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Effects of Dietary Ascorbic Acid on Blood Haematological Profile, Serum Biochemical Components and Tonic Immobility Reaction of Male Turkeys under Summer Condition

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This experiment was performed to determine the effects of dietary ascorbic acid (ASA) supplementation on blood components, leukocyte and tonic immobility of male turkeys under summer conditions. A total of 120 one-day-old male turkeys were fed with three different diets. The experimental diets were: control without ASA (ASA-0), 150 mg of ASA/kg (ASA-150) and 300 mg of ASA/kg (ASA-300) of diet. The turkeys received the experimental diets for 18 weeks. Diets in mash form and water were provided *ad libitum*. In the ASA-150 and ASA-300 groups, the eosinophile and basophile ratios were higher than those of the ASA-0 group at 12 weeks of age ($P<0.05$ and $P<0.001$), but lymphocyte (L), heterophil (H), monocyte, H: L ratio and tonic immobility were not influenced by the ASA levels ($P>0.05$). Both dietary ASA levels caused a quadratic decrease in serum cholesterol, low density lipoprotein (LDL) and glutamic-oxaloacetic transaminase (SGOT) ($P<0.05$), whereas dietary ASA caused a liner increase in serum iron concentration of turkeys ($P<0.05$). Serum glucose, triglyceride, high density lipoprotein (HDL), total protein, albumin, globulin, lactate dehydrogenase (LDH), glutamic-pyruvate transaminase (SGPT), urea, uric acid and alpha amylase were not affected by the addition of ASA to the diets ($P>0.05$). As a conclusion, blood haematological profile, serum biochemical components and tonic immobility of turkeys were not significantly affected by dietary ASA levels at chronic high summer temperatures in general, and, therefore, there is no need for ASA supplementation in turkeys' diets under summer temperatures that do not exceed 31.7℃.

Key words: ascorbic acid, summer condition, turkey

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Introduction

Turkeys in modern environments can experience a variety of stressors in environmental, pathological, or nutritional forms. The global environmental temperature is also showing an upward trend. In the turkey industry of the world, huge economic losses occur every year because of mortality and decreased production in relation to high environmental temperatures.

New turkey strains are more sensitive to high ambient temperatures than earlier strains (Veldkamp *et al.,* 2002). Fast-growing turkeys produce more heat and have greater difficulty in dissipating heat in hot temperatures, which leads to higher body temperatures and reduced performance (Veldkamp, 2002).

Earlier reports showed that turkeys could be reared in environmental temperatures between 10 and 26℃ if dietary energy was regulated (Albuquerque *et al.,* 1978). Yahav (1999) reported a significant decline in weight gain and feed intake when turkeys were raised at 35℃ versus 25℃ and body temperatures were at normal levels. High ambient temperatures depressed feed intake, resulting in reduced BW gain and breast meat yield of turkeys when compared to optimum temperatures (Veldkamp *et al.,* 2000).

A number of methods are available to alleviate the negative effects of high environmental temperatures on the performance of poultry in terms of feed consumption, body weight gain and feed efficiency. As it is expensive to cool the buildings in which the animals are housed, such methods are focused mostly on dietary manipulation. Studies have shown that antioxidant nutrient supplementation, especially the addition of ascorbic acid (ASA), can be used to diminish the negative effects of environmental stress in broilers (Sahin *et al.,* 2003). There has been considerable interest in the possible nutritional role for ASA on the basis that endogenous synthesis may not

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be adequate to meet the full needs of poultry at all times or that requirements for ASA may be increased under certain circumstances, such as stressful conditions (Whitehead and Keller, 2003). Dietary ASA increases resistance to infections and stresses of all kinds in poultry (John, 1992). Also, dietary ASA may alter serum cholesterol, glucose, total protein and albumen concentrations in stressed broilers (Sahin *et al.,* 2003; Kutlu and Forbes, 1993). We did not find any literature related to the effect of dietary ASA supplementation on blood traits in turkeys.

Stressful conditions can generate a stage of tension that evokes behavioral and physiological chain reactions in broilers (Mahmoud *et al.,* 2004). The effects of environmental stressors on avian leukocytes have been widely considered in numerous studies (Altan *et al.,* 2000; Campo and Davila, 2002; Yalçin *et al.,* 2003). Nathan *et al.* (1976) reported that heat stress caused a significant decrease in leukocyte counts in cocks. The heterophil: lymphocyte (H : L) ratio in the avian blood is related to stress status. H : L ratio of about 0.2, 0.5 and 0.8 are characteristics of low, medium and high degrees of stress, respectively (Gross and Siegel, 1993). Heterophil numbers increase during mildly or moderately stressful conditions; consequently, the H: L ratio can be used to detect the presence of physiological stress and extreme stress conditions resulted inheterophenia and basophilia in fowl (Maxwell and Robertson, 1998). Özkan *et al.* (2004) reported that turkeys exposed to acute heat stress (30.5℃), compared to turkeys in the control group (20℃), experienced increased body temperature, heterophil, basophile, and monocyte proportions and H : L ratio but a decreased number of lymphocytes.

Tonic immobility (TI) has been described as an adaptive psycho-physiological response which is characterized by reduced responsiveness induced by physical restraint; it has been used as a measure of fearfulness and heat stress status in poultry (Jones, 1996; Yalçin *et al.,* 2003). Yalçin *et al.* (2003) reported that exposing birds to heat stress increased the duration of TI. However, there was no research found that examined TI measured according to heat stress and dietary ASA supplementation in turkeys.

To the best of our knowledge, the physiological demand for dietary ASA is not well documented in turkeys under optimum and high environmental temperatures. Therefore, the present study was conducted to determine the effects of dietary inclusion levels of ASA concentrations on blood biochemistry, leukocyte cell profile and TI duration under summer heat conditions.

Materials and Methods

Experimental birds and diets

A total of 120 male one-day-old BUT6 turkey chicks were obtained from a local commercial hatchery. A standard brooding program was applied for the first three weeks. Turkey toms were reared at natural summer temperatures from 4 to 18 weeks of age. Poults were divided into 3 treatments groups. Each group consisted of five replications with eight birds per replicate. The experimental diets were: control without ASA (ASA-0), 150 mg of ASA per kg of feed (ASA-150) and 300 mg of ASA per kg of feed (ASA-300) (L-ascorbic acid, Rovimix® Stay C® 35, Roche, Levent-Istanbul, Turkey). Feed was formulated at four week intervals according to NRC (1994) recommendations. Fresh feed with ASA was mixed at 7 day intervals. Small amounts of the basal diets were first mixed with the respective amounts of ASA as a small batch and then with a larger amount of the basal diets until they were homogeneously mixed with the total amount of the respective diets. Body weights (BW) of toms were obtained individually at 0, 4, 8, 12, 16 and 18 weeks of age, and body weight gain (BWG) was calculated from these data. Feed intake (FI) was measured on pen bases on the same days and corrected for mortality. The feed conversion ratio (FCR) was calculated as the ratio between total BWG to total FI of all birds in each pen. Lighting included 24 h of light from 0 to 3 d and 16 h of light and 8 h of darkness from 4 to 126 d of age. This study was conducted during the months of May through September 2006 in Izmir, Turkey. This region is one of the hottest regions of Turkey in the summer season. The experimental diets used are provided in Table 1. The temperature and humidity of the house were recorded three times daily at 8 : 00, 15 : 00 and 20 : 00 o'clock. Feed and water were provided *ad libitum.*

Feed chemical analyses

The dry matter content of feed samples was determined by oven-drying at 105℃ for 18 h. The ether extract content of feed samples was obtained by the Soxhlet extraction using anhydrous diethyl ether. The Kjeldahl method was used for the analysis of the total nitrogen content of feed samples, and crude protein was expressed as nitrogen × 6.25 (AOAC, 1980). The crude ash content was determined after heating the feed in a muffle furnace at 550℃ for 16 h. The crude fibre content of the feed was determined using 12.5% H_2SO_4 and 12.5% NaOH solutions (Nauman and Bassler, 1993).

Tonic immobility

Tonic immobility was observed at 12 and 16 weeks of age, but we failed in the observation due to heavy body weight and seriously injury of birds at 16 weeks of age. Therefore, we presented only 12 weeks of age TI observations in the table. The test was performed on 20 poults for each group. Chicks were transferred to an empty room and direct eye contact between the observer and bird was avoided. The duration of TI, the time interval until the bird righted itself, and the number of inductions (10 s periods of restraint) necessary to attain TI were recorded. If TI could not be induced after 5 attempts, the bird was considered to be unsusceptible and a score of 0 was recorded. If the bird remained in TI after 10 min, the test was stopped and a maximum score of 600 s was given for duration (Campo and Davila, 2002).

Leukocyte cell counts

Blood samples of turkeys were collected at 12 and 16

Table 1. **The composition of diets at 0 to 18 weeks (%)**

Ingredients	Age, week				
	0 to 4	4 to 8	8 to12	12 to 16	16 to 18
Maize	43.76	40.00	40.00	40.00	40.00
Wheat	—	9.81	21.25	29.42	36.04
Soybean meal	51.48	45.41	33.98	25.51	18.55
Vegetable oil	0.18	0.95	1.34	2.00	2.78
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25	0.25
Limestone	2.04	1.72	1.49	1.32	1.16
Mono calcium phosphate	1.85	1.45	1.18	1.08	0.87
Coccidiostat	0.10	0.10	0.10	0.10	0.10
L-Lysine	0.14	0.06	0.06	0.05	—
DL-Methionine	—	0.05	0.15	0.07	0.05
NaCl	0.20	0.20	0.20	0.20	0.20
Chemical composition (%)[2]					
Dry matter	90.73	90.46	90.70	90.65	90.52
Crude protein	28.37	24.58	20.87	19.07	16.32
Crude fiber	4.08	3.40	3.14	3.35	2.64
Crude ash	7.78	6.85	5.37	5.72	4.94
Crude fat	2.46	3.11	3.20	3.66	3.97
Calcium	1.24	1.04	0.88	0.78	0.66
Available phosphorus	0.57	0.48	0.41	0.37	0.32
Lysine	1.56	1.40	1.12	0.91	0.74
Methionine	0.46	0.41	0.39	0.34	0.27
ME (MJ/kg)	11.19	11.65	12.18	12.68	13.17

[1] Supplied per kg of the feed: Vitamin A, 15000 I.U; Vitamin D_3, 2000 I.U; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B_1 (thiamin), 3.0 mg; Vitamin B_2 (riboflavin) 6.0 mg; Vitamin B_6, 5.0 mg; Vitamin B_{12}, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg; Colin Chloride, 400 mg; Manganese, 80.0 mg; Iron, 35.0 mg; Zinc, 50.0 mg; Copper, 5.0 mg; Iodine, 2.0 mg; Cobalt, 0.4 mg; Selenium, 0.15 mg.

[2] Calcium, available phosphorus, lysine, methionine and metabolizable energy (ME, MJ/kg) contents of feed was calculated according to NRC (1994).

weeks of age. A total of 60 birds were used (20 birds from each group) to determine blood cell count. In the differential leukocyte cell counts, two drops of blood were collected from the wing vein, and blood smears were made on duplicate glass slides. Blood smears were stained using Wright stains approximately 4 h after preparation with methyl alcohol fixation. Leucocyte cells [heterophils (H), lymphocytes (L), eosinophiles, monocytes, and basophils] were counted by the same person to 100 cells per individual smear, and the H: L ratios were calculated (Gross and Siegel, 1986).

Blood biochemical analysis

A sample of blood was obtained by vena puncture of the left wing vein from two toms in each pen (total 30 birds) at 18 weeks of age for blood chemical analysis. Then blood samples were kept on ice and transferred to the laboratory and centrifuged at 1500 g for 10 min.; the serums were then removed and stored at −20℃ until analyzed. The serum components, such as glucose, triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin, globulin, lactate dehydrogenase (LDH), alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT) urea and iron, were measured by using the kits from the same manufacturer with an auto-analyzer (model BT 3000 plus, Biotechnica, Rome, Italy).

Statistical Analysis

The data were analyzed using the GLM procedure of SAS (1996). Linear and quadratic polynomial contrasts were used to evaluate treatment effects. The results of the statistical analysis are shown as mean values and standard error of the means (SEM) in the tables.

Results

Average house temperatures throughout the experiment were 26.1±0.6, 31.5±0.3, and 31.7±0.3℃ and measures of mean house humidity were 50.8±1.2; 38.2±0.9 and 36.6±0.7 (%), determined at 8: 00, 15: 00 and 20: 00 o'clock, respectively. The average livability of turkeys was 96.7±2.87 (%) for the experiment, and there were no statistically significant differences among the groups.

The effects of ASA on lymphocyte, heterophil, eosinophile, basophile, monocyte counts and H: L ratio at 12 and 16 weeks of age are given in Table 2. Dietary ASA supplementation (ASA-150 and ASA-300) caused a quadratic increase in eosinophile ($P<0.05$) and a linear increase in basophile ($P<0.001$) counts at 12 weeks of age. Basophile count in the ASA-0 group was significantly

Table 2. **Effects of ascorbic acid on lymphocyte, heterophil, eosinophile, basophile, monocyte and H : L ratio at 12 and 16 weeks of age**

Leukocyte components	Ascorbic acid levels (mg/kg)				Probabilities	
	Week	0	150	300	SEM	Linear	Quadratic
Lymphocyte, %	12	66.60	65.05	64.50	1.20	0.216	0.442
	16	64.35	63.30	65.90	1.34	0.417	0.390
Heterophil, %	12	21.85	20.95	20.85	1.04	0.498	0.758
	16	21.00	21.75	19.10	1.12	0.237	0.234
Eosinophile, %	12	7.30	8.70	8.35	0.34	0.040*	0.016*
	16	8.40	8.45	8.30	0.47	0.879	0.974
Basophile, %	12	2.75	3.85	4.75	0.26	<0.001**	<0.001**
	16	4.90	4.85	5.25	0.27	0.352	0.513
Monocyte, %	12	1.50	1.45	1.55	0.14	0.794	0.873
	16	1.35	1.65	1.45	0.13	0.582	0.238
Heterophil: Lymphocyte ratio	12	0.33	0.33	0.33	0.02	0.974	0.991
	16	0.34	0.35	0.33	0.02	0.253	0.248

SEM: pooled standard error of the means; *: $P<0.05$; **$P<0.01$.

Table 3. **Effects of ascorbic acid on some blood components at 18 weeks of age**

Blood components	Ascorbic acid levels (mg/kg)			SEM	Probabilities	
	0	150	300		Linear	Quadratic
Glucose, mg/dL	254.3	255.2	257.3	7.75	0.783	0.961
Triglyceride, mg/dL	57.2	45.0	50.2	5.90	0.848	0.353
Cholesterol, mg/dL	135.7	116.8	121.3	5.36	0.591	0.049*
HDL, mg/dL	58.80	51.10	55.13	2.61	0.868	0.257
LDL, mg/dL	67.90	52.84	58.87	3.80	0.751	0.047*
Total protein, g/dL	3.56	3.82	3.61	0.12	0.221	0.391
Albumin, g/dL	1.69	1.82	1.73	0.05	0.572	0.158
Globulin, g/dL	1.87	2.00	1.88	0.03	0.860	0.552
LDH, U/L	517.0	615.2	576.4	39.72	0.308	0.231
SGOT, U/L	2.67	0.60	1.50	0.45	0.058	0.018*
SGPT, U/L	14.3	13.8	11.5	1.04	0.065	0.147
Urea, mg/dL	3.90	4.20	4.41	0.33	0.272	0.550
Iron, ug/dL	75.6	102.80	109.90	8.79	0.033*	0.088

SEM: pooled standard error of the means; *: $P<0.05$.

lower than those of ASA-150 and ASA-300 groups, and basophile count of the ASA-150 group was lower than that of ASA-300 group at 12 weeks of age. Furthermore, the heterophil, lymphocyte, monocyte numbers and H: L ratios of chicks were not affected by the dietary treatment at 12 weeks of age. On the other hand, dietary ASA did not affect the leukocyte cell profile at 16 weeks of age ($P>0.05$).

The addition of 150 and 300 mg of ASA/kg to the diets caused a quadratic decrease in serum cholesterol, LDL and SGOT ($P<0.05$). But levels of serum iron concentration were increased linearly by supplementation of 150 and 300 mg of ASA/kg to the diets ($P<0.05$, Table 3). Dietary ASA did not affect serum levels of glucose, triglyceride, HDL, total protein, albumin globulin, LDH, SGPT or urea ($P>0.05$).

TI durations in the turkeys fed with the control, 150 mg of ASA/kg and 300 mg of ASA/kg diets were 278.0±38.3, 302.7±38.3 and 332.9±39.3 seconds ($P>0.05$), respectively. Dietary ASA supplementation to the basal diets slightly increased TI durations, but these increases were not significant among the groups ($P>0.05$).

Body weight, body weight gain, feed intake and feed conversion ratio for the period from 0 to 18 weeks are presented in Table 4. Dietary ASA supplementation did not affect body weight, body weight gain, feed intake or feed conversion ratio of the turkeys ($P>0.05$).

Discussion

Results of the present experiment indicate that the dietary ASA supplementation (ASA-150 and ASA-300) resulted in a significant increase in the eosinophile and

Table 4. **Effects of ascorbic acid levels on body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of male turkeys**

Item	Week	Ascorbic acid levels (mg/kg)			SEM	Probabilities	
		0	150	300		Linear	Quadratic
BW, kg	0	0.059	0.057	0.058	0.001	0.840	0.925
	18	13.25	13.26	13.54	0.21	0.329	0.546
BWG, g/day	0 to 18	104.5	106.9	107.0	0.47	0.328	0.546
FI, g/day	0 to 18	283.6	287.2	282.8	0.81	0.834	0.650
FCR, feed, g: gain, g	0 to 18	3.10	3.01	3.10	0.81	0.988	0.810

SEM: pooled standard error of the means.

basophile ratio at 12 weeks of age, but dietary ASA levels did not affect leukocyte cell counts at 16 weeks of age. Özkan *et al.* (2004) reported that heterophil, basophile, and monocyte proportions and H : L ratio were increased and lymphocyte levels were lowered by acute heat exposure (30.5℃) compared to 20℃ in turkeys at 16 wks of age. The current results agreed with Aengwanich *et al.* (2003), who reported that dietary ASA supplementation did not affect lymphocyte cell counts in broilers. There is a relationship between ASA and the H : L ratio that has been documented; ASA reduced the H : L ratio in birds under heat, cold, sound, fasting or managemental stress in broilers (Gross, 1992; McKee and Harrison, 1995; Zulkifli *et al.,* 2000; Lohakare *et al.,* 2005).

Ascorbate is necessary for the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation. As this reaction is the rate-limiting step of the cholesterol catabolism in the liver, ASA deficiency induces a marked deceleration of this reaction, leading to cholesterol accumulation in the liver and the blood (Naidu, 2003). In the present study, dietary ASA supplementation decreased serum cholesterol and LDL concentrations but did not affect serum levels of triglyceride and HDL. The reduction of blood cholesterol concentrations due to the addition of ASA has been demonstrated in broilers (Kutlu and Forbes, 1993; Sahin *et al.,* 2003; Ahmed *et al.,* 2005). However, Clegg *et al.* (1976) and McKee *et al.* (1997) noted that dietary ASA in broilers did not affect plasma cholesterol levels. In this study, dietary ASA supplementation numerically decreased levels of serum triglyceride, but these differences were not significant among the groups. These results support those of McKee *et al.* (1997), who found that 150 ppm of dietary ASA did not affect plasma triglycerides in broilers. However, it is reported that dietary ASA decreased serum triglycerides in broilers (Clegg *et al.,* 1976) and quails (Gursu *et al.,* 2004; Seyrek *et al.,* 2004). Sahin *et al.* (2002a) reported that dietary ASA supplementation (100 and 200 mg/kg of diet) resulted in a decrease in serum triglycerides and cholesterol concentrations, but protein and albumin concentrations were increased compared to control groups in Japanese quails.

Levels of serum total protein, albumin and globulin concentrations were higher in the ASA-150 and ASA-300 groups than those of the control group, but differences were not significant. In contrast, it is reported that dietary ASA supplementation increased serum total protein in broilers (Sahin *et al.,* 2003) and quails (Sahin *et al.,* 2002 b). Seyrek *et al.* (2004) noted that dietary ASA increased serum albumin in quails. Also, they reported that the increase of serum albumin concentrations in the experimental groups could be partially explained by the reduction of synthesis and secretion of corticoids in birds fed diet-supplemented ASA. However, according to these results, there is discrepancy in terms of the effectiveness of ASA on serum protein.

ASA has an important role in the absorption of iron from the gut (John, 1992). In the present study, increasing dietary ASA supplementation also resulted in the linear increase in serum iron concentration. This result is supported by Criste *et al.* (1996), and they concluded that dietary supplementation of 1.5 g of ASA/kg increased iron absorption from the gut and serum iron concentration in growing chickens.

In the current study, TI durations were not influenced by dietary ASA supplementation. Birds fed with ASA-0 had the shortest TI duration (278.0 second) but were not statistically different from the birds fed the ASA-150 (302.7 second) and ASA-300 diets (332.9 second). There have no research in the literature on TI in turkeys. However, this observation agreed with Campo and Carnicer (1993), who did not find a significant effect of dietary ASA (400 mg/kg diet) on the period of TI in laying hens. In contrast to our study, Jones (1996) reported that dietary ASA supplementation shorted TI duration in laying hens.

In conclusion, there is a possibility that turkeys adapted to these chronic high summer temperatures and, therefore, the ambient temperature was not too high to cause significant changes when examining some criteria in turkeys. On the other hand, it is difficult to establish the degree of stress experienced by birds under practical conditions due to species, strain, degree of environmental temperature, humidity, diet, etc. Therefore, responses to dietary ASA may be more variable in poultry. Furthermore, additional and more detailed research is required to explore the effects of ASA supplementation on the diets on turkeys in different conditions.

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