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Effects of Polyphenols Extracted from Tamarind (*Tamarindus indica* L.) Seed Coat on Differential White Blood Cell Count in Broilers (*Gallus domesticus*) Exposed to High Environmental Temperature

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Abstract: Tamarind (*Tamarindus indica* L.) is a plant that belongs to the Leguminosae family and grows naturally in tropical and subtropical regions. Tamarind seed coat is composed of polyphenols especially procyanidin oligomers. The objective of this experiment was to determine the effect of polyphenols extracted from tamarind seed coat on differential white blood cell counts of broilers maintained at high environmental temperature. Broilers were divided into 2 groups. In group 1, broilers were maintained in environmental temperature at 26±2°C throughout experimental period, and in group 2, broilers were maintained in environmental temperature at 38±2°C and received polyphenols i.e. 0, 100, 200, 300, 400 and 500 mg/kg in diets. Differential white blood cell counts were investigated on days 1, 7, 14 and 21 of experimental period. The results revealed the following information: Lymphocyte and basophil levels of broilers maintained in the environmental temperature at 38±2°C and received polyphenols at 400 mg/kg in diet were increased ($p<0.05$). On the other hand, the heterophil and monocyte levels of broilers maintained in the environmental temperature at 38±2°C and received polyphenols at 400 mg/kg in diet were decreased ($p<0.05$). This occurrence indicated that polyphenols extracted from tamarind seed coat could reduce heat stress in broilers.

Key words: Lymphocyte, heterophil, monocyte, basophil, eosinophil, heat stress

INTRODUCTION

Heat stress is one of the most important factors adversely affecting overall poultry production in the tropics (Zulkifi *et al.*, 2000; Marshaly *et al.*, 2004; Naseem *et al.*, 2005). Exposure of chickens to heat stress causes significant behavioral and physiological response resulting in poor growth performance, immunosuppression and high mortality rates (Mujahid *et al.*, 2005). Scientists are attempting to search for methods to reduce the adverse effects of this condition. Many methods have been studied: supplemented substances in water and/or diet i.e. electrolyte (Borges *et al.*, 2003; Borges *et al.*, 2004), ascorbic acid (Puron *et al.*, 1994; Mckee *et al.*, 1997; Puthongsiriporn *et al.*, 2001; Lohakare *et al.*, 2005; Roussan *et al.*, 2008), vitamin E (Puthongsiriporn *et al.*, 2001), acetylsalicylic acid (Puron *et al.*, 1994; Roussan *et al.*, 2008), sodium bicarbonate (Puron *et al.*, 1994; Puron *et al.*, 1997; Roussan *et al.*, 2008), potassium chloride (Smith and Teeter, 1992; 1993; Beker and Teeter, 1994; Roussan *et al.*, 2008), phosphoric acid (Daskiran *et al.*, 2004), citric acid (Daskiran *et al.*, 2004), manganese proteinate (Sands and Smith, 1999), chromium picolinate (Sands and Smith, 1999), 2-hydroxy-4-(methylthio) butanoic acid

(Ribeiro and Penz, Jr., 2001), DL-methionine (Ribeiro and Penz, Jr., 2001), carbon dioxide (Smith and Teeter, 1993) and ammonium chloride (Smith and Teeter, 1993) etc.

Corticosteroid concentrations in blood have been used as a measure of environment stress in birds. In addition, the relationship between Adrenocorticotrophic Hormone (ACTH) and leukocytes response has been widely examined (Altan *et al.*, 2000). White blood cell parameters i.e. heterophil (Altan *et al.*, 2000; Post *et al.*, 2003; Borges *et al.*, 2003; Borges *et al.*, 2004; Lien *et al.*, 2007), lymphocyte (Altan *et al.*, 2000; Borges *et al.*, 2003; Borges *et al.*, 2004; Yalcin *et al.*, 2004; Lien *et al.*, 2007), monocyte, basophil and eosinophil (Altan *et al.*, 2000) are used for indication of heat stress in chicken. Altan *et al.* (2000) reported that when broilers were exposed to high environmental temperature, their lymphocyte and monocyte levels decreased. On the other hand, heterophil and basophile levels increased, but eosinophil was not changed. At present, these parameters are generally used as an indicator of heat stress in chickens.

The tamarind (*Tamarindus indica* L.) is a tree-type of plant which belongs to the Leguminosae family, grows

naturally in tropical and subtropical regions and is one of the most important plant resources for food materials (Tsuda *et al.*, 1995). The pulp is used in spices and seasoning and it is accepted as a herbal medicine in the world. The flower and leaf are eaten as vegetables. The germ obtained from the seed is used for manufacturing tamarind gum (Tsuda *et al.*, 1994). Other parts of the plant present antioxidant, antihepatotoxic, antiinflammation, antimutagenic and antidiabetic (Martinello *et al.*, 2006). Pumthong (1999) reported that tamarind seed coat was composed of polyphenols including tannins, anthocyanidin and oligomeric anthocyanidins. Moreover, Gu *et al.* (2003) found that the seed of tamarind contained 29.32 procyanidin oligomers and 101.89 g/kg high molecular weight tannins, respectively. We have hypothesized that the polyphenols extracted from tamarind seed coat could reduce heat stress in broilers maintained in high environmental temperature. Therefore, the aim of this experiment was to examine the effect of polyphenols extracted from tamarind seed coat on lymphocyte, heterophil, monocyte, basophil and eosinophil levels of broilers maintained in high environmental temperature. Results from this study would provide fundamental knowledge for using the polyphenols from natural products as feed additives in the poultry production in tropical region.

MATERIALS AND METHODS

Chemical: Fresh tamarind fruits were purchased from a local market in Maha Sarakham Province, in the northeastern part of Thailand. The pericarp and seeds were carefully separated from the fruit. The seeds were heated in a hot air oven at 140°C, for 45 min, cooled and cracked to separate their outside brown layer. Only brown-red seed coats were collected and these were then ground into fine powder (Komutarin *et al.*, 2004). The polyphenols in the tamarind seed coat powder were extracted one time by using 95% ethanol as a solvent (1:5, w:v). Tamarind seed coat powder and ethanol were mixed and pH adjusted to 4 by using 5% acetic acid. The mixture rested at room temperature for 72 h (was shaken every 12 h) and the upper solution was collected for further processing. The high molecular weight tannin in the extract was precipitated by using protein from non-fat milk. The protein was prepared by mixing fresh non-fat milk with 5% acetic acid (5:1, v/v). It was then held at room temperature overnight and then the supernatant was discarded. The extracts with high molecular weight tannin and protein from non-fat milk were mixed, held at room temperature overnight and the upper solution was collected. The pH of the extract was adjusted to 6 by using 3M of NaOH, rested at room temperature overnight and the upper solution was collected. The polyphenols in the solution were dried by using a spray drying method. Total phenolic compounds in each gram of

tamarind seed coat extract powder were analyzed by using the Folin-Ciocalteu method (Kahkonen *et al.*, 1999).

Animal welfare: This experiment was performed under the care and use of experimental animals committee of Mahasarakham University.

Animals and experimental design: One hundred and forty seven 1 day old broilers (*Gallus domesticus*) were obtained from a local commercial farm near the laboratory of the Faculty of Veterinary Medicine and Animal Science, Mahasarakham University. The chickens were brooded and fed a standard ration. A completely randomized design (3 replications/ treatment and 7 broilers/experimental unit) was used. When they were 18 days old, broilers were divided into 2 groups. In group1, broilers were maintained in the environmental temperature at 26±2°C throughout experimental period. In group 2, broilers were maintained in environmental temperature at 38±2°C and received polyphenols extracted from tamarind seed coat i.e. 0, 100, 200, 300, 400 and 500 mg/kg in diets *ad libitum* (proximate composition of diet: CP = 21.4%, ME = 3,110 kcal/kg) with continuous light and water supplies.

Sample collection and differential white blood cell count procedure: On days 1 (initial experimental day, on which over a period of 8 h, broilers received tamarind seed coat extract before blood sample collection), 7, 14 and 21 of the experimental period, blood sample collections were performed after the chickens were subjected to high environmental temperatures for 2 h. Chickens were restrained manually and two milliliters of blood sample were collected from the cervical vein using a 3-ml syringe, 23-gauge needle 1.5 inches in length then placed in microtube with EDTA. The samples were cooled to approximately 4°C, using icepacks and transferred to the laboratory within 2 h after blood collection. Blood films were made, air dried then stained with Giemsa-Wright's stain. Differential white blood cell counts were performed using the standard avian guidelines of Ritchie *et al.* (1994).

Statistical analysis: All data were analyzed using the ANOVA procedure. Means were separated by Duncan's multiple range tests. The level of significance was determined at $p < 0.05$.

RESULTS

Lymphocyte: On day 1 of experimental period, the lymphocyte levels of broilers maintained at 38±2°C and received polyphenols at 100, 200, 300, 400 and 500 mg/kg in diets were significantly higher than broiler that received polyphenols at 0 mg/kg diet ($p < 0.05$). Lymphocyte levels of broilers that received polyphenols

Table 1: Heterophil, lymphocyte, monocyte, basophil and eosinophil of broilers were maintained at 26±2°C and broilers maintained in the environmental temperature at 38±2°C and received polyphenols extracted from tamarind seed coat at 0, 100, 200, 300, 400 and 500 mg/kg in diets

Parameters	Days	Environmental temperature							SEM
		26±2°C		38±2°C					
		0 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	500 mg/kg		
Heterophil (%)	1	10.74 ^a	11.28 ^a	5.71 ^b	9.33 ^a	4.95 ^b	4.06 ^b	5.28 ^b	1.14
	7	9.45	8.57	9.48	12.28	13.71	10.06	8.19	1.52
	14	7.67 ^{abc}	13.59 ^a	12.04 ^{ab}	12.71 ^{ab}	4.84 ^c	6.44 ^b	10.57 ^{abc}	1.95
	21	10.11	9.24	9.95	7.44	9.07	8.09	6.24	1.51
Lymphocyte (%)	1	74.13 ^{bc}	65.34 ^c	75.86 ^{ab}	75.14 ^{ab}	82.29 ^{ab}	84.46 ^a	80.47 ^{ab}	2.97
	7	76.37 ^a	75.85 ^a	74.08 ^{ab}	67.67 ^{bc}	65.80 ^c	71.62 ^{abc}	68.51 ^{abc}	2.45
	14	66.05	65.40	67.85	66.66	75.27	76.96	71.95	3.95
	21	69.83	68.80	67.96	73.00	68.98	73.04	71.76	3.83
Monocyte (%)	1	11.66 ^b	20.15 ^a	12.10 ^b	12.05 ^b	9.43 ^b	8.00 ^b	11.43 ^b	2.09
	7	11.95	12.71	13.05	14.67	15.43	14.25	19.76	1.69
	14	21.44	16.80	17.01	16.56	15.96	13.17	13.52	2.81
	21	15.89	16.54	17.38	14.85	18.43	16.27	18.00	3.00
Basophil (%)	1	2.38	2.20	2.57	1.86	2.53	3.01	2.38	0.38
	7	1.41 ^d	2.48 ^{cd}	2.57 ^{bcd}	4.36 ^a	4.24 ^{ab}	3.25 ^{abc}	3.09 ^{abcd}	0.52
	14	3.52	3.07	2.43	2.91	3.35	2.26	3.33	0.57
	21	3.22	3.73	3.08	3.87	2.78	1.72	3.38	0.44
Eosinophil (%)	1	1.09 ^{bc}	1.03 ^{bc}	3.76 ^a	1.62 ^b	0.81 ^{bc}	0.47 ^c	0.33 ^c	0.33
	7	0.82	0.38	0.71	1.03	0.81	0.81	0.44	0.24
	14	1.30	1.15	0.68	1.15	0.58	1.18	0.62	0.39
	21	0.94	1.69	1.68	0.84	0.75	0.88	0.62	0.28

^{a,b,c} and ^{bc} within row, mean with no common superscript differ significantly (p<0.05); SEM = Standard Error of the Mean

at 400 mg/kg in diet were significantly higher than that of broilers maintained at 26±2°C (p<0.05). On day 7, lymphocyte levels of broilers maintained at 26±2°C and broilers maintained in the environmental temperature at 38±2°C and received polyphenols at 0 mg/kg in diet were significantly higher than broilers that received polyphenols at 200 and 300 mg/kg in diet (p<0.05), but not significantly different with broiler received polyphenols at 100, 400 and 500 mg/kg in diets (p>0.05). On days 14 and 21 of experimental period, the lymphocyte count of broilers maintained in both conditions were not significantly different (p>0.05) (Table 1).

Heterophil: On day 1, heterophil of broilers maintained at 38±2°C and received polyphenols at 100, 300, 400 and 500 mg/kg in diets was significantly lower than that of broilers that received polyphenols at 0 and 200 mg/kg in diets and broilers maintained at 26±2°C (p<0.05). On day 14, the heterophil of broilers maintained at 38±2°C and received polyphenols at 0 mg/kg in diet were significantly higher than broilers that received polyphenols at 300 and 400 mg/kg in diets (p<0.05). On days 7 and 21 of experimental period, the heterophil of broilers maintained in both conditions were not significantly different (p>0.05) (Table 1).

Monocyte: On day 1, the monocyte level of broiler maintained in environmental temperature at 38±2°C and received polyphenols at 0 mg/kg in diet were significantly higher than that of broilers that received polyphenols at 100, 200, 300, 400 and 500 mg/kg in diets and broilers maintained at 26±2°C (p<0.05). On days 7, 14 and 21 of experimental period, monocyte count of broilers in both conditions were not significantly different (p>0.05) (Table 1).

Basophil: On day 7, basophil level of broilers maintained at 38±2°C and received polyphenols at 200 and 300 mg/kg in their diets was significantly higher than broilers that received polyphenols at 0 mg/kg in diet and broilers maintained at 26±2°C (p<0.05). On days 1, 14 and 21 of experimental period, basophile count of broilers in both conditions were not significantly different (p>0.05) (Table 1).

Eosinophil: On day 1, eosinophil level of broilers maintained at 38±2°C and received polyphenols at 100 mg/kg in diet was significantly higher than that of broiler that received polyphenols at 0, 200, 300, 400 and 500 mg/kg in diets and broilers maintained at 26±2°C (p<0.05). The eosinophil level of broilers that received polyphenols at 200 mg/kg in diet was significantly higher than that of broilers that received polyphenols at 400 and

500 mg/kg in diets ($p < 0.05$). On days 7, 14 and 21 of experimental period, the basophil count of broilers in both conditions was not significantly different ($p > 0.05$) (Table 1).

DISCUSSION

On days 1 and 7 of experimental period, the lymphocyte count of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 0 mg/kg in their diet was not different than that of broilers at $26 \pm 2^\circ\text{C}$. This result showed that the environmental temperature at $38 \pm 2^\circ\text{C}$ had no effect on the lymphocyte level of the broilers. The results of this study were different from the report of Altan *et al.* (2000), Borges *et al.* (1999), Campo and Davila (2002), Borges *et al.* (2004). They reported that when birds were under heat stress, lymphocyte levels decreased. Whereas, on day 1, after broilers received polyphenols for 8 h, the lymphocyte level of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 100, 200, 300, 400 and 500 mg/kg in their diet was higher than that of broiler that received polyphenols at 0 mg/kg diet. The lymphocyte level of broiler maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 400 mg/kg in diet, was higher than that of broiler maintained at $26 \pm 2^\circ\text{C}$. Moreover, on day 7, the lymphocyte count of broilers maintained at $26 \pm 2^\circ\text{C}$ and broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 0 mg/kg in their diet was not different from the count of broilers that received polyphenols at 100, 400 and 500 mg/kg in their diet. This study showed that polyphenols extracted from tamarind seed coat could increase the lymphocyte level of broilers maintained at $38 \pm 2^\circ\text{C}$ and the most effective amount of polyphenol extract from tamarind seed coat to lymphocyte level was 400 mg/kg in the diet.

On day 1, after broilers were exposed to the high environmental temperature at $38 \pm 2^\circ\text{C}$ for 8 h, the heterophil of broiler that received polyphenols at 0 mg/kg in diet was not different from broilers at $26 \pm 2^\circ\text{C}$. Whereas, on day 14, heterophil of broilers that received polyphenols at 0 mg/kg in diet was higher than broilers at $26 \pm 2^\circ\text{C}$ by nearly two times, but not statistically significantly different. In a clinical diagnosis, this result concluded that the heterophil of heat stressed broilers on day 14 increased. This was in accordance with reports of Maxwell *et al.* (1992), Cooper and Washburn (1998), Borges *et al.* (1999), Altan *et al.* (2000) and Borges *et al.* (2004). They reported that when chickens were under heat stress, the heterophil in blood circulation increased. Jain (1993) suggested that glucocorticoid, which marks released when chickens are under stress, causes heterophilia primarily by inducing an increased release of heterophil from the bone marrow reserve through the circulation. The heterophil of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 100, 300, 400 and 500 mg/kg in diets was lower than that of broilers maintained at $38 \pm 2^\circ\text{C}$ and received

polyphenols at 0 and 200 mg/kg in diets and broilers maintained at $26 \pm 2^\circ\text{C}$. Moreover, on day 14, the heterophil of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 300 and 400 mg/kg in diets was higher than that of broilers that received polyphenols at 0 mg/kg in diet. These results showed that polyphenols from tamarind seed coat at 300 and 400 mg/kg in diets could reduce the heterophil of broilers maintained at $38 \pm 2^\circ\text{C}$ on days 1 and 14 after heat exposure and the most effective level of polyphenols to heterophil was 400 mg/kg in diet.

Bush (1991) explained that when animals were under stress causing release of endogenous glucocorticoid, this condition caused monocytosis. It arises because monocytes move from a marginal pool into the circulation. On day 1, the number of monocytes of broilers maintained in the environmental temperature at $38 \pm 2^\circ\text{C}$ and received tamarind seed coat extract at 0 mg/kg in diet was higher than that of broilers maintained at $26 \pm 2^\circ\text{C}$. Therefore, broilers maintained at $38 \pm 2^\circ\text{C}$ were under heat stress. When broilers maintained at $38 \pm 2^\circ\text{C}$ for 8 h and received polyphenols at 100, 200, 300, 400 and 500 mg/kg in diet, their monocyte level was lower than that of broilers that received polyphenols at 0 mg/kg in diet. These results indicated that polyphenols extracted from tamarind seed coat could reduce heat stress in broilers maintained at $38 \pm 2^\circ\text{C}$. The most effective level of polyphenols from tamarind seed coat to monocyte was 400 mg/kg in diet.

On day 7, the basophil count of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 200 and 300 mg/kg in diets was higher than that of broilers that received polyphenols at 0 mg/kg in diet and broilers maintained at $26 \pm 2^\circ\text{C}$. The basophil count of broilers maintained at $38 \pm 2^\circ\text{C}$ and received polyphenols at 200 and 300 mg/kg in diets was not different from that of broilers that received polyphenols at 400 and 500 mg/kg in their diets. Jain (1993) found glucocorticoid induced basopenia in different species of animals. Moreover, Bush (1991) suggested that glucocorticoid can decrease the number of basophil in cases of basophilia. In this study, polyphenols increased the number of basophils in blood circulation. Therefore, the result from this study showed that polyphenols from tamarind seed coat could reduce the effect of high environmental temperature to broilers that used the number of basophils as a parameter and the most effective level of polyphenols from tamarind seed coat to basophil was 200 mg/kg in diet.

Bush (1991) found that in cases of acute stress, the release of adrenaline (epinephrine) causes first a mild eosinophilia followed by a moderate eosinopenia 'peaking' after about 4 h. This was in accordance with Jain (1993). He reported that a transient eosinophilia may occur during the endogenous release of epinephrine under physiological stress. Besides, Bush

(1991) suggested that the release of endogenous glucocorticoids which cause an eosinopenia by several mechanisms: inducing lysis of eosinophil, increasing phagocytosis of eosinophils by macrophage, stimulating eosinophil migration into the small intestine and lymphoid tissues and reducing bone marrow production of eosinophils. In this study, on day 1, the eosinophil count of broilers maintained at $38\pm 2^{\circ}\text{C}$ and received polyphenols at 100 mg/kg in diet was higher than that of broilers that received polyphenols at 0, 200, 300, 400 and 500 mg/kg in diets, and broilers maintained at $26\pm 2^{\circ}\text{C}$. This result showed that polyphenols from tamarind seed coat at 100 mg/kg in diet had increased the number of eosinophil or eosinophilia at the early period of heat exposure, like the effect of endogenous epinephrine that reported by Bush (1991) and Jain (1993). On the other hand, the eosinophil count of broilers that received polyphenols at 400 and 500 mg/kg in diets was lower than that of broilers that received polyphenols at 200 mg/kg in their diet. This phenomenon showed that polyphenols at 400 and 500 mg/kg in diets had decreased number of eosinophil or eosinopenia in the early period of heat exposure like the effect of endogenous glucocorticoid that reported by Bush (1991). However, the mechanism of polyphenols that produces these effects is unknown.

Conclusion: Polyphenols extracted from tamarind seed coat increased lymphocyte and basophil levels and decreased heterophil monocyte and both increased and decreased levels of eosinophil. The most effective level of polyphenols to broilers maintained at high environmental temperature $38\pm 2^{\circ}\text{C}$ was 400 mg/kg in diet. Finally, the polyphenols had the main effect to the differential white blood cell count of broilers maintained at high environmental temperature during days 1-7 of experimental period.

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