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The Effects of Ascorbic Acid and Seasonal Temperatures on Meat Quality Characteristics of Broiler Chickens Maintained in Open-Sided and Closed Houses

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Abstract: High ambient temperature is one of the prominent stressors that elicits low meat quality characteristics in broiler meat. The effect of ascorbic acid supplementation in drinking water on meat quality of broiler chickens reared in closed and open-sided houses during hot (ambient temperature 36°C) and cool (ambient temperature 23°C) seasons were studied. Four hundred and thirty two, one-day-old chicks were used for each house across the two seasons. Birds were maintained in 23 h light and 1 h dark cycles and offered *ad libitum* access to water and commercial broiler diets. Broilers in both houses were randomly subjected to four drinking water treatments (9 birds in each 6 replicates/treatment): 0, 100, 200 and 300 ppm ascorbic acid. *Pectoralis* muscles were taken at 24 h postmortem and analyzed for ultimate pH, expressed juice, cooking loss, Warner-Bratzler shear force value, myofibril fragmentation index, sarcomere length and colour. *Pectoralis* muscles collected during the hot season had significantly ($p<0.05$) higher pH, lower expressed juice, darker colour meat (L*) than those collected during cool season group. During the hot season, meat samples from chicken reared in an open-sided house had significantly ($p<0.0$) higher pH and lower lightness value (L*) than those reared in a closed house. Supplementation of drinking water with various levels of ppm ascorbic acid did not significantly improve meat quality characteristics of broiler chicken reared in open-sided or closed housing at high ambient temperatures.

Key words: Seasonal temperature, ascorbic acid, meat quality, open-sided house

INTRODUCTION

The poultry industry constitutes an integral part of the Sultanate of Oman's growing animal sector and is an important supplier of high quality protein. High ambient temperature is one of the main constraints confronting the poultry industry. Sensitivity of broiler chickens to heat stress is often a serious problem. Stress is manifested by increased motor activity and behavioral changes, accompanied by symptoms such as disturbances in respiration, blood circulation, hyperthermia, cyanosis and muscle rigidity (Ruiter, 1985). As a result of metabolic changes in the muscle, heat stress often results in a decrease in meat quality (Kadim *et al*., 2006; 2007; 2008a). Responses of birds to hot environments are in part mediated through changes in circulating levels of hormones, glucose, electrolytes and leucocytes and the function of organs (Blalock and Smith, 1985; Mitchell and Kettlewell, 1998). As the heat load increases, the resulting increase in body temperature will lead to tissue damage and release of intracellular vitamin and mineral components into the circulation (Whitehead and Keller, 2003). Much of the tissue damage, particularly those involving cell membranes, arises from lipid peroxidation which is enhanced in acute hot ambient temperatures. High ambient temperatures can also affect the quality of the meat by altering the physiology and metabolism of muscle (Northcutt *et al*., 1994; Froning *et al*., 1978). However, during the hot season, the poultry industry reports substantial losses in yield due to poor water-holding capacity, poor texture and pale colour (McCurdy *et al*., 1996). The loss of protein functionality due to extensive protein denaturation is considered to be the primary factor associated with low meat quality characteristics (Santos *et al*., 1994).

While the adverse physiological effects of high temperatures might be lessened by measures such as increasing ventilation rate or the use of cooling devices, chronic exposure might be addressed through nutritional adjustments. High ambient temperature causes oxidative stress and impairs antioxidant status *in vivo* (Sahin *et al*., 2001; Whitehead and Keller, 2003). Lower plasma concentrations of antioxidant minerals and vitamins, such as vitamin C and increased oxidative damage have been observed in stressed birds (Feenster, 1985; Sahin *et al*., 2002). Antioxidant nutrient supplementation; especially vitamins C has been reported to attenuate the negative effects of environmental temperatures (Kafri and Cherry, 1984; Njoku, 1986). A previous study at our Animal Experimental Station revealed a beneficial effect of vitamin C supplementation on the performance of heat stressed broiler chickens (Kadim *et al*., 2008b). The objective of this study was therefore to ascertain whether ascorbic acid added to the drinking water during periods

of high seasonal temperature has an effect on broiler meat quality.

MATERIALS AND METHODS

Environmental parameters: Climatic data including average temperature and relative humidity were recorded by a weather monitoring station at the Agricultural Experiment Station of Sultan Qaboos University. The experimental period was divided into two seasons. The cool season (January-February) with an average temperature of 23°C and a relative humidity of 58% and the hot season (August-September) with an average temperature of 33°C and a relative humidity of 50%.

Birds and housing: A total of 432 Cobb 500 broiler chickens (one-day old) were obtained from a commercial hatchery Nizaw Modern Poultry farms and used for each season. Chicks were transported to the Sultan Qaboos University Poultry Research Unit, where they were randomly distributed to 24 pens, with nine birds each. The broiler chickens were housed in litter-covered floor pens and provided a commercial ration (23% crude protein and 3,000 kcal ME/kg) *ad libitum*. Two different houses were used: a closed and an open-sided house. The closed house was well insulated by double aluminum layer with fiberglass in between. Cooling water pad system with expel fans were used. The open-sided house was a natural ventilated shed constructed from galvanized iron with profiled steel shed roofing. Chicken mesh panels and a block work protection up to one meter high were fixed on all sides. Electrical wall fans were positioned to circulate air and minimize temperature differences within and between pens. Canvas sheets were used to screen direct sunrays during midday. Each house was subdivided into 24 pens bedded with wood-shaving litter. Each pen was equipped with a plastic feeder and drinker to provide *ad libitum* access to feed and water. Lighting of 23 h light and 1 h dark was provided in the closed house. The house temperature and relative humidity were recorded 3 times daily (8:00, 1300 and 23:00 h) at the level of the birds' head in the centre of each growing area.

Ascorbic acid preparation and meat sampling: Four levels of ascorbic acid (0, 100, 200, 300-ppm) were prepared by mixing of ascorbic acid (BDH Laboratory Supplies Poole, England) with fresh water. Each pen was randomly assigned to one of four treatments (6 replicates per treatment). At the end of each experiment and following a 12 h period of feed withdrawal, 20 birds from each treatment were hung on shackles and killed by bleeding for 90s from a single neck cut severing the carotid arteries and jugular veins. After bleeding, birds were subscalded at 63°C for 45s, manually defeathered and eviscerated. At 15 min postmortem, carcasses were placed in a manually agitated tap water and the carcasses were transferred to a chiller (2-4°C) for 24 h before running meat quality measurements. A lengthwise incision was made in the skin covering both side breast muscles and *pectoralis* muscles were dissected out to measure meat quality parameters.

Carcass pH and temperature measurements: The central *pectoralis* muscle pH and mid-muscle temperatures of both sides were monitored using a portable pH meter (Hanna waterproof pH meter, Model Hi 9025) fitted with a polypropylene spear-type gel electrode (Hanna Hi 1230) and a temperature adjusting probe. The pH meter was calibrated at room temperature (24°C) before each measurement. Measurements, designated as pH_i and T_i (40 min., 2, 4, 6, 8, 10, 12, 24 h postmortem) were recorded. For each measurement, the pH probe and the thermometer were inserted into carcasses with a similar depth (0.5 cm).

Meat quality evaluation: The *pectoralis* muscle was selected because it allowed an adequate sample size for quality measurements. Meat quality measurements, including, ultimate pH, expressed juice, cooking loss, Warner-Bratzler shear force, sarcomere length, myofibrillar fragmentation index and colour L*, a* b* were determined. The ultimate pH was assessed in homogenates (using a Ultra Turrax T25 homogeniser at about ¼ speed with 3 x 5 second bursts) of duplicate 1.5-2 g muscle tissue in 10 ml of neutralized 5-mM sodium iodoacetate (pH 7.0, 150 mM KCl). The pH of slurry measured using a Metrohm pH meter (Model No. 744) with a glass electrode. Triplicate 25 mm-thick slices were cut from each muscle at the time of muscle preparation. The slices were weighed and stored in a chiller (2-4°C) in plastic bags until cooked by immersing the bags in a water bath at 70°C for 90 min. The cooked meat was kept at 2-3°C overnight and 12 cores (13 mm x 13 mm cross-section) were cut from the centre of each slice. Each core was then sheared perpendicularly to the fibres in 2 places, with digital Dillon Warner-Bratzler shear device (kg). Cooking loss of meat samples was determined by difference between raw and cooked meat sample weights. Sarcomere length was determined by a laser diffraction method (Spectra-physics helium-neon laser, 2 mW 0.49 mm diameter beam, with a wavelength of 632.8 nm), similarly to the procedure described by Cross *et al.* (1980/1981). Myofibrillar fragmentation index was measured using a modification of the method of Johnson *et al.* (1990). Expressed juice was assessed using a filter paper method, as the total wetted area less the meat area (cm^2) relative to the weight of the sample (g), following the procedure of Hamm (1986). *Pectoralis* muscle slices (1-1.5 cm thick) were allowed to "bloom" for 30 min at room temperature (25±2°C) and then L*, a*, b* of the CIE Lab colorimetric from each samples were recorded twice using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Japan).

Statistical analysis: A complete factorial analyzed design of 2 (house) x 4 ascorbic acid levels was used. Statistical analysis was carried out using analysis of variance procedure (Ott, 1993), to evaluate the effect of seasonal temperature and ascorbic acid levels and their interaction on quality characteristics of meat chickens maintained in closed and open-sided houses using GLM procedures of SAS (1993). Significant differences between treatment means were assessed using the least-significant-difference procedure.

RESULTS AND DISCUSSION

Ambient temperature: During the first 2 weeks of the experiment, there was no difference in the average temperatures between closed and open-sided houses during the two seasons (Fig. 1). It was determined that there was a much greater fluctuation in ambient temperatures and humidity from night to day and from day to day in an open-sided house than in a closed house. However, the average temperatures in the open-sided house between weeks 3-6 were 7-8°C higher in the hot season than during the cool season, while the relative humidity throughout ranged 60-65%. The recorded temperatures in the open-sided house indicated that the range of temperatures during the hot season was well above the birds' thermo-neutral zone. During the hot season in the open-sided house, birds often exhibited signs of heat stress such as panting and wing lifting. Birds receiving 200-300 ppm ascorbic acid exhibited less behavioural changes of heat stress. Similarly Kadim *et al.* (2008b) reported that heat-stressed birds supplemented with 250-ppm ascorbic acid exhibited relatively less panting than their un-supplemented counterparts. This suggests that ascorbic acid improves the ability of the bird to tolerate higher heat loads.

Temperature and kinetics of pH decline: Average temperature-time and pH-time relationships for the *pectoralis* muscle with various levels of ascorbic acid are shown in Table 1 and 2 for cool and hot seasons, respectively. There are implicit interrelationships between temperature and pH because glycolysis is exothermic and the effects of pH are severe when a muscle is still near body temperature (Swatland, 2008). The temperature-time data of the *pectoralis* muscle indicate that there was no significant difference between different levels of ascorbic acid within each season.

Table 1 and 2 illustrate that as early as 20 min post-mortem the *pectoralis* muscle of the closed housed birds had a significantly lower pH than that of the open-sided housed counterparts across both seasons. Specifically, maximum pH differences between hot and cool season birds were observed as early as 20 min postmortem and persisted through 1 h postmortem, after which the differences in pH began to decrease (Table 1 and 2). Ma and Addis (1973) and Vanderstoep and Richards (1974) demonstrated that maximum differences in pH declined between fast and slow glycolyzing in bird muscle occurred during the first hour postmortem. The accelerated pH decline in birds during the hot season persisted for 24 h postmortem in closed-sided house. The overall muscle pH decline followed a similar trend to that observed by Ma and Addis (1973) and Vanderstoep and Richards (1974). The current study indicated that the broilers reared during the cool season exhibited accelerated postmortem glycolysis compared to the birds raised during hot season. A rapid decline in pH, when the muscle temperature is high, can result in protein denaturation, which affects the colour and water-holding capacity of the meat (Warris and Brown, 1987). This may probably be due to heat stress impairing energy utilization (Bhattacharya and Hussain, 1974) and consequently reducing glycogen storage before slaughter. These findings are in accordance with those of Apple *et al.* (1995) which showed that stressful conditions lead to depletion of muscle glycogen reserves before slaughter which subsequently increases the pH of meat. Glycogenolysis in skeletal muscle is regulated by the activity of glycogen phosphorylase (Apple *et al.*, 1995). Increased metabolism of muscle glycogen during stress may be a direct result of the calcium release into the myofibril associated with muscle contraction (Rosell and Saltin, 1973). On the other hand, increasing catecholamines levels can also activate glycolysis (Drummond *et al.*, 1969). Therefore, in the present study, both of these factors may have played a role in the activation of glycolysis.

Meat quality: The present study revealed that the meat quality characteristics of the broiler meat were not significantly affected by ascorbic acid levels (Table 3 and 4). This might be due to the fact that rearing broiler chickens for 5-6 weeks may not be long enough for the ascorbic acid to have an effect on muscle metabolism. Table 4 shows significant ($p<0.05$) differences in the *pectoral*muscle ultimate pH between the closed and open-sided house during the hot season (5.79 vs. 5.67). The variation in ultimate pH between the two housing systems during hot season would have been a result of combination of high temperature and relative humidity. High ambient temperatures reduce the bird's feed intake and impose physiological stresses, which activate glycogenolysis in skeletal muscle (Kreikemeier *et al.*, 1998). Physiologically stressed birds use glucose and gluconeogenic precursors as their major oxidative fuel. Low muscle glycogen content resulting from exhaustion or chronic stress before death results in high pH values and minimal rigor shortening, which could be one of the significant factors causing deterioration of meat quality characteristics.

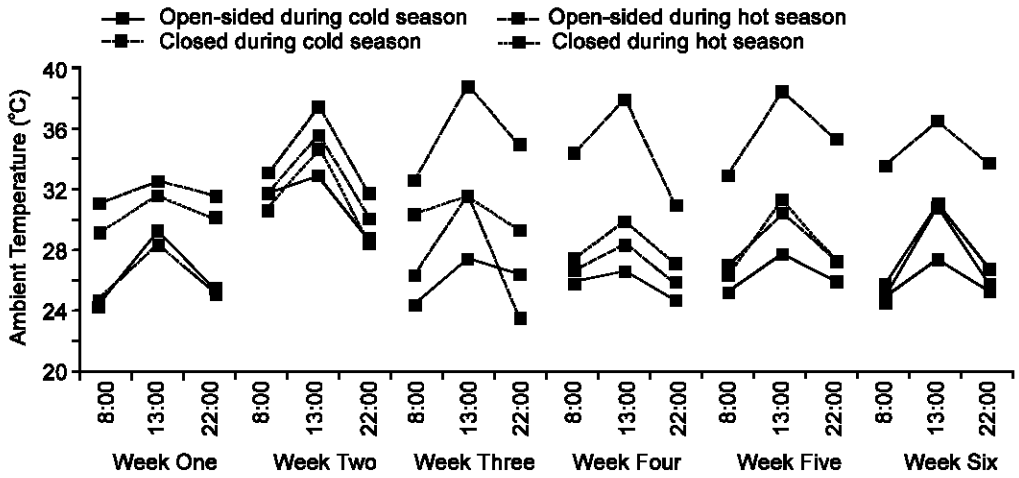

Fig. 1: Weekly average closed and open-sided house's ambient temperatures measured at 8, 13 and 22 h

Table 1: Effects of Ascorbic Acid (AA) supplementation on *pectoralis* muscle temperature °C and pH mean values of broiler chickens reared in closed and open-sided houses during cool season

Time Post-mortem) (min	Temp. °C	Open-sided house Ascorbic acid (ppm) 0	100	200	300	Closed house Ascorbic acid (ppm) 0	100	200	300	SEM	Significance[1] H[2]	AA	H x AA
No. of samples	20	20	20	20	20	20	20	20	20				
20	37.4	6.46^{b}	6.43^{b}	6.43^{b}	6.43^{b}	6.28^{a}	6.23^{a}	6.21^{a}	6.20^{a}	0.04	*	NS	NS
40	34.8	6.38^{b}	6.36^{b}	6.35^{b}	6.36^{b}	6.19^{a}	6.15^{a}	6.14^{a}	6.13^{a}	0.03	*	NS	NS
60	32.1	6.15^{b}	6.12^{b}	6.11^{b}	6.10^{b}	6.09^{a}	6.07^{a}	6.05^{a}	6.00^{a}	0.05	*	NS	NS
120	23.9	5.96	5.97	5.94	5.95	5.92	5.89	5.86	5.82	0.04	NS	NS	NS
240	19.5	5.86	5.83	5.84	5.82	5.80	5.78	5.76	5.72	0.03	NS	NS	NS
360	10.4	5.79	5.76	5.70	5.7^{0}	5.74	5.73	5.72	5.70	0.05	NS	NS	NS
480	6.7	5.72	5.70	5.69	5.66	5.68	5.67	5.65	5.63	0.03	NS	NS	NS'

[1]*p<0.05, NS = Not-significant. [2] H = housing system. [abc]Means within the same row with different superscripts were significantly different (p<0.05)

Table 2: Effects of Ascorbic Acid (AA) supplementation on *pectoralis* muscle temperature °C and pH mean values of broiler chickens reared in closed and open-sided Houses (H) during hot season

Time Post-mortem (min)	Temp. °C	Open-sided house Ascorbic acid (ppm) 0	100	200	300	Closed house Ascorbic acid (ppm) 0	100	200	300	SEM	Significance H	AA	H x AA
No. of Samples	20	20	20	20	20	20	20	20	20				
20	38.2	6.72^{b}	6.61^{b}	6.60^{b}	6.62^{b}	6.53^{a}	6.47^{a}	6.33^{a}	6.32^{a}	0.06	*	NS	NS
40	35.1	6.51^{b}	6.56^{b}	6.55^{b}	6.56^{b}	6.42^{a}	6.30^{a}	6.31^{a}	6.30^{a}	0.07	*	NS	NS
60	33.5	6.39^{b}	6.32^{b}	6.35^{b}	6.31^{b}	6.29^{a}	6.17^{a}	6.09^{a}	6.07^{a}	0.08	*	NS	NS
120	24.8	6.25^{b}	6.17^{b}	6.15^{b}	6.14^{b}	5.98^{a}	5.91^{a}	5.89^{a}	5.88^{a}	0.06	*	NS	NS
240	20.1	6.07^{b}	5.99^{b}	5.98^{b}	5.97^{b}	5.86^{a}	5.82^{a}	5.78^{a}	5.75^{a}	0.06	*	NS	NS
360	12.9	5.91^{b}	5.86^{b}	5.86^{b}	5.85^{b}	5.80^{a}	5.78^{a}	5.72^{a}	5.70^{a}	0.07	*	NS	NS
480	7.2	5.83^{b}	5.82^{b}	5.79^{b}	5.78^{b}	5.72^{ab}	5.70^{a}	5.69^{a}	5.67^{a}	0.06	*	NS	NS

[1]*p<0.05, NS = Not-significant. [2]HS = housing system. [abc]Means within the same row with different superscripts were significantly different (p<0.05)

Shear values were slightly (p>0.05) lower in the muscles during the hot (3.6 kg) than in the cool season (4.0 kg), with no differences between the closed and open-sided houses within each season (Table 3 and 4). Similarly, the sarcomere length and myofibrillar fragmentation index in breast muscle were similar between the closed and open-sided houses during both seasons.

Cooking loss and expressed juice were measured to obtain an overall assessment of the water-holding capacity of meat. The expressed juice from hot season

Table 3: Effects of Ascorbic Acid (AA) supplementation on *pectoralis* muscle quality characteristics of broiler chickens reared in closed and open-sided houses during cool season

	Open-sided house				Closed house							
	Ascorbic acid (ppm)				Ascorbic acid (ppm)					Significance		
Parameters	000	100	200	300	000	100	200	300	SEM	Housing	AA	Interaction
Ultimate pH	5.70	5.68	5.67	5.65	5.65	5.66	5.64	5.65	0.17	NS	NS	NS
Cooking Loss (%)	21.9	20.9	19.1	18.5	20.9	19.1	18.4	18.4	1.09	NS	NS	NS
Expressed Juice (cm^2/g)	26.8	26.6	25.6	25.5	26.4	26.4	24.5	24.7	4.29	NS	NS	NS
W-B Shear value (kg)	4.7	4.1	3.9	3.7	4.3	4.1	3.8	3.6	0.36	NS	NS	NS
Myofibrillar Fragmentation Index (%)	88.9	88.9	90.6	91.3	88.7	88.9	90.1	91.0	12.1	NS	NS	NS
Sarcomere Length (µm)	1.61	1.71	1.62	1.64	1.63	1.62	1.57	1.60	0.70	NS	NS	NS
Colour Lightness L^*	57.7	58.4	58.6	59.5	58.1	59.2	60.7	61.5	1.55	NS	NS	NS
Colour Redness a^*	14.9	14.7	13.3	13.9	14.5	14.1	13.6	14.6	0.27	NS	NS	NS
Colour Yellowness b^*	10.4	11.1	11.9	11.4	9.5	10.1	10.7	10.4	0.82	NS	NS	NS

[abc]Means within the same row with different superscripts were significantly ($p<0.05$)

Table 4: Effects of Ascorbic Acid (AA) supplementation on *pectoralis* muscle quality characteristics of broiler chickens reared in closed and open-sided houses during hot season

	Open-sided house				Closed house							
	Ascorbic acid (ppm)				Ascorbic acid (ppm)					Significance		
Parameters	000	100	200	300	000	100	200	300	SEM	Housing	AA	Interaction
Ultimate pH	5.80[b]	5.81[b]	5.79[b]	5.77[b]	5.70[a]	5.69[a]	5.68[a]	5.68[a]	0.16	*	NS	NS
Cooking Loss (%)	23.8	23.6	23.6	22.5	23.4	24.5	21.5	21.7	0.78	NS	NS	NS
Expressed Juice (cm^2/g)	27.4[b]	27.3[b]	26.6[b]	25.6[b]	24.6[ab]	24.2[ab]	22.8[a]	22.5[a]	4.45	*	NS	NS
W-B Shear value (kg)	3.9	3.8	3.6	3.1	3.8	3.6	3.4	3.2	0.36	NS	NS	NS
Myofibrillar Fragmentation Index (%)	90.2	90.6	91.5	91.9	91.1	91.5	91.5	91.9	15.53	NS	NS	NS
Sarcomere Length (µm)	1.90	1.82	1.91	1.89	1.82	1.91	1.88	1.92	0.76	NS	NS	NS
Colour Lightness L^*	50.4[a]	50.4[a]	51.1[a]	52.3[a]	54.9[b]	55.1[b]	56.5[b]	57.2[b]	2.12	*	*	*
Colour Redness a^*	16.9	16.2	16.1	16.3	15.6	15.2	15.3	15.2	0.96	NS	NS	NS
Colour Yellowness b^*	11.9	12.0	12.4	12.6	11.3	11.7	11.9	12.1	0.74	NS	NS	NS

[ab]Means within the same row with different superscripts were significantly different ($p<0.05$)

samples were 26.7 and 23.5 cm^2/g for open-sided and closed houses, respectively, while the values of 26.1 and 25.5 cm^2/g for their counterparts during cool season (Table 3 and 4). These values did not exhibit levels of statistical significance. An increase in muscle pH has been shown to increase expressed juice by increasing the electrostatic repulsions between myofibrillar proteins (Offer and Knight, 1988). The solubility of myofibrils from breast muscle is greatly diminished as a slight decrease in pH (Xiong and Brekke, 1991). A small decline in pH in this range has the capacity to reduce expressed juice, gel strength and emulsifying capacity of breast muscle (Daum-Thunberg *et al*., 1992).

The colour of fresh meat is an extremely important characteristic influencing the consumer's purchase decision (Fletcher, 2002). Rearing broiler in an open-sided house during the hot season resulted in significant changes in colour values of the breast meat (Table 3 and 4). Muscles from open-sided house carcasses were significantly darker (higher L* values) than muscles from closed house carcasses. The ranges in meat samples a* and b* values (redness and yellowness) between open-sided house carcasses were not significantly different. In the present study, the low ultimate pH values from the closed house during hot season samples might have led to more protein degradation resulting in higher colour values. These findings are in agreement with previous reports (Barbut, 1993; Fletcher, 1995; 1999; Allen *et al*., 1997; Yang and Chen, 1993; Froning, 1995), in which dark-coloured meat had a high ultimate pH. Postmortem glycolysis decreases muscle pH, making muscle surface brighter and superficially wet (Swatland, 2004). If the ultimate meat pH is high, the physical state of the proteins will be above their iso-electric point. Proteins will associate with more water in the muscle and therefore, fibers will be tightly packed, causing more light to be absorbed by the muscle and the meat appears darker in colour (Kauffman and Marsh, 1987; Cornforth, 1994). This might be due to a decrease in the rate of oxygen diffusion which is associated with mitochondrial activity (Lawrie, 1958). Indeed, mitochndria survive and function better in postmortem muscle tissue at elevated pH values which results in elevated oxygen consumption by the tissue and accelerated darkness (Ashmore *et al*., 1972). Levels of ascorbic acid had no significant effect on the chromaticity coordinate L*, b* and a* of the lab colour space (CIE Lab). There were no significant interactions between housing system and level of ascorbic acid for the L*, b* and a* values.

Conclusion: The season had a significant effect on broiler meat quality characteristics. Birds reared in an open-sided house during the hot season (average temperature: 35°C) had elevated ultimate pH and darker meat colour than those reared during cool season (average temperature: 21°C). Open-sided house broiler rearing resulted in darker colour than that from closed house carcasses during the hot season. The administration of various concentrations of ascorbic acid had no marked effect on quality characteristics of broiler muscles.

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