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Effect of Acetic Acid Supplementation on Egg Quality Characteristics of Commercial Laying Hens during Hot Season

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Abstract: This study was conducted to determine the effects of acetic acid supplement at four levels (control, 200, 400 and 600-ppm in drinking water) on egg production and quality in commercial Brown Leghorn reared birds during the hot season (32°C). One hundred and sixty 30 week-old laying birds were randomly divided into 4 groups and subjected to four levels of acetic acid (5 birds in each of the 8 replicates/treatment) and fed *ad libitum* a commercial layers mash ration (17% protein and 2800 Kcal/Kg metabolizable energy). The birds were maintained under 14 h light and 8 h dark cycles and offered water and feed *ad libitum*. Egg production, egg weight; length; diameter; specific gravity; shell weight; shell thickness and colour, yolk, albumen unit, albumen height and colour were daily recorded for 70 consecutive days. The drinking acetic acid significantly improved egg production, external and internal egg quality parameters. Acetic acid supplementation at 600, 400 and 200 ppm groups significantly ($p<0.01$) increased average egg production by about 20, 15 and 10% compared with the control group. An increase in the supplemental acetic acid produced a linear increase in external egg qualities such as egg weight ($p<0.01$), egg length ($p<0.05$), egg diameter ($p<0.05$) and egg shell colour (L*, a*, b*) ($p<0.05$). Internal egg quality was also significantly ($p<0.01$) improved by increasing the organic acid supplementation. Albumen height, yolk pH and Haugh unit were increased with increasing acetic acid concentrations in the drinking water. The most marked results were obtained with 600 ppm acetic acid supplementation. This level may be considered as a protective management practice in commercial egg production that reduces the negative effects of heat stress.

Key words: Acetic acid, egg weight, egg size, shell thickness, egg yolk color

Introduction

High temperatures adversely impacts on many aspects of laying hen performance, including increased mortality as well as reduced appetite, egg production and quality (Marsden *et al*., 1987; Donkoh, 1989; Siegel, 1995). The negative effects of heat stress on egg production and quality is mainly due to a reduction in the feed intake (Savory, 1986; Balnave, 1998). 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; Sahin *et al*., 2003). Moreover, effects of heat stress can be ameliorated by an increase in water intake and thereby more efficient maintain respiratory evaporative heat loss, reported by Belay and Teeter (1993). As the heat load increases, the resulting elevation body temperature will lead to tissue damage and the release of intracellular components into the circulation (Whitehead and Keller, 2003).

Several alterative methods have been used available to alleviate the effects of high environmental temperature on bird performance due to the high cost associated with cooling bird houses. Such methods focus mainly on manipulating the diet or drinking water. Organic acid (e.g., acetic acid) and their slats inhibit microorganism growth in the gastrointestinal tract, modifying intestinal pH and improving feed utilization (Vogt *et al*., 1981; Patten and Waldroup, 1998; Owings *et al*., 1990; Skinner *et al*., 1991; Adams, 1999). Kadim *et al*. (2008) observed that supplemental ascorbic acid significantly alleviated the heat-related stress effects on broiler chickens' performance. This suggests that adding acetic acid to drinking water may alleviate heat stress conditions in laying hens and improve egg production and quality. The objective of this study was to evaluate the effects of acetic acid supplementation on egg production and the quality of commercial hens reared under conditions of heat stress (35°C).

Materials and Methods

Environmental parameters: Weather data including average temperature and relative humidity were recorded by a weather monitoring station at the Agricultural Experiment Station in Sultan Qaboos University. The experimental period was carried out during the hot season with an average temperature of 35°C and a relative humidity of 65% (Fig. 1).

Birds, acetic acid and experimental design: One-hundred and sixty commercial Brown Leghorn hens that have been in lay for about 10 weeks were randomly

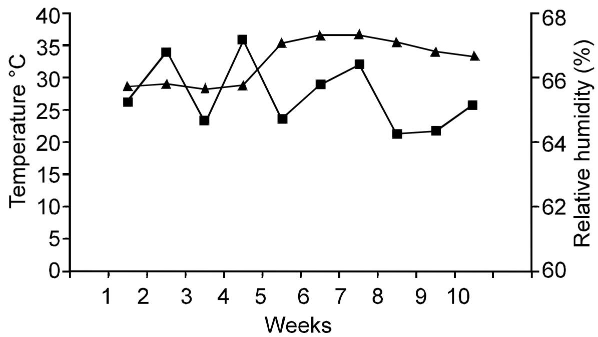

Fig. 1: Average weekly ambient temperature and relative humidity of the layer shed

divided into four equal groups. The birds were housed in commercial battery operation located in a closed house. The closed house was well insulated by double aluminum layer with fiberglass between them. Cooling water pads system with expel fans were used. The house temperature and relative humidity were recorded 3 times daily at 8:00, 1300 and 23:00 h. Five birds were housed in individual cages (20.6×30.5×35.6 cm). Each level of acetic acid was fed to eight replicates for a total of 40 birds per treatment. Artificial lights were set from 0500-1900 h when necessary to provide at least 14 h of light. Approximately 5.4 1× light intensity was allowed at bird level. Birds were fed *ad-libitum* a commercial layers mash ration (Oman Flour Mill, Muscat, Sultanate of Oman) and water. Four levels of acetic acid (control (0), 200, 400, 600 ppm) were prepared by mixing acetic acid (Vinegar: BDH Laboratory Supplies Poole, England) with fresh water. Each pen was randomly assigned to one of 4 treatments. Temperature and humidity of the house were measured 3 times a day (0800, 1200 and 2000 h). The average ambient temperature and relative humidity were 32.5±3.7°C and 40.8±6.3%, respectively.

Egg production and quality: Daily egg collections were carried out at the same time every morning for ten consecutive weeks (30-40 weeks). Egg weight, egg length and width and egg shell weight were recorded for all eggs. Length and width of eggs were measured using a caliper. The shells were dried at 60°C in an oven for 3 days and then weighed. Egg quality measurements including specific gravity, egg shell thickness, weight, colour and Haugh unit were conducted daily for all eggs from all treatments.

Specific gravity of eggs was determined using the saline flotation method of Hempe *et al*. (1998). Salt solutions were made in incremental concentrations of 0.005 in the range from 0.1065-0.1120 g/L. Haugh units were calculated using the Haugh unit formula (Eisen *et al*., 1962) based on the height of albumen determined by a micrometer and egg weight (Saginomiya, TLM-N1010, Tokyo, Japan). Shell thickness was determined by taking the mean value of the thickness measured at three locations on the egg (air cell, equator and sharp end) using a dial pipe gauge (Mitutoyo, 0.01-20 mm, Tokyo, Japan). At sampling, eggs were weighed and broken onto a flat surface where the height of the inner thick albumen was measured with an electronic albumen height gauge. The yolk was separated from the albumen and weighed. The pH of the albumen and yolk were measured immediately with a pH meter (Metrohm pH meter; Model No. 744 with a glass electrode). The weight of the albumen was calculated by the difference between the weight of the egg and the weight of the yolk plus the shell. CIE L*, a*, b* light reflectance coordinates of the egg shell surface and yolk were measured at room temperature (25±2°C) using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Japan). The albumen height gauge instrument was used to measure eggs albumin height. The Haugh unit reading (internal quality) was determined by using a micrometer to measure the height of albumin by gently emptying the egg contents onto a glass table without disturbing the albumin. The formula for using the weight of the egg and albumin thickness to determine Haugh unit was"

$$\text{Haugh unit} = 100 \log (H-1.7\, W^{0.37} + 7.57)$$

where,

H = Albumen height.

W = Weight of the egg.

Albumen height is measured with micrometer gauge (Albumen height gauge) mounted on a tripod placed over the egg broken out on to a glass plate.

The average of two quality measurements from each sample was recorded.

Statistical analysis: Statistical analysis was carried out using the analysis of variance procedure (Ott, 1993), to evaluate the effects of acetic acid levels on egg quality characteristics of laying birds using GLM procedures of SAS (1996). Significant differences between treatment means were assessed using the least-significant-difference procedure.

Results

Egg production: The effect of supplementation of the drinking water at various levels of acetic acid on egg production of laying hens during the period between 30-40 weeks of age is presented in Table 1. Egg production increased with the increasing level of acetic acid supplementation. The average egg production for the whole period significantly increased by approximately 20, 15 and 10% for the 600, 400 and 200 ppm acetic acid groups, respectively compared with the control group. The number of egg per hen also increased significantly with the increasing acetic acid level.

Table 1: Effect of acetic acid supplementation on overall egg production, external and internal quality parameters in laying commercial hens reared under conditions of heat stress

	Acetic acid levels (ppm)				
Parameters	000	200	400	600	SEM
No. of egg	25.9[a]	28.7[b]	30.3[c]	32.3[d]	0.44
No. of egg/hen	0.81[a]	0.83[b]	0.87[c]	0.90[d]	0.01
Egg Weight (g)	58.9[a]	60.2[b]	61.2[bc]	62.3[c]	0.23
Egg Length (cm)	5.5[a]	5.6[a]	5.7[ab]	5.8[b]	0.01
Egg Diameter (cm)	4.3[a]	4.4[a]	4.5[ab]	4.6[b]	0.01
Egg shell colour					
Light (L*)	62.3[a]	63.1[b]	63.9[b]	63.9[b]	0.19
Red (a*)	14.6[b]	14.3[ab]	14.1[a]	14.2[a]	0.11
Yellow (b*)	30.6[b]	29.6[ab]	29.0[a]	28.5[a]	0.14
Shell Weight (g)	7.4	7.6	7.5	7.5	0.03
Shell Weight%	12.6	12.6	12.7	12.5	0.13
Yolk colour					
Light (L*)	60.0	62.2	63.3	64.4	0.16
Red (a*)	9.6	9.1	8.72	8.4	0.60
Yellow (b*)	49.9	48.5	48.8	49.3	0.59
Albumin Height (mm)	12.5[a]	12.9[b]	13.3[c]	13.9[c]	0.06
Albumin pH	8.31	8.32	8.36	8.31	0.016
Yolk pH	6.19[b]	6.16[b]	6.08[a]	6.06[a]	0.020
Haugh Unit (mm)	109.2[a]	110.5[a]	111.8[b]	112.0[b]	0.37

SEM: Residual Standard Error. Means with the same row with different letters were significant (p<0.05)

Egg external parameters: Mean egg weight, length, width, shell thickness, shell weight and percent shell weight of laying hens subjected to various level of acetic acid supplementation are given in Table 1. The egg weight of experimented layers was significantly ($p<0.05$) influenced by the level of acetic acid. The 400-600 ppm acetic acid groups had significantly ($p<0.05$) heavier eggs compared to the control group. Figure 2 demonstrates that from day 40 of the experiment period, the 400-600 ppm acetic acid groups had a significantly ($p<0.05$) heavier egg weight than the control group. The other acetic acid level groups showed an increase in the egg weight during the first 40 days and then a decline towards the end of the experimental period.

There were also significant ($p<0.05$) differences in the length of eggs between the four treatment groups (Fig. 3 and Table 1). The 400-600 ppm acetic acid group had a significantly ($p<0.05$) higher egg length than the control group. Similarly, the 600 ppm acetic acid group had a significantly ($p<0.05$) higher egg diameter than the control and 200 ppm acetic acid groups (Fig. 4 and Table 1). The 400 ppm acetic acid group showed an increase in egg diameter until 40 day, then a decline towards the end of the experiment.

The effects of acetic acid supplements on egg shell quality and egg shell colour is presented in Table 1, Fig. 5. Acetic acid levels had no significant effect on the egg shell weight. The 400-600 ppm acetic acid groups had a significantly higher L* value and lower a* and b* values than the control group (Fig. 5).

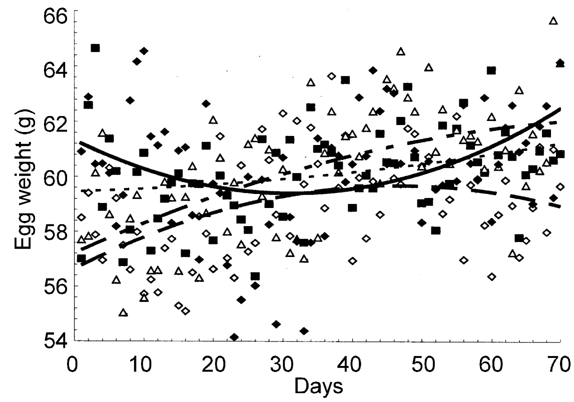

Fig. 2: Effect of acetic acid concentrations on egg weight of layer hens reared during the hot season (': 0 o acetic-acid (----), •: 200ppm acetic acid (- - -), ª: 400ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

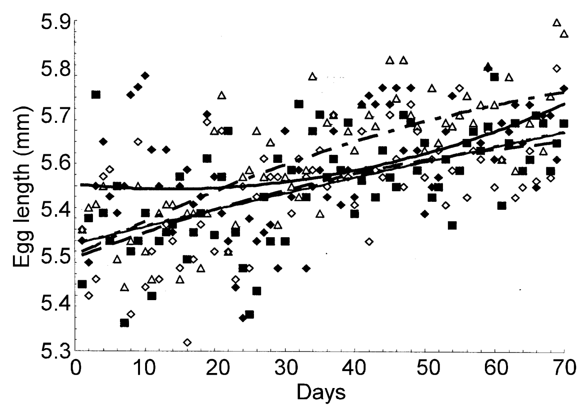

Fig. 3: Effect of acetic acid concentrations egg length of hens reared during the hot season (': 0 acetic acid (----), •: 200 ppm acetic acid (- - -), ª: 400 ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

Egg internal parameters: The effect of acetic acid supplementation on Haugh unit, albumen height and the yolk colour of eggs are presented in Table 1. The statistical analysis of the present study revealed that acetic acid supplementation at 400-600 ppm levels significantly ($p<0.05$) improved albumen height, yolk pH and Haugh unit of the egg.

Figure 7 show the relationship between the redness (a*) of the shell color and various level of acetic acid, respectively. The redness and yellowness of shell color was significantly affected by the various level of acetic acid. The highest yellowness and lowest redness colour values were found in the 600 ppm acetic acid group compared to control group.

Figure 8 shows the relationship between the albumin height in the eggs and various levels of acetic acid

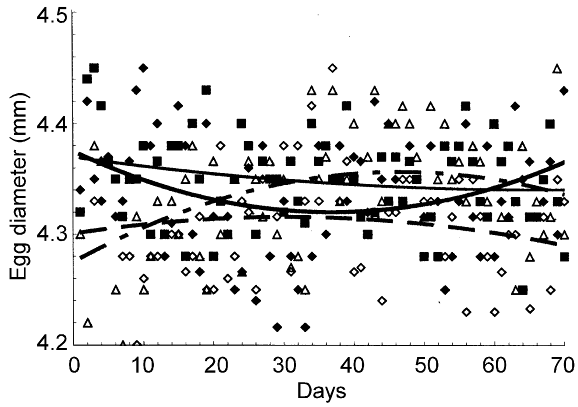

Fig. 4: Effect of acetic acid concentrations on egg diameter of hens reared during the hot season (‘: 0 acetic acid (----), •: 200ppm acetic acid (- - -), ª: 400ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

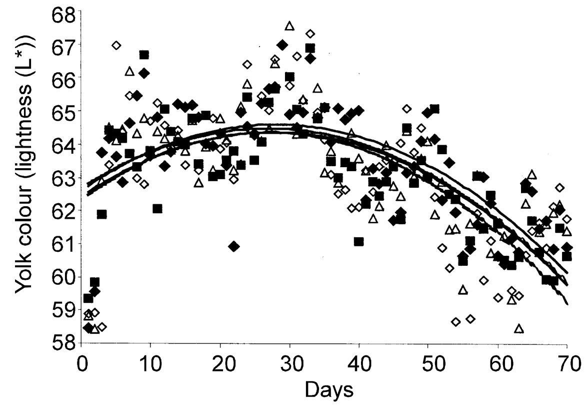

Fig. 6: Effect of acetic acid concentrations on yolk colour (lightness L*) of hens reared during the hot season (‘: 0 acetic acid (----), •: 200 ppm acetic acid (- - -), ª: 400 ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

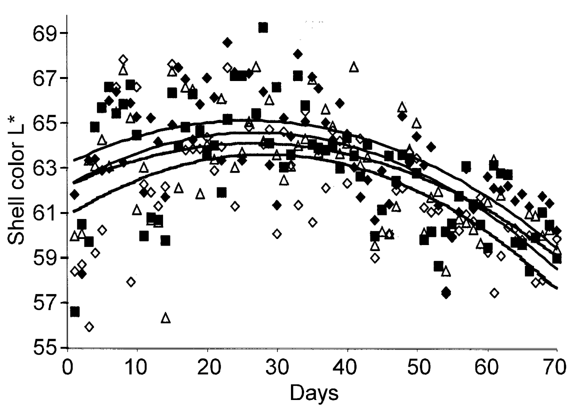

Fig. 5: Effect of acetic acid concentrations on shell color (lightness L*) of commercial hens reared during the hot season (‘: 0 acetic acid (----), •: 200 ppm acetic acid (- - -), ª: 400 ppm acetic acid (!·!·!) and ,: 600 acetic c acid (——)

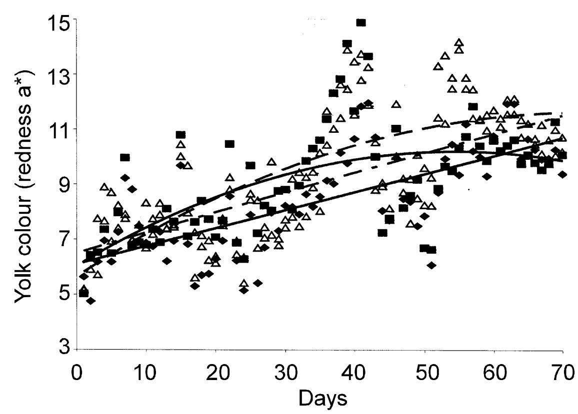

Fig. 7: Effect of acetic acid concentrations on yolk colour (redness a*) of hens reared during the hot season (‘: 0 acetic acid (----), •: 200ppm acetic acid (- - -), ª: 400ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

within days. The highest effect of acetic acid was at the 400 ppm level and the lowest level was at 200 ppm. The 600 pm level was between 0.00 (control) and 200 pm levels of acetic acid. It can be therefore, concluded that, the 400 pm level was the more effect to increase the albumin height in the egg. Figure 9 shows the relationship between the Haugh unit in eggs and various levels of acetic acid. The highest level was 200 ppm of acetic acid. At 600 ppm of acetic acid, the albumin height began increasing from the first day of the experiment until day 35 when it decreased till the end of the experiment.

Discussion

Administration of acetic acid significantly improved egg production in laying hens reared during the hot season (32EC) under Omani conditions. In agreement with the present study, Jensen and Chang (1976), Gama *et al*. (2000), Yesibag and Colpan (2006) and Soltan (2008) found that organic acid supplementation has positive effects on egg production in laying hens. According to Mahdavi *et al*. (2005), the influence of organic acid on poultry production depends on gut flora and environmental temperatures. High ambient temperatures decrease serum and tissue vitamin and mineral concentrations in poultry (Anderson, 1987), which consequently causes a reduction in egg production. It has been suggested that organic acid effects include an improvement in the digestibility and retention of nutrients, as well as modifying the microbial population in the digestive tract (Caja *et al*., 2000). Moreover, organic acid may be important to facilitate

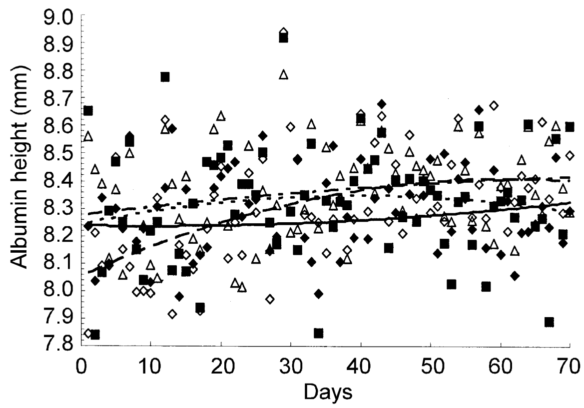

Fig. 8: Effect of acetic acid concentrations on albumin height of hens reared during the hot season (‘: 0 acetic acid (----), •: 200ppm acetic acid (- - -), ª: 400ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

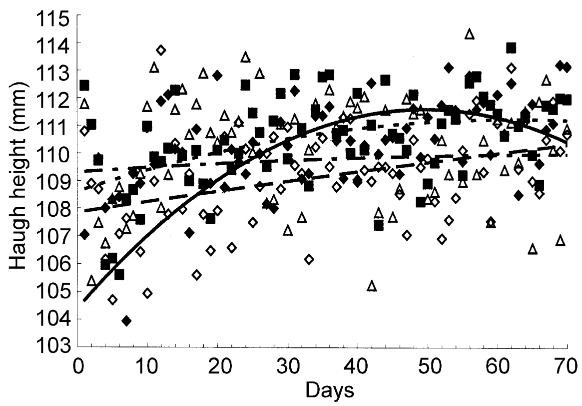

Fig. 9: Effect of acetic acid concentrations on Haugh unit of hens reared during the hot season (‘: 0 acetic acid (----), •: 200ppm acetic acid (- - -), ª: 400ppm acetic acid (!·!·!) and ,: 600 acetic acid (——)

utilization of nutrients under hot environments by bringing non-enzymatic scission of plant cell wall polysaccharides to induce hydroxyl radicals (Fry, 1998). This may lead to better nutrient digestion. Marron *et al*. (2001) observed a 20% decrease in *in vivo* viscosity of ileal digesta in birds fed a diet supplemented with 250 mg organic acid/kg diet, which indicates that it may influence pathways of energy metabolism (Runho *et al*., 1997; McKee *et al.*, 1997). In addition, organic acid was suggested to improve digestive enzyme activity, microbial phytase activity, increased pancreatic secretion increased growth of the gastrointestinal mucosa (Dibner and Buttin, 2002) and promotes enteric absorption of nutrients in ligated duodenal loops (Combs and Pesti, 1976).

The present findings are in agreement with those reported by Jafar and Blaha (1996), Kafri and Cherry (1984), Njoku (1986), Orban *et al*. (1993), Mckee *et al*. (1997), Blaha *et al*. (2000), Marron *et al*. (2001), Pardue (1983) and Aengwabich *et al*. (2003). In contrast, Puron *et al*. (1994) found that dietary 200 mg ascorbic acid/kg did not improve broiler performance reared under 36°C. However, the amount of heat stress experienced by birds is not only dependent on ambient temperature but also on air movement and humidity.

In the present study, 400-600 ppm acetic acid supplementation showed a significant ($p<0.05$) improvement in egg weight, diameter and length of eggs from the laying hens between 30-40 weeks of age. This indicates that acetic acid has a potentially ameliorating effect on some stressors, which allowed for an improvement in the weight of the eggs. The beneficial effect of ascorbic acid supplementation upon egg weight, during the hot season, has also been reported in white Leghorn by Perek and Kendler (1962, 1963). However, Jensen and Chang (1976), Gama *et al*. (2000), Yalcin *et al*. (2000), Chen and Chen (2004), Mahdavi *et al*. (2005), Yesibag and Colpan (2006) and Soltan (2008) reported that supplementation of organic acids did not improve egg weight in laying hens.

The present study demonstrated that supplementation with acetic acid significantly improved shell weight and thickness of eggs. The differences in egg shell quality may be a consequence of the increased mineral and protein absorption (Soltan, 2008). The phenomenon of increased absorption is reflected in the increased calcium and protein deposits of the shell and contributes to improving the quality which may result in increased shell weight and thickness. Several studies reported a reduction in shell weight and thickness of layer eggs as a result of high ambient temperatures (Thornton and Moreng, 1959), which have been significantly improved following supplementation with organic acid (Zeidler, 2001; Rodriguez-Navarro *et al*., 2002; Chen and Chen, 2004; Soltan, 2008). It has been suggested that this response to organic acid may be influenced by factors such as dietary protein level, calcium level and the bird's metabolic rate. However, Mahdavi *et al*. (2005) and Yesibag and Colpan (2006) reported that supplementation of lactic acid to the laying hen did not significantly improve egg shell quality. The lack of consistent findings of the present study and Mahdavi and Colpan's findings may be due to management factors, such as the method of organic acid preparation, type of organic acid used and the vitamin's innate instability. Moreover, in the present study, using the drinking water for supplementing may have been a more suitable choice because water consumption increases during high ambient temperatures. Organic acid supplementation in drinking water improved internal egg quality, which is in agreement with Gama *et al*. (2000), Yalcin *et al*. (2000) and Soltan (2008) who also found a significant improvement in albumen and yolk in layer hens used a diet supplemented with 1% organic acid. However, Yesibag and Colpan (2006) stated that dietary organic acid has no effect on internal egg quality parameters.

The present study demonstrated beneficial effects of supplementation with acetic acid on Haugh unit. Detrimental effects of supplementation with ascorbic acid have been reported in chickens (Thornton and Moreng).

Conclusion: The present study indicated that acetic acid supplementation at level 400 or 600 ppm in drinking water improved egg quality characteristics during the hot season. High levels of acetic acid significantly increased egg weight, egg diameter and egg length. Albumin height increased which causes the yolk amount to decrease in the egg and the egg weight to increase as the shell weight is not affected by the various levels of acetic acid. An increase in the albumin and a decrease in the yolk components of the egg indicates a healthy egg being produced due to the lower levels cholesterol because of lower yolk. This study indicated that 400-600 ppm acetic acid can be used during the hot months to decrease heat stress and improve egg production.

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