup>ab</sup>	21243 <sup>a</sup>	22756 <sup>a</sup>	19031 <sup>ab</sup>	1225.78
	7	13477 <sup>c</sup>	27200 <sup>ab</sup>	31437 <sup>a</sup>	29851 <sup>ab</sup>	28326 <sup>ab</sup>	23927 <sup>b</sup>	28809 <sup>ab</sup>	1242.91
	14	15472 <sup>d</sup>	26590 <sup>c</sup>	29557 <sup>bc</sup>	30193 <sup>bc</sup>	36776 <sup>ab</sup>	33001 <sup>abc</sup>	41317 <sup>a</sup>	1746.72
	21	18353 <sup>c</sup>	18088 <sup>c</sup>	19136 <sup>c</sup>	28697 <sup>a</sup>	25216 <sup>ab</sup>	20597 <sup>c</sup>	21492 <sup>bc</sup>	1003.69
	Mean	14073 <sup>b</sup>	21343 <sup>ab</sup>	24094 <sup>ab</sup>	26175 <sup>a</sup>	27890 <sup>a</sup>	25070 <sup>ab</sup>	27662 <sup>a</sup>	1734.78

<sup>a,b</sup> and <sup>c</sup> within row, mean with no common superscript differ significantly ( $p < 0.05$ ); SEM = Standard Error of the Mean

Table 3: Weight and lesion scores of bursa of Fabricius of the broilers in the first group and all six sub-groups of the second group

Environmental temperature									
38±2°C									
Parameters	Days	26±2°C	0 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	500 mg/kg	SEM
Final BW (kg)	28	2.51	2.38	2.45	2.56	2.45	2.45	2.23	0.07
Relative BurW (g/kgBW)	28	2.08 <sup>a</sup>	0.83 <sup>b</sup>	1.05 <sup>b</sup>	1.36 <sup>ab</sup>	0.86 <sup>b</sup>	0.72 <sup>b</sup>	1.00 <sup>b</sup>	0.25
LSBF	28	0.33 <sup>c</sup>	2.67 <sup>a</sup>	2.00 <sup>a</sup>	0.66 <sup>bc</sup>	0.33 <sup>c</sup>	1.77 <sup>ab</sup>	2.33 <sup>a</sup>	0.22

<sup>a,b</sup> and <sup>c</sup> within row, mean with no common superscript differ significantly ( $p < 0.05$ ); Final BW = Final Body Weight; Relative BurW = Relative Bursa of Fabricius Weight; LSBF = Lesion Scores of Bursa of Fabricius; SEM = Standard Error of the Mean

**Lymphocyte count:** On day 1, 7 and 14 of the experimental period, the lymphocyte counts of the broilers in all sub-groups of the second group were higher than that of the broilers in the first group ( $p < 0.05$ ). However, on day 1 the lymphocyte count of the broilers receiving polyphenols at 0 mg/kg in their diet was not different from that of the broilers maintained at 26±2°C ( $p > 0.05$ ). When comparing the results among the six sub-groups of the second group, it was found that, on day 1, the lymphocyte counts of the broilers fed with polyphenols at 300 and 400 mg/kg were higher than that of the broilers receiving polyphenols at 0 mg/kg ( $p < 0.05$ ). On day 7, the lymphocyte counts of the broilers receiving polyphenols at 100 mg/kg in their diet was higher than that of the broilers receiving polyphenols at 400 mg/kg in the diets ( $p < 0.05$ ). On day 14, the lymphocyte count of the broilers fed with polyphenols at 500 mg/kg in their diet was higher than those of the broilers receiving polyphenols at 0, 100 and 200 mg/kg in the diets ( $p < 0.05$ ). The lymphocyte count of the broilers with 300

mg/kg polyphenols in the diet was higher than that of the broilers with 0 mg/kg polyphenols in the diet ( $p < 0.05$ ) (Table 2).

**Final body weight, relative weight and lesion scores of bursa of Fabricius:** On day 28 of the experimental period, final body weight of broilers in all groups were not different ( $p > 0.05$ ). The relative bursa of Fabricius weight of the broilers maintained at 26±2°C was higher than those of the broilers receiving polyphenols at 0, 400 and 600 mg/kg in their diets ( $p < 0.05$ ). The relative bursa of Fabricius weight of the broilers fed with 200 mg/kg polyphenols in the diets was not different from that of the broilers maintained 26±2°C ( $p > 0.05$ ). The lesion scores of the bursa of Fabricius of the broilers maintained at 26±2°C and those fed with 200 and 300 mg/kg polyphenols in the diets were not different ( $p < 0.05$ ), but lower than those of the broilers fed with 0, 100, 400 and 500 mg/kg polyphenols in their diets ( $p < 0.05$ ) (Table 3).

Table 4: NDV-HI titer of the broilers in the first group and all six sub-groups of the second group

Environmental temperature									
38±2°C									
Parameter	Days	26±2°C	0 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	500 mg/kg	SEM
NDV-HI titer (log <sub>2</sub> )	7	2.44	2.11	1.67	2.5	2.11	1.94	2.61	0.57
	14	3.50	1.75	1.67	2.5	2.00	2.00	1.00	0.35
	21	<1	<1	<1	<1	<1	<1	<1	-
	Mean	2.97	1.93	1.67	2.5	2.06	1.97	1.81	0.46

NDV-HI titer = Newcastle Disease Virus-Hemagglutination Inhibition titer; SEM = Standard Error of the Mean

**NDV-HI titer:** On days 7, 14 and 21 of the experimental period, the NDV-HI titers of the broilers in all groups were not different ( $p>0.05$ ) (Table 4).

## DISCUSSION

As the final body weights of the broilers in all groups were not different, polyphenols extracted from tamarind seed coat had no effect on the broilers' body weight. The result was in accordance with the report of Aengwanich *et al.* (2009b), which stated that polyphenols extracted from tamarind seed coat did not help improve the broilers' body weight at the later stage of the experimental period. On days 1, 7 and 14 of experimental period, the total white blood cell counts of the broilers maintained at 26±2°C were lower than those of the broilers maintained at 38±2°C. It can be said that total white blood cells of broilers increase when the body temperature increases. This result is similar to the report of Davis *et al.* (2008) and Aengwanich *et al.* (2003), who observed that when broilers were under stress and heat stress, their total white blood cells increased. Jain (1993) explained that glucocorticoid caused leukocytosis by inducing the increased release of leukocyte from the bone marrow reserve through the circulation. This was in accordance with the report of Puvadolpirod and Thaxton (2000a) who found that after broilers received ACTH, their total white blood cells increased.

When examining the 38±2°C broilers with different polyphenols feeds, it was found that on day 7, the total white blood cell count of the broilers fed with polyphenols at 200 mg/kg in their diets was higher than that of the broilers receiving polyphenols at 400 mg/kg in their diets. On day 14, total white blood cell count of the broilers fed with polyphenols at 500 mg/kg in their diets were higher than that of the broilers receiving polyphenols at 0 mg/kg in their diets. These phenomena showed that polyphenols extracted from tamarind seed coat also caused the increase of the total white blood cells in the broilers under heat stress. Furthermore, the different levels of polyphenols in their diets had various effects on the total white blood cells of heat stress broilers. However, the mechanism of polyphenols that produces this effect is still unknown.

On day 21, the total white blood cell count of the broilers maintained at 26±2°C was not different from those of the broilers maintained at 38±2°C and fed with polyphenols at 0, 100, 400 and 500 mg/kg in their diets. This means that temperature and polyphenols feed have no effect on the number of white blood cells in the later stage of the experiment. The results are similar to the reports of Puvadolpirod and Thaxton (2000b) and Aengwanich (2007) who found that broilers could adapt to stress and heat stress. Moberg and Mench (2000) also explained that when animals were subjected to repeated stresses, in the first few days after the exposure, they usually showed an increased response, but later the response decreased.

In terms of lymphocyte counts, on day 1, the lymphocyte counts of the broilers maintained at 26±2°C and the broilers maintained at 38±2°C and fed with polyphenols at 0 mg/kg in their diets were not different. This result indicated that early exposure to high environmental temperature had no effects on the number of lymphocytes in broilers. However, this result is different from the report of Davis *et al.* (2008) who observed that stress condition could cause alteration in leukocyte counts within 1-2 h. Borges *et al.* (1999), Altan *et al.* (2000), Campo and Davilla (2002) and Borges *et al.* (2004) reported that when broilers were under heat stress, the number of their lymphocytes decreased. Jain (1993) explained this phenomenon that glucocorticoid, released in broilers under stress causing lymphopenia, is attributed to lympholysis in blood, DNA damage and lymphoid tissue atrophy. It also increases the shift of lymphocytes from blood to other body compartments causing the decrease of lymphocytes in blood circulation (Compton *et al.*, 1990; Heckert *et al.*, 2002).

Moreover, on days 1, 7 and 14 of the experimental period, lymphocyte counts of the broilers maintained at 38±2°C increased and the lymphocyte counts of the broilers receiving polyphenols at 300, 400 and 500 mg/kg in their diets were higher than those of the broilers receiving polyphenols at 0, 100 and 200 mg/kg in their diets. For example, on day 1, the number of the lymphocytes in the broilers fed with polyphenols at 300 and 400 mg/kg in their diets were higher than that of the broilers receiving polyphenols at 0 mg/kg in their diets.

On day 14, the lymphocyte counts of the broilers fed with polyphenols at 300 and 500 mg/kg in their diets were higher than that of the broilers receiving polyphenols at 0 mg/kg in diet. In addition, the numbers of the lymphocytes in the broilers fed with polyphenols at 500 mg/kg in their diets were higher than those of the broilers receiving polyphenols at 100 and 200 mg/kg in their diets. However, on day 7, there was a reverse result. That is, the lymphocyte counts of the broilers fed with polyphenols at 0, 100 and 200 mg/kg in their diets were higher than that of the broilers receiving polyphenols at 400 mg/kg in their diets. These results indicated that polyphenols could increase the number of lymphocytes in the blood circulation. Aengwanich *et al.* (2009a,b) tentatively concluded that polyphenols extracted from tamarind seed coat could reduce heat stress in broilers.

On day 21, the total white blood cells of broilers fed with polyphenols at 200 mg/kg in their diets were higher than those of the broilers that received polyphenols at 0, 100, 300, 400 and 500 mg/kg in their diets. Furthermore, the number of the lymphocytes of the broilers fed with polyphenols at 200 and 300 mg/kg in diets were higher than those of the broilers with the other feeds. These results indicated that on day 21 of the experimental period, the diets with the polyphenols extracted from tamarind seed coat at 200 mg/kg could increase the number of white blood cells and lymphocytes. When considering the relationship between total white blood cells and lymphocytes, it was found that when the number of the total white blood cells increased, the amount of lymphocytes in blood circulation also increased.

Generally, after broilers were under heat stress, the bursa of Fabricius was atrophied. The number of lymphocytes in the bursa of the broilers under heat stress decreased (Aengwanich, 2008). This phenomenon explained that after broilers were exposed to high ambient temperature, corticosterone stored in the adrenal cortex was released into the blood circulation to help increase the broilers' metabolism. Corticosterone caused bursa of Fabricius atrophy and decreased lymphocytes (Aengwanich, 2009). Moreover, lesion scores of bursa of Fabricius increased (Pamok *et al.*, 2009). When heat stressed broilers received polyphenols extracted from tamarind seed coat at 200 mg/kg in their diets, it increased the lymphocyte level in their blood circulation and relative bursa of Fabricius weight and decreased lesion scores. Besides, the relative bursa of Fabricius weight and lesion scores of the broilers maintained at  $26\pm 2^{\circ}\text{C}$  and the broilers maintained at  $38\pm 2^{\circ}\text{C}$  and fed with polyphenols at 200 mg/kg in their diets were not different. These occurrences showed that polyphenols extracted from tamarind seed coat at 200 mg/kg in their diets could reduce the effect of heat stress on lymphocyte, relative

bursa of Fabricius weight and lesion scores of bursa of Fabricius in broilers. This was in accordance with the report of Aengwanich and Suttajit (2010), which stated that polyphenol extracted from tamarind seed coat could reduce heterophil/lymphocyte ratio, indirect parameter of corticosterone and serum malondialdehyde in heat stress broilers. Therefore, mode of action of polyphenols extracted from tamarind seed coat reduced corticosterone and oxidative stress in heat stressed broilers. Besides, when comparing the relative bursa of Fabricius weight and the lesion scores of bursa of Fabricius in the broilers receiving polyphenols at 300, 400 and 500 mg/kg in their diets with the broilers receiving polyphenols at 200 mg/kg in their diets, it was found that the relative bursa of Fabricius weight of the formers were seemingly lower than the latter. On the other hand, the lesion scores of bursa of Fabricius of the broilers receiving polyphenols at 400 and 500 mg/kg in their diets were likely to be higher than that of the broilers fed with polyphenols at 300 mg/kg in their diets. These phenomena indicated that after broilers received polyphenols extracted from tamarind seed coat over 200 mg/kg in their diets, the ability of the extract in reducing heat stress in broilers decreased. Therefore, the suitable level of polyphenols extracted from tamarind seed coat for reducing the impact of heat stress to the immunity of broilers was at 200 mg/kg in diet. In addition, when examining the level of NDV-HI titer, it was found that throughout the experimental period, the NDV-HI titer levels of the broilers maintained at  $38\pm 2^{\circ}\text{C}$  in all feed groups were not different. This phenomenon indicated that polyphenols extracted from tamarind seed coat has no effect on humoral immunity in heat stressed broilers.

In conclusion, the results clearly indicated that polyphenols extracted from tamarind seed coat increased total white blood cells, lymphocytes and relative bursa of Fabricius weight. Moreover, the extracts could reduce lesion scores of bursa of Fabricius, but has no effect on humoral immunity. The most effective level of polyphenols extracted from tamarind seed coat that can help reduce the impact of heat stress to broilers' immune system is at 200 mg/kg in diet.

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