

EFFECT OF ANTANAN (*CENTELLA ASIATICA*) AND VITAMIN C ON THE BURSA OF FABRICIUS, LIVER MALONALDEHIDE AND PERFORMANCE OF HEAT-STRESSED BROILERS

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ABSTRACT

High environmental temperatures may cause heat stress in poultry. This may increase water consumption, decrease feed consumption and in rum, decrease productivity level. In addition, high temperature contributes to oxidative stress, a condition where oxidant activity (free radicals) exceeds antioxidant activity. In our research, antanan (*Centella asiatica*) and vitamin C were utilized as anti heat-stress agents for heat-stressed broilers. We used 120 male broilers 2-6 weeks old, kept at 31.98 ± 1.94 °C during the day and 27.36 ± 1.31 °C at night. The data collected were analyzed with a completely randomized factorial design of 2 x 3 (2 levels of vitamin C, 3 levels of antanan at 4 replications) and continued with the contrast-orthogonal test when significantly different. The results indicate that the treatments of 5 and 10% of antanan with or without 500 ppm of vitamin C and vitamin C alone significantly ($P < 0.05$) decreased the heterophil/lymphocyte (H/L) ratio and liver malonaldehyde (MDA). These treatments, however, significantly ($P < 0.05$) increased the bursa of Fabricius weight, feed consumption and body weight gain. It could be concluded that basal ration administered with 5% antanan and 500 ppm vitamin C could effectively prevent broilers from heat stress. The results support the conclusion that a basal ration supplemented with 5% antanan and 500 ppm of vitamin C or their combinations, effectively reduces heat stress in broilers.

Key words : Heat stress/ *Centella asiatica* / Vitamin C

INTRODUCTION

High environmental temperatures may result in the accumulation of body heat load so that the body suffers from heat stress. As one of the homeothermic species, poultry could maintain their body temperature relatively constant by increasing respiration rate and water consumption and/or decreasing feed consumption. As a result, their growth rate and productivity will decrease.

May and Lott (2001) showed that body weight gain of 3 to 7-week-old male broilers raised at a temperature of 30°C was 1869 g significantly lower than for those raised at 22°C with body weight gains of 2422 g and feed conversion decreased from 3.28 to 2.54. The lower performances of broilers raised at high temperatures may have occurred as a result of lowered secretion of thyroid hormones (Geraert *et al.* 1996), decreased blood hemoglobin and hematocrit levels (Yahave/a. 1997), or increased excretion of some minerals (Belay *et al.* 1992) and some amino acids (Tabiri *et al.* 2001).

In addition, heat stress may also cause oxidative stress in the body and develop abundant free radicals, promoting the occurrence of peroxidation of membrane lipids and hence attacking DNA and protein membranes (Rahman 2003). Takahashi and Akiba (1999) indicated that feeding oxidized lipids to broilers significantly decreased feed consumption, body weight gain, plasma vitamin C, and plasma α -tocopherol. In fact, the results were followed by an increase in plasma malonal-dehyde (MDA) and blood heterophyl/lymphocytes (H/L) ratios as biological indices of stress in avian species.

Antanan/pegagan (*Centella asiatica* (L.) Urban), one of the medicinal plants containing active materials such as asiatic acid, asiaticoside, and madecasic, is readily available and evidently eliminates stress in rats (Kumar and Gupta 2003). Shukla *et al.* (1999) reported that placing asiaticoside on rats wound increases curability and accelerates enzymatic and non-enzymatic antioxidant activities of new-growing tissues. In addition, vitamin C reportedly eliminated cold stress (Sahin and Sahin 2002) and heat stress in poultry (Puthongsiriporn *et al.* 2001) and showed synergism with some active materials contained in antanan (Bonte *et al.* 1994). With this in mind, we have examined the effect of antanan (*Centella asiatica*) and vitamin C on the bursa of Fabricius, liver malonaldehyde and performance in heat-stressed broilers.

MATERIALS AND METHODS

This research used 2 to 6-week-old male broilers placed in several pens located in an open poultry house. Each pen was flitted with a 40-Watt lamp and a zinc-plate backing functioning as a heat reflector. Temperature and relative humidity measurements obtained at noon and afternoon were $31.98 \pm 1.28^\circ\text{C}$ and $78.82 \pm 5.43\%$, respectively. Temperature and relative humidity measurements at night and early morning were $27.36 \pm 0.88^\circ\text{C}$ and $86.23 \pm 3.93\%$, respectively. The levels of 500 ppm vitamin C and 10% antanan (all plant parts) of ration used were established during preliminary trials. Vitamin C was dissolved in drinking water and served in the morning, two hours after the broilers received their last waterfeeding.

One-hundred-and-twenty-two-week-old male broilers were randomly allocated into 24 pens, 5 broilers each. Antanan (5% and 10%) was mixed with other ingredients to make three different rations as follows: 1) The control ration contained the calory as metabolizable energy (ME) 3245.02 kcal/kg and 20.84% crude protein , 2) A5 = 5% antanan contained ME 3222.95 kcal/kg and crude protein 20.91%, and 3) A10 = 10% antanan contained ME 3202.87 kcal/kg and crude protein 20.99%. The ration formulation and nutrient composition of treatments are presented in Table 1.

Table 1. Ration formulation and nutrient composition

Ingredients	A0 (%)	A5 (%)	A10 (%)
Ground corn	63.00	57.60	52.35
Soybean meal	17.00	17.00	17.00
Fish meal	11.20	11.20	11.20
Feather meal	4.80	4.80	4.80
Antanan	0.00	5.00	10.00
Coconut oil	2.25	3.05	3.50
Dicalcium phosphate	0.10	0.10	0.00
Calcium carbonate	0.90	0.75	0.65
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Calculated nutrient content :			
Metabolizable energy(kkal/kg)	3245.02	3222.94	3202.87
Protein (%)	20.84	20.91	20.99
Lipid (%)	6.16	6.15	6.96
Phosphor (%)	0.65	0.65	0.63
Calcium (%)	1.03	1.28	1.02
Crude fiber (%)	2.46	3.28	4.09
Lysine (%)	1.39	1.38	1.36
Methionine (%)	0.51	0.49	0.48
Analyzed Vitamin C (mg/100 g)	20.21	22.92	25.88

A0 = 0% antanan as control (K); A5 = 5% antanan and A10 = 10% antanan

The broilers were subjected to six treatments, 20 broilers each, as follows:

- 1) K (Control)/ration neither contained antanan nor vitamin C.
- 2) A5/ration was supplemented with 5% antanan
- 3) A10/ration was supplemented with 10% antanan
- 4) C, drinkwater contained 500 ppm vitamin C
- 5) A5C, combination of A5 and C, and
- 6) A10C, combination of A10 and C

Variable measurements:

- 1) Relative bursa of Fabricius weight taken from 4-week-old broilers, by weighing the organ and divided by body weight (Puvadolpirod and Thaxton 2000).
- 2) Heterophyl/lymphocyte ratio was taken from 4-week-old broilers, by hemo-cytometer method. Blood was diluted 1:101 in a red blood cell pipette with Nat and Herrick diluent. The total leucocyte count includes heterophils, lymphocytes, monocytes, basophils, and eosinophils is divided by the number of lymphocytes.
- 3) Liver malonaldehyde (MDA) taken from 6-week old broilers, by measuring the thiobarbituric acid (TEA) value using Tarladgis method (Apriyantono *et al.* 1989). Distillate from liver sample with pH: 1.5, is added to the TBA reagents, covered, mixed, and incubated in boiling water bath for 35 minutes. After cooling, the absorbance of filtrate (D) was determined at 528 nm wave length. The TBARS values = 7.8 D were expressed as mg/kg of malonaldehyde per kg of tissue.
- 4) Feed consumption, body weight gain, and feed conversion are measured for 4 weeks (from 2 to 6-week-old broilers). Feed consumption was determined by weighing the given ration minus the leftover. Body weight gain was measured by weighing the final body weight at 6 weeks old minus the body weight at 2 weeks old. Feed conversion was measured by dividing feed consumption with body weight gain.

Statistical analysis

The collected data were analyzed using a completely randomized factorial design (CRD) 2 x 3 (2 levels of vitamin C i.e. 0 and 500 ppm and 3 levels of antanan i.e. 0, 5 and 10% of rations at 4 replications), and where applicable, continued with orthogonal contrast test according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The results of the effect of antanan and vitamin C administration on bursa of Fabricius weight, H/L ratio and liver MDA contents are presented in Table 2. The data for feed consumption (FC), body weight gain (BWG), and feed conversion are presented in Table 3.

From Table 2, the average relative of 4 - week bursa of Fabricius weight for controls (K) is significantly lower than those treated with A5, A10, C, A5C, or A10C. The bursa of Fabricius weight is similar for all treatments from A5 to A10C.

These results suggest that antanan, vitamin C, or their combinations increase bursa of Fabricius weight of broilers that suffered heat stress.

The ability of antanan to stimulate lymphoid gland weight was reported in stressed rats by Sharma *et al.* (1996). Antanan contains phenol compounds that potentially can prevent peroxidation of lipid membranes including T- and B lymphocyte membranes. T-lymphocytes produce cellular immunities while B lymphocytes produce humoral immunities produced by the bursa of Fabricius in bird species. Phenol compounds of tea extracts were potentially capable of stimulating the production of lymphoid cells in rats (Murtini *et al.* 2003). On the other hand vitamin C acts, as a water-soluble antioxidant capable of protecting lymphocyte from suffering heat stress (Puthongsiriporn *et al.* 2001). As a result, the number of circulating lymphocytes increased so that the H/L ratio decreased.

Table 2 shows a relationship between the increase of the bursa of Fabricius weight and decrease in H/L ratio because the bursa of Fabricius is a lymphoid organ producing lymphocytes. Thus, the smaller the bursa of Fabricius size, the fewer lymphocytes will be produced, and in turn, the higher the H/L ratio will be. Increase of the relative lymphoid organ weight occurred as a result of antanan feeding which was demonstrated by Sharma *et al.* (1996), while increase of the relative bursa of Fabricius weight as a result of supplementing vitamin C to broilers was reported by Anim *et al.* (2000).

Table 2. Effects of the administration of antanan and vitamin C on the weight of bursa of Fabricius, H/L ratio and liver MDA levels of male broilers 2 to 6 weeks old.

Variables	Treatments					
	K	A5	A10	C	A5C	A10C
Bursa of Fabricius (g/kg BW)	1.45±0.63 ^a	2.89±0.85 ^b	2.80±0.29 ^b	2.38±0.73 ^b	2.73±1.08 ^b	2.88±0.32 ^b
H/L ratio	0.93±0.41 ^b	0.49±0.20 ^a	0.39±0.16 ^a	0.79±0.41 ^a	0.46±0.34 ^a	0.43±0.11 ^a
MDA (mg/100g)	15.09±4.00 ^d	14.16±4.70 ^c	6.40±2.9 ^b	2.79±1.04 ^a	9.96±8.17 ^b	8.46±6.54 ^b

^{a-d} Means ± SE in a row with no common superscript differ significantly (P < 0.05)

K= control; A5= 5% antanan; A10= 10% antanan; C= 500ppm vitamin C; A5C= 5% antanan + 500 ppm vitamin C and A10C= 10% antanan + 500 ppm vitamin C

Heat stress generating oxidative stress may increase MDA content as a result of lipid peroxidation, especially for unsaturated fatty acids of membrane cells. Feeding

antioxidants, i.e. antanan and vitamin C, significantly decreases liver MDA content. Besides of being able to relieve free radicals by releasing an electron and a proton (a hydrogen ion), phenol compounds are also able to provide a chelating effect in such a way that phenols bind to transition ions. Unbound metals might increase free radicals (Pietta 2000). In addition, phenol compounds are characterized by flavonoids that are able to decrease fluidity of cell membranes so that it may decrease diffusion of free radicals and MDA contents. This is also true for vitamin C, a water-soluble antioxidant with 2 hydroxyl groups at C_2 and C_3 that are readily oxidized (Sediaoetama 1987).

Table 3. Effects of the administration of antanan and vitamin C on feed consumption (FC), body weight gain (BWG), and feed conversion (FC/BWG)

Variables	Treatments					
	K	A5	A10	C	A5C	A10C
FC (g)	2711±196 ^a	3026±229 ^b	3071±14 ^b	2883±36 ^b	3156±247 ^b	2935±198 ^b
BWG (g)	1181±66 ^a	1297±113 ^b	1347±11 ^b	1254±35 ^b	1376±135 ^b	1330±100 ^b
Feed conversion	2.30±0.16 ^a	2.35±0.31 ^a	2.42±0.30 ^a	2.30±0.23 ^a	2.31±0.32 ^a	2.22±0.29 ^a

^{a,b} Means ± SE in a row with no common superscript differ significantly (P < 0.05)

K= control; A5= 5% antanan; A10= 10% antanan; C= 500ppm vitamin C; A5C= 5% antanan + 500 ppm vitamin C and A10C= 10% antanan + 500 ppm vitamin C

In addition, it is unmistakable that feeding antanan and vitamin C increases feed consumption and body weight gain of broilers over four weeks (2-6 weeks of age) but not for feed conversion (Table 3). This agrees with the reports of Anim *et al.* (2000) for broilers and Sharma and Sharma (2002) for rats suffering from stress. Antanan contains antioxidants such as phenol compounds that are capable of eliminating oxidative stress processes (Blokhina 2000), as is apparent from the decrease of H/L ratios, liver MDA levels and increase of bursa of Fabricius weights reported here.

CONCLUSIONS

Feeding antanan or vitamin C or the combination of antanan and vitamin C increases bursa of Fabricius weight, feed consumption, and body weight gain but decreases H/L ratio and liver MDA levels. The combination of 5% antanan and

vitamin C tends to increase feed consumption and body weight gain and , therefore this treatment tends to be very effective in alleviating heat stress in broilers.

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