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Effects of Dietary Ascorbic Acid Supplementation on Growth Performance, Carcass, Bone Quality and Blood Parameters in Broilers During Natural Summer Temperature

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Abstract: This experiment was conducted to determine dietary supplementation of ascorbic acid (ASA) on the performance, carcass, bone traits and, some serum indices of broilers. A total of 180 day-old chicks were distributed into 3 treatment groups with 6 replicate containing 10 chicks each. The experimental diets were: (1) control, no dietary ASA supplementation (ASA0), (2) dietary ASA supplementation 150 mg kg^{-1} (ASA150) of diet and (3) 300 mg kg^{-1} of diet (ASA300). The experiment was lasted up to 42 days of age. Dietary ASA did not affect body weight and gain and feed conversion ratio but quadratically changed daily feed intake of broilers at 21-42 and 0-42 days of age ($p<0.05$). The carcass and parts yields, dry matter, crude protein and pH of meat and bone traits were not affected ($p>0.05$) but crude fat and thigh meat colour were linearly changed ($p<0.05$) by the dietary supplement. Dietary ASA supplementation quadratically changed the serum alanine aminotransferase and linearly decreased aspartate amino transferase ($p<0.05$) but did not affect other serum constituents. To conclude, dietary ASA supplementation have some beneficial effects on broiler meat composition and colour and serum AST and ALT levels during natural summer temperature.

Key words: Broiler, summer heat stress, ascorbic acid

INTRODUCTION

The deleterious effects of high environmental temperature on broiler production have been well documented. Body weight gain, feed consumption and feed efficiency were significantly lowered by subjecting chicks to heat stress (Kutlu, 2001). Seasonal heat stress may negatively influence the performance of poultry. The Aegean Region is one of the hottest regions of the Turkey. Several methods are available to alleviate the negative effects of high environmental temperature on performance of poultry. Because of the high cost and impractical of cooling animal buildings, interest in dietary manipulations has been increased. Studies have shown that antioxidant nutrient supplementation, especially ascorbic acid (ASA) can be used to attenuate the negative effects of environmental stress (Sahin *et al*., 2003). There has been considerable interest in the possible nutritional role for ASA on the basis that endogenous synthesis may not be adequate to meet the full needs of poultry at all times or requirements for ASA may be increased under certain circumstances as stressful conditions (Whitehead and Keller, 2003). Dietary ASA supplementation improved

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performance of heat challenged broiler chickens and lowered corticosterone level in the blood (Pardue *et al*., 1985). Thus, substantial attention has been paid to the role of nutritional additives to minimize the effect of heat stress. Supplementation of ASA to broiler diet may alleviate effect of heat stress on the performance of broiler chicks reared under heat stress (Kutlu and Forbes, 2000; Kutlu, 2001; Çelik and Öztürkcan, 2003). Broiler chicken seems to have a special appetite for ASA and tends to consume more diet supplemented with ASA at high temperature (Kutlu and Forbes, 2000). However, there are some reports that dietary ASA supplementation did not positively affect broiler performance (Stilborn *et al*., 1988; Marron *et al*., 2001), or decreased live weight of broilers (Njoku, 1986).

The beneficial effects of ASA on poultry performance in heat stress condition are well documented (Pardue and Thaxton, 1986; Whitehead and Keller, 2003). However, effectiveness of ASA on carcass, bone and blood parameters were a little examined compared to performance traits. Dietary ASA supplementation may improve carcass traits (Kutlu, 2001; Sahin *et al*., 2003; Lohakare *et al*., 2005). Ascorbic acid may influence bone development by mediating the biosynthesis of 1,25-dihydroxycholecalciferol and collagen (Farquharson and Jefferies, 2000; McCormack *et al*., 2001) and also, may be involved calcium mobilization in bone (Dorr and Balloun, 1976). Several investigators observed positive effects of ASA on some bone traits (Lohakare *et al*., 2004; Lohakare *et al*., 2005) but others failed to show effect of ASA (Franchini *et al*., 1993; McCormack *et al*., 2001). On the other hand, dietary ASA supplementation may affect blood constituents (Clegg *et al*., 1976; Kutlu and Forbes, 1993a; Sahin *et al*., 2003). However, the responses of poultry performance, carcass, bone and blood parameters to supplemental ASA have been inconsistent. Therefore, the aim of this study was determine the effects of dietary ASA supplementation on growth performance, carcass and bone and blood parameters of broilers under natural summer hot condition.

MATERIALS AND METHODS

Animals, Diets and General Procedures

A total of 180 day-old male Ross-308 broiler chicks were individually weighed, wing banded and distributed as randomly into three treatments with 6 replicate pens and 10 chicks per pen (10 birds m^{-2}) and fed from 0 to 42 days of age. Floor pen was furnished with fresh wood shavings litter. Three experimental diets based on maize-wheat-soybean were formulated according to NRC (1994). The treatments were: (1) control without any addition (C, ASA0), (2) ascorbic acid (L-ascorbic acid, Rovimix® Stay C®35, Roche, Levent-Istanbul, Turkey) was added 150 mg kg^{-1} of diet (ASA150) and (3) 300 mg kg^{-1} of diet (ASA300). Fresh feed with ASA was prepared at 7 days intervals. Small amounts of the basal diet were first mixed with the respective amounts of ASA as a small batch and then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. Chicks were fed a starter diet throughout 0 to 21 days of age and fed a grower diet throughout 22 to 42 days of age. The experimental diets used in this study are given in Table 1. The experiment was performed throughout from June 08 to July 18 2006 at the Ege University, Odemis Vocational School and Poultry Research Unit. The house was artificially heated to provide standard brooding temperatures (28 to 34°C) from hatch to 7 days of age. The lighting schedule was 24L: 0D from 0 to 3 day and 23L: 1D (darkness from 24:00 to 01:00) from 4 to 42 days of age. Feed and water were consumed *ad libitum*.

Determination of Performance Traits

The individual body weight (BW) of birds was determined at 0, 21 and 42 days of age. Body weight gain (BWG) was calculated from 0 to 21, 22 to 42 and 0 to 42 days. Total feed intake at subgroup base was measured at 21 and 42 day. Mortality was recorded daily and feed intake was

Table 1: The composition of starter diet at 0 to 3 weeks and grower diets at 3 to 6 weeks (g kg^{-1})

	Diets (day)	
Composition of starter diets	0 to 21	22 to 42
Ingredient		
Yellow maize	433.18	400.00
Wheat	90.27	185.95
Soybean meal	207.71	195.36
Soybean cake	150.00	150.00
Fish meal	50.00	-
Vegetable oil	40.00	36.76
Ground limestone	12.24	14.74
Dicalcium phosphate	10.06	10.89
Sodium chloride	2.00	2.00
DL-Methionine	1.04	0.80
Anticoccidial (cocistac)	1.00	1.00
Vitamin and mineral premix[1]	2.50	2.50
Analyzed composition (g kg^{-1})		
Dry matter	911.80	909.80
Crude protein	223.30	193.60
Crude fiber	55.20	36.40
Crude ash	56.80	53.30
Calculated composition (g kg^{-1})		
Total calcium	86.70	92.00
Available phosphorus	40.00	34.00
Lysine	12.20	10.40
Methionine	4.50	3.80
Metabolizable energy (MJ kg^{-1})	13.39	13.34[1]

Supplied mg kg^{-1} of diet: Vitamin A, 15000 I.U; Vitamin D3, 2000 I.U; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin) 6.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg; Choline 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]Dry matter, crude protein, crude fiber, ether extract and crude ash contents of diets were analyzed, total calcium, available phosphorus, lysine, methionine and metabolizable energy values of diets were calculated

adjusted for mortality. Daily feed intake (DFI) and feed conversion ratio (DFI g / BWG g) (FCR) was calculated for 0 to 21, 22 to 42 and 0 to 42 days of age. Average ambient temperatures were 28.1±0.47, 33.1±0.27 and 31.7±0.25°C and humidity 50.31±1.03, 38.6±0.81 and 37.03±0.69% for 8 am, 3 pm and 8 pm, between 8 to 42 days, respectively.

Determination of Carcass and Meat Traits

Twelve males were selected according to their BW from each dietary treatment and killed by cervical dislocation at 42 days of age. The carcass, abdominal fat pat (excluding gizzard fat), empty gizzard, liver, heart, kidney weights were recorded individually. Then breast and thigh portions were separated from carcass and weighted. Relative body part weights were obtained by calculation as weight of part *100: total carcass weight. The cold carcass weight was recorded after carcasses were kept at +4°C for 18 h.

Six breast and thigh meat samples each group were collected in plastic trays, weighed and stored in an air tight plastic bag in a freezer (-20°C) until samples were required for analysis. They were homogenized using a blender and analyzed for dry matter, nitrogen, ether extract and crude ash. The dry matter, crude protein, ether extract, crude ash of feeds and meat samples were analyzed according to AOAC (1980). The crude fibre content of feedstuffs was determined by using 12.5% H_2SO_4 and 12.5% NaOH solutions (Naumann and Bassler, 1993). The pH value of the samples was determined with a pH meter (Hanna Instruments-8413) by thrusting the probe into the breast and thigh. The colors of breast and thigh were measured using a Minolta colorimeter (CM508d) to measure CIE Lab values (L* measures relative lightness, a* relative redness and b* relative yellowness).

Determination of Bone Traits

All of the chicks left shank length and width were measured with a caliper rule on day 42. After chicks slaughter, twelve left tibia bones each group were removed and cleaned of adheral tissues and then weighed; length and width (at midpoint) were measured with a digital caliper. Bones were frozen at -20°C until analyses. After thawing to room temperature bone breaking strength was determined by Instron Testing Machine and the bones were subjected to test at the midshaft of each bone until they fractured (Norgaard-Nielsen, 1990). The centre of each bone was aligned with the breaking probe (10 mm diameter) which approached at 30 mm min^{-1}. The supports for each bone were 30 mm apart. The breaking strength was determined from the failure point (peak) of each loading curve. The ash content of tibia bone was determined after heating in a muffle furnace at 550°C for 16 h.

Determination of Blood Components

Twelve blood samples per group were obtained by venepuncture of left wing vein at 42 days of age for blood biochemical analysis. The blood samples were kept on ice and transferred to the laboratory where they were centrifuged at 1500 g for 10 min. and serums were removed and stored at -20°C until analyzed. The serum total protein, albumin, glucose, triglyceride, total cholesterol, high density lipoprotein (HDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using kit from the same manufacturer with an auto analyzer (model BT 3000 plus, Biotechnica, Rome, Italy).

Statistical Analyses

The data were analyzed using the GLM procedure of SPSS (1998). Linear and quadratic polynomial contrasts were used to evaluate treatment effects. Because no biologically significant cubic effects were detected, only the results of linear and quadratic contrasts are reported in tables. The results of statistical analysis were shown as mean values and standard error of the means (SEM) in tables.

RESULTS AND DISCUSSION

Performance Traits

The BW, BWG and FCR of broilers were not statistically influenced by the dietary ASA supplementation at the experimental period (Table 2). However, in the ASA150 group DFI quadratically decreased ($p<0.05$) and FCR was the best in this group. It is generally assumed that dietary ASA supplementation increased feed intake of birds at the heat stress condition. However in the present study there is no difference in terms of DFI between control and ASA150 or ASA300. On the other hand, DFI in ASA300 group was higher than that of ASA150 group. Dietary ASA300 have more efficient on DFI than ASA150 level. The daily protein consumption of groups were calculated as 10.19, 9.93 and 10.54 g; and 25.93, 24.72 and 26.67 g for control, ASA150 and ASA 300 groups at 0 to 21 and 22 to 42 days of age respectively. Also daily energy consumption of groups were calculated as 0.61, 0.60 and 0.63 MJ kcal/day; and 1.79, 1.70 and 1.84 MJ kcal/day for control, ASA150 and ASA 300 groups at 0 to 21 and 22 to 42 days of age, respectively. Due to lacking marked differentiates in feed intake and feed protein and energy content, consumed daily protein and energy values were similar. It is well known heat stress begins to occur when ambient temperature rises above 27°C in poultry and it causes a decrease in feed intake and BWG (Kutlu, 2001). The present study was carried out under practical condition and daily temperature range of 28.1 to 33.1°C. However, Balnave (2004) reported that birds grown in semitropical and tropical areas have a better acclimate ability than moderate temperature. In these areas, temperatures below 32°C are not considered a seriously stress factor. On the other hand, birds adapt better to a high daily maximum temperature when the night

Table 2: Effects of ascorbic acid levels on body weight, body weight gain, feed intake and feed conversion ratio

	Ascorbic acid (mg kg^{-1})				p-value	
Characteristics	0	150	300	SEM	Linear	Quadratic
Body weights (g bird^{-1})						
0 day	43.01	43.64	42.29	0.49	NS	NS
21 day	752.20	714.14	734.04	10.9	NS	NS
42 day	2137.80	2075.22	2155.60	33.29	NS	NS
Body weight gain (g day^{-1})						
0 to 21	33.80	31.93	32.94	0.55	NS	NS
21-42	66.00	64.84	67.68	1.52	NS	NS
0 to 42	49.89	48.37	50.32	0.87	NS	NS
Feed intake (g day^{-1})						
0 to 21	45.62	44.48	47.19	1.23	NS	NS
21 to 42	133.92	127.70	137.74	1.77	NS	*
0 to 42	112.57	107.14	116.06	1.44	NS	*
Feed conversion ratio (feed/gain)						
0 to 21	1.36	1.37	1.39	0.02	NS	NS
0 to 42	2.04	1.99	2.03	0.03	NS	NS
0 to 42	1.80	1.78	1.81	0.01	NS	NS

ASA0, ASA150, ASA300: ascorbic acid, 0, 150 and 300 mg per kg of diet, *:p<0.05; SEM: standard error of the means (Pooled)

temperature falls to 25°C or lower (Balnave, 2004). This explains present results, because the experimental area is semitropical and night house temperature around 20 to 25°C. Therefore, chicks consumed enough feed in night and BW is not affected by the diurnal heat stress. Since, it is known that effectiveness of ASA in poultry diets have a rise value in heat stress but not normal condition (Kutlu, 2001). The studies with ASA showed that 0 to 1000 mg kg^{-1} doses of ASA supplementation to the diets did not affect body weight, body weight gain and feed conversion ratio in broilers (Pardue *et al*., 1985; Marron *et al*., 2001). However, Kutlu and Forbes (1993a, b), Sahin *et al*. (2003) and Lohakare *et al*. (2005) reported that dietary ASA (150 to 1000 mg kg^{-1}) improved performance traits of broilers under heat stress. Contrary to the results above, Njoku (1986) noted that ASA 300 mg kg^{-1} reduced the body weights of broilers.

Carcass Traits

Dietary ASA supplementation did not improve carcass and parts, liver, gizzard, heart, kidney and abdominal fat yields (p>0.05) (Table 3). The current results are in agreement with Fletcher and Cason (1991) and Celik and Ozturkcan (2003), who found that ASA supplementation have no effect on carcass and abdominal fat. However, some studies suggested that dietary ASA supplementation increased carcass (Kutlu, 2001; Sahin *et al*., 2003; Lohakare *et al*., 2005), breast (Lohakare *et al*., 2005), liver, heart, spleen and empty gizzard weight (Sahin *et al*., 2003) and decreased abdominal fat pad (Kutlu, 2001; Sahin *et al*., 2003).

The chemical composition and pH of meat samples are shown in Table 3. Dry matter, crude protein and crude ash content of the thigh and meat chemical composition were not statistically affected by the dietary ASA supplementation (p>0.05). However, dietary ASA supplementation linearly decreased (p<0.05) crude fat content of thigh meat. Low fat content meat is preferred due to costumers demand. These results in agreement with Kutlu (2001), who found that dietary ASA supplementation decreased fat content in broilers. The lightness (L*), redness (a*) and yellowness (b*) values of breast were not affected by the dietary ASA supplementation (p>0.05, Table 3). However, L* values linearly increased (p<0.05) and b* value quadratically changed but a* values did not statistically affected by the treatments. While Lohakare *et al*. (2004) noted that dietary ASA did not affect colour of broiler meat, but their one study, Lohakare *et al*. (2005) found ASA positively affected meat color and caused an increase in L* and a* values but low b* values in broilers. In this study, pH values of breast and thigh meat did not influenced by the dietary ASA levels. Young *et al*. (2003) reported that providing ASA before slaughtering did not affect meat pH and colour of broilers.

Table 3: Effects of ascorbic acid levels on slaughter and carcass weight (g) and yield (%) and carcass part weights (g) and relative weights[1] (%) of parts of male broilers at 6 weeks of age

	Ascorbic acid (mg kg^{-1})				p-value	
Traits (%)	0	150	300	SEM	Linear	Quadratic
Carcass	74.60	74.90	74.70	0.48	NS	NS
Thigh	40.70	40.60	41.40	0.42	NS	NS
Breast	41.70	41.80	40.70	0.47	NS	NS
Wing	10.80	11.20	11.00	0.20	NS	NS
Liver	2.36	2.42	2.51	0.09	NS	NS
Gizzard	1.67	1.72	1.59	0.09	NS	NS
Heart	0.65	0.63	0.69	0.03	NS	NS
Kidney	0.82	0.86	0.83	0.05	NS	NS
Abdominal fat	2.27	2.31	2.10	0.15	NS	NS
Thigh meat composition						
Dry matter	25.82	24.65	25.78	0.92	NS	NS
Crude protein	21.68	21.17	20.70	0.46	NS	NS
Crude fat	3.48[a]	2.96[b]	2.94[b]	0.46	*	NS
Crude ash	0.97	0.99	0.98	0.05	NS	NS
Breast meat pH	6.09	6.09	6.03	0.04	NS	NS
Thigh meat pH	6.44	6.47	6.39	0.04	NS	NS
Meat pigmentation						
Breast						
L*	50.64	50.36	50.64	0.94	NS	NS
a*	2.45	3.61	3.12	0.33	NS	NS
b*	10.39	11.36	11.91	0.61	NS	NS
Thigh						
L*	52.72	57.63	56.69	2.07	*	NS
a*	2.72	3.75	4.57	0.37	NS	NS
b*	10.73	10.28	12.81	0.69	NS	**

ASA0, ASA150, ASA300: ascorbic acid, 0, 150 and 300 mg per kg of diet, P: probability, *:p<0.05; **:p<0.01; SEM: standard error of the means (Pooled).; L*: lightness, a*: redness, b*: yellowness

Table 4: Effects of ascorbic acid levels on shank and tibia measurements

	Ascorbic acid (mg kg^{-1})				p-value	
Traits	0	150	300	SEM	Linear	Quadratic
Tibia						
Weight (g)	18.95	18.98	19.32	0.42	NS	NS
Length (mm)	105.81	106.32	105.79	0.98	NS	NS
Width (mm)	9.83	9.67	9.62	0.17	NS	NS
Breaking strength, kg-force	32.01	31.82	33.24	2.64	NS	NS
Ash (%)	41.50	41.24	40.02	0.80	NS	NS
Shank						
Length (mm)	65.81	65.30	65.82	0.35	NS	NS
Width (mm)	12.92	12.71	12.61	0.12	NS	NS

ASA0, ASA150, ASA300: ascorbic acid, 0, 150 and 300 mg kg^{-1} of diet, SEM: Standard error of the means (Pooled)

Bone Traits

Shank length and width and tibia weight, length, breaking strength and ash were not affected by the dietary ASA supplementation (Table 4, p>0.05). Similarly, Roberson and Edwards (1994), Franchini *et al*. (1993) and McCormack *et al*. (2001) reported that dietary ASA supplementation did not affect bone properties in broilers. In contrast, Lohakare *et al*. (2005) showed that dietary ASA addition increased tibia breaking strength and ash. It is known that ASA plays a crucial role in the hydroxylation of proline residues necessary for the synthesis of procollagen, (Leeson and Summers, 2001) and also it is required for bone development as a cofactor for the bioconversion of vitamin D_3 to its active form of 1,25 (OH) D_3 (McDowell, 2000). However, Orban *et al*. (1993) reported that effect of ASA in bone metabolism is limited to hydroxylation of proline for formation of collagen and bone matrix during the growth phase of bone. Moreover, Thornton (1968) reported that ASA seemed

Table 5: Effects of ascorbic acid levels on blood serum constituents

	Ascorbic acid (mg kg^{-1})				p-value	
Items	0	150	300	SEM	Linear	Quadratic
Total protein (g dL^{-1})	3.30	3.46	3.66	0.15	NS	NS
Albumin (g dL^{-1})	1.51	1.50	1.56	0.06	NS	NS
Glucose (mg dL^{-1})	156.71	161.86	173.88	7.80	NS	NS
Triglyceride (mg dL^{-1})	37.14	40.71	37.50	2.99	NS	NS
Cholesterol (mg dL^{-1})	128.40	122.30	123.30	5.95	NS	NS
HDL (mg dL^{-1})	53.86^{b}	59.86^{ab}	62.50^{a}	7.64	*	NS
SGOT (AST) (U L^{-1})	3.86^{ab}	10.57^{a}	2.50^{b}	1.39	NS	*
SGPT (ALT) (U L^{-1})	14.86^{a}	8.14^{ab}	2.25^{b}	1.81	*	

*: $p<0.05$, SEM: Standard error of the means (Pooled)

to enhance the reduction in ossification of the epiphysical plate, mineralization of new bone and the reduction in the degrees of calcification of both types. So, this might partially explain why bone traits were not appreciably affected by dietary ASA supplementation in the present study.

Blood Parameters

Dietary ASA supplementation did not affect serum total protein, albumin, glucose, triglyceride and total cholesterol levels of broilers ($p>0.05$, Table 5). However, dietary ASA supplementation caused a linear increase in serum HDL concentration ($p<0.05$). Similar results were obtained by Clegg *et al.* (1976) and McKee *et al.* (1997), who reported that dietary ASA supplementation did not affect blood cholesterol and plasma triglyceride (McKee *et al.*, 1997) in broilers and albumin, creatine and glucose (Seyrek *et al.*, 2004) in quails. On the contrary these findings the reduction of blood cholesterol (Kutlu and Forbes, 1993a; Sahin *et al.*, 2003), triglyceride (Clegg *et al.*, 1976) and glucose (Kutlu and Forbes, 1993a; Sahin *et al.*, 2003) concentrations by feeding ASA have been demonstrated in broilers and quails (Gursu *et al.*, 2004; Sahin *et al.*, 2002). Also, Kutlu and Forbes (1993a) and Sahin *et al.* (2003) reported that dietary ASA supplementation increased serum protein in broilers. In the current study, dietary ASA supplementation caused a quadratic change in the AST and a linear and quadratic decrease in the serum ALT levels ($p<0.05$). The ALT catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. It is used as a liver function test and elevated levels monitored liver malfunction (Murray *et al.*, 1990). On the contrary to those results, Sahin *et al.* (2002) and Gursu *et al.* (2004) reported that dietary ASA did not affect serum ALT and AST levels in quails.

In conclusion, this experiment results suggested that additional ASA in broiler diets had some beneficial effect on some of examined parameters such as crude fat of meat, colour of thigh in broiler chicks, but most of parameters did not influenced by the dietary ASA supplementation. However, there were a number of fundamental differences among reports, including the basal diets, genetic stocks used and environmental conditions of the experiments. Further experiments should need to be conducted to determine whether the effect of ASA at different condition in broilers.

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