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Effect of Vitamin C, Acetylsalicylic, $NaHCO_3$ and KCL supplementation on the performance of broiler chickens under heat stress condition

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This experiment was conducted to evaluate the effects of supplementation of vitamin C (Vit.C; 62.5mg/L), acetylsalicylic acid (ASA; 62.5 mg/L), sodium bicarbonate ($NaHCO_3$; 75 mg/L), and potassium chloride (KCl; 125 mg/L) in drinking water on heat-exposed broilers. A total of 225 male Cobb 500 broiler chicks, 33 d of age, were randomly assigned to 3 treatment groups, with 3 replicates of 25 birds each. The chicks in the first group were exposed to cyclic temperatures (31 to 34°C for12h and 22 to 24°C for12h) and supplemented with Vit C, ASA, KCl and $NaHCO_3$(HS-SUP), the chicks in second group were exposed to cyclic temperatures (31 to 34°C for 12 h, and 22 to 24°C for 12 h) [heat-stress non supplemented (HS-NON); the other chicks were kept under thermoneutral condition (22 to 24°C) and not supplemented as control(C). At the end of the experiment chicks in the control group had better (P<0.05) live body weight, daily gain, total feed intake and less mortality rate (%) than chicks in the HS-SUP and HS-NON groups, whereas chicks in the HS-SUP group had better (P<0.05) live BW and gain, total feed intake, feed conversion ratio (FCR), and mortality rate than chicks in the HS-NON group. The results of this study suggest that ascorbic acid; ASA, KCl, and $NaHCO_3$ in combination offer a potential protective management practice for preventing heat stress-related depression in the performance of broiler chickens.

Key words: Acetylsalicylic acid, broiler, heat stress, potassium chloride, sodium bicarbonate, ascorbic acid

Introduction

Heat stress is of major concerns for poultry production. Biochemical and physiological changes associated with hyperthermia can potentially promote reactive oxygen species (ROS) formation. The impaired muscle membrane integrity in breast muscle of heat-stressed broiler chickens (Sandercock *et al*.,

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2001) was also considered to be related with the changed redox balance, because broiler chickens that were exposed to acute heat stress exhibited more than a 2-fold increase of MDA as an indicator for lipid peroxidation, in the skeletal muscle (Mujahid *et al*., 2007; Sahin *et al*., 2002). High ambient temperature negatively influences the performance of broilers. Several methods are available to alleviate the effect of high environmental temperature on the performance of poultry. Because it is expensive to cool animal buildings, such methods are focused mostly on dietary manipulations. An ambient temperature above 30°C is considered to have an adverse effect on the performance of broiler chicks. Earlier findings have suggested that reduced feed intake, body weight, and feed conversion efficiency is caused by high environmental temperatures (Azad *et al*., 2004; Mujahid *et al*., 2009). However, supplementing the diet with vitamins and minerals can alleviate some of these adverse effects on growth performance, attributed to high ambient temperatures (Sahin and Kucuk, 2001; Sahin *et al.*, 2002). The negative effects of high temperatures on poultry performance can be minimized by the use of appropriate housing design, installation of cooling systems, feed formulations designed according to feed intake and weather conditions, and the use of some minerals, electrolytes, ascorbic acid, or acetylsalicylic acid (ASA) in the drinking water of birds (Branton *et al*., 1986; Naseem *et al*., 2005 a,b; Smith and Teeter, 1992). Several studies indicated that heat stress reduces the bodyweight (Al-Neemy and Hassan, 2002), immune response and also causes mortality Younis (2007) and differenttherapeutic measures are used to minimize the harmful effects of heat stress on performance of broiler chickssuch as ascorbic acid Younis (2007), vitamin E (Sahin *et al.*, 2002), acetylsalicylic acid (Naseem *et al*., 2005b), potassium chloride (Al-Khateeb and Al-DdinSalih, 2005), sodium bicarbonate, acetic acid Hassan (2009) and organic and inorganic chromium Moeini *et al*. (2011).

Poultry have the ability to synthesize ascorbic acid, but this ability is inadequate under stress conditions, such as high environmental temperatures, high humidity, a high productive rate, and parasitic infestation Pardue and Thaxton (1986) documented evidence that particular environmental stressors can alter the use or synthesis of ascorbic acid in poultry. Therefore, supplementation of ascorbic acid in water appears necessary during heat stress. This study was conducted to evaluate the effects of combined supplementation of ascorbic acid, ASA, $NaHCO_3$, and KCl in water on the performance of heat-exposed broilers.

Materials and methods

A total of 225 healthy male Cobb 500 broiler chicks at 21 d of age were used in this study. Broiler chickens were obtained from a local hatchery, reared in litter pens from 21 to 33 d of age under temperature conditions recommended by the Cobb Broiler Management Guide Cobb (2008), and monitored to determine whether there were any apparent clinical signs before initiation of the experiment. The chicks were provided with a basal grower feed from 21 to 28 d of age, followed by a finisher feed from 29 to 41 d of age (Table 1). At 33 d of age, broiler chickens were randomly assigned to 3 treatment groups, with 3 replicates of 25 birds each. Each group was supplied with a trough feeder and an automatic cup drinker. Feed and water were supplied libitum during the experimental period. The birds were exposed to cyclic temperature (31 to 34°C for 12 h, and 22 to 24°C for 12 h) and were supplemented with ascorbic acid (62.5 mg/L), ASA (62.5 mg/L), KCl (125 mg/L), and $NaHCO_3$ (75 mg/L) [10] in the water throughout the experimental period, and the water was changed every day [heat-stress supplemented (HS-SUP) group], or exposed to cyclic temperatures (31 to 34°C for 12 h, and 21 to 23°C for 12 h) and not supplemented [heat-stress non supplemented (HS-NON) group]. The other birds were kept under thermoneutral conditions (21 to 24°C) and not supplemented (control group). The duration of the experiment was 10 days. The lighting program before and during the experiments was as recommended by the Cobb Broiler Management Guide Cobb (2008).The feed intake, BW, and body gain were recorded at the end of experiment, and mortality was recorded daily. Feed intake values were adjusted for mortality to the nearest day. The FCR was calculated at the end of the experiment as total feed consumed divided by the weight of live and dead birds. Blood samples were collected from chicks (4 chicks per treatments per environmental chamber), the chicks were sacrificed, and portions of the pectoralissuperficialis muscle were rapidly excised. Tissues were immediately frozen in liquid nitrogen and powdered. Meanwhile sera were collected by centrifuging blood samples at 1500×g for 20 min. Sera and tissues were stored at −20 and −80°C, respectively. Pectoralissuperficialis muscle was used for MDA measurements after 1 week of storage at −80°C.

Lipid peroxidation was assayed colorimetrically as a 2-thiobarbituric acid reactive substance (TBARS) using the modified method of Ohkawa *et al*. (1979) described by Mujahid *et al*. (2007). The TBARS content was assayed by using a spectrophotometer (Hitachi U-2001, USA) at 532 nm and expressed as nmol of MDA per mg protein. Protein concentration was determined by the method of Bradford (1976) using crystalline bovine serum albumin as a standard.

The data were analyzed using the general linear model procedure of SAS software (SAS, 2003) as a complete randomized design. Differences among treatment means were determined using the Duncan's multiple-range test.

Table 1. Ingredients and chemical analyses of the grower and finisher diets fed to broiler chickens

Diet composition (%)	Grower (%)	Finisher (%)
Soybean meal (45%)	33.53	27.10
Ground corn	57.86	63.92
Lys	0.16	0.25
Soybean oil	4.73	5.20
Monocalcium phosphate	1.42	1.36
Salt	0.27	0.23
Limestone، ground	1.47	1.40
Sodium bicarbonate	0.07	0.092
Dl-met	0.28	0.23
Vitamin and mineral premix[1]	0.20	0.20
Calculated analysis		
ME (kcal/kg)	3.05	3.15
Met and Cys (%)	0.94	0.83
Lys (%)	1.25	1.15
Met (%)	0.60	0.52
CP (%)	21.00	18.5
Calcium (%)	0.90	0.85
Available phosphorus (%)	0.44	0.42

[1]Each kilogram of feed (SUP javandaro Co., tehran, iran) contained the following: iron, 44 mg; copper, 5 mg; zinc, 75 mg; manganese, 6 mg; iodine, 1.306 mg; selenium, 0.225 mg; folic acid, 0.6 mg; biotin, 100 g; pantothenic acid, 10 mg; niacin, 39.994 mg; vitamin A (retinyl acetate), 12,500 IU; vitamin D3, 2,500 IU; vitamin E, 50 IU; vitamin K3, 3.5 g; vitamin B1, 1 g; vitamin B2, 5.5 g; vitamin B6, 2.5 g; vitamin B12, 20.0 g

Results and discussions

The results of this study indicated that birds in the control group exhibited better ($P<0.05$) live BW and gain, total feed intake, FCR, and mortality rate (%) than birds in the HS-SUP and HS-NON groups (Table 2). The growth rates of broilers exposed to cyclic temperatures were depressed by 149 g/bird in HS-SUP group) and 256 g/bird in HS-NON group, compared with birds in the control group. This result was consistent with the general trend observed in heat-stressed chicks (Austic, 1985; Chen *et al*., 2005; Geraert *et al*., 1996; Pardue *et al*., 1985; Sahota *et al*., 1998; Siegel, 1995; Yahav *et al*., 1996). It is believed that for every 10°C increase in ambient temperature above 20°C, there is a 17% reduction in feed intake (Austic, 1985). The depression in growth rate

and in BW gain at high environmental temperatures of 31 to 34°C (Table 2) might have been caused by many factors, including decreased feed intake (Emmans and Charles, 1989), inefficient digestion (Har *et al*., 2003), impaired metabolism (Farrell and Swain, 1977), and genetic background (Cahaner *et al*., 1995). However, the growth rate of birds in the HS-SUP group was significantly ($P<0.05$) better than that of birds in the HS-NON group. The total mortality rate and FCR of birds in the treatments groups were significantly ($P<0.05$), greater than those of the control group, whereas the FCR and total mortality rate of birds in the HS-NON group were significantly ($P<0.05$) greater than those in the HS-SUP group. This clearly indicates ($P<0.05$) a lower mortality rate and FCR under cycling heat stress temperatures when ascorbic acid, ASA, KCl, and $NaHCO_3$ were supplemented. This result was similar to the reports from earlier studies (Leeson *et al*., 1992; Smith, 1993; Teeter and Smith, 1986). The poor FCR obtained during cyclic heat stress in this experiment might be related to decreased feed intake, decreased feed utilization (insufficient digestion), or both. The high mortality of broilers in hot environments might have been due to inefficient evaporative cooling, which may have led to an increased body heat load. This accumulation of heat may have caused a continued increase in body temperature until the birds died from heat prostration (Branton *et al*., 1986; Kutlu, 1996). Poultry have the ability to synthesize ascorbic acid, but this ability is inadequate under stress conditions, such as high environmental temperatures, high humidity, a high productive rate, and parasitic infestation (Pardue and Thaxton, 1986) documented evidence that particular environmental stressors can alter the use or synthesis of ascorbic acid in poultry. Therefore, supplementation of ascorbic acid in water is necessary during heat stress, based on the results of their study. Table 2 shows that broilers in the HS-SUP group had significantly ($P<0.05$) better live BW and gain, total feed intake, FCR, and mortality rate (%) than birds in the HS-NON group. These results support those of Keskin and Durgan (1997), and Naseem et al. [21], who reported that KCl and $NaHCO_3$ improved the performance of birds during heat stress. Niokue (1986); Sahota *et al*. (1998), and Naseem *et al*. (2005) reported that ascorbic acid improved the FCR. Sharma and Bhatti (1998) reported that ASA improved the FCR.

Table 2. Mean ± SE of live BW (g/chick), live BW gain (g/chick), total feed intake (g/chick), accumulated FCR (g/g), and mortality rate (%), from 33-d-old (beginning of the experiment) to 42-d-old (end of the experiment)

Group [1]	Live BW		BW gain	Feed intake	FCR	Mortality
	33 d	42 d				
CONTROL	1,429^{a}± 0.12	2,021^{a}± 0.18	592^{a}± 0.06	910.2^{a}± 0.31	1.538^{c}± 0.02	1.33^{c}± 1.8
HS-SUP	1,432^{a}± 0.19	1,875^{b}± 0.11	443^{b}± 0.01	844.9^{b}± 0.29	1.907^{b}± 0.05	8.0^{b}± 1.5
HS-NON	1,455^{a}± 0.21	1,765^{c}± 0.23	310^{c}± 0.12	792.1^{c}± 0.35	2.458^{a}± 0.09	17.3^{a}± 1.2

a–c Means with different superscripts in the same column are different (P<0.05).

The effect of supplementation with vitamin C, ASA, KCl and $NaHCO_3$on Serum albumin, glucose, uric acid, Malondialdehyde (MDA) concentrations in skeletal muscle of 42-day-old broilers under heat stress is shown in Table 3. Geraert *et al*. (1996) reported that constant high temperature (32 °C, 14 days) did not affect plasma triacylglycerol and uric acid levels, but did affect the glucose level. In the present study the serum concentrations of both glucose, and uric acid were not significantly changed by chronic heat exposure (P>0.05). These results are in accordance with those of Azad *et al*. (2010).

Moreover, chronic heat stress in this experiment caused no change in serum albumin levels (P>0.05). These results suggest that the experimental conditions employed here are suitable for the identification of physiological and biochemical characteristics of broiler chickens under chronic heat stress conditions.Thisfinding is in accordance with those reported earlier by others, whoshowed that the supplementation of Ascorbic acid leadto increase the immunity of birds and tolerance heat andpotassium chloride increased the water consumptionsof bird decreased and body temperature andfinally mortality rate is reduced (Al-Neemy and Hassan, 2002; Younis, 2007).

Mujahid *et al*. (2009) reported that 12 h of acute heat exposure produced a 2-fold increase of MDA levels in skeletal muscle.

Table 3. Mean ± SE of broilers Serum concentrations of albumin, glucose, and uric acid (mmol/L), and malondialdehyde (MDA) concentration (nmol/mg protein) of pectoralis muscle of 49-d-old broilers

Group[1]	Albumin	Glucose	Uric Acid	MDA
CONTROL	145.33± 1.69	22.53± 0.52	59.48± 2.07	0.81± 0.09
HS-SUP	148.64± 1.72	23.60± 0.62	55.88± 1.75	0.71± 0.07
HS-NON	142.93± 1.64	23.02± 0.60	53.30± 1.62	0.78± 0.08

Means within a column showing different superscripts are not significantly different (P>0.05).
1HS-SUP = heat-stress group supplemented with ascorbic acid (62.5 mg/L), acetylsalicylic acid (62.5 mg/L), sodium bicarbonate (75 mg/L), and potassium chloride (125 mg/L) and exposed to cyclic temperatures (31 to 34°C for 12 h, and22 to 24°C for 12 h); HS-NON = heat-stress group not supplemented but exposed to cyclic temperatures (31 to 34°C for12 h, and 22 to 24°C for 12 h).

It has also been demonstrated by Wang *et al*. (2009) that broiler chickens exposed to acute heat stress (3 and 5 h) exhibited a 4-fold increment of MDA in the pectoralis majors. In this chronic heat stress experiment, we observed no increase in MDA levels in skeletal muscle (P>0.05): the degree of changes was not similar to those observed in the 'acute' heat stress model of previous studies (Mujahid *et al*., 2009; Wang *et al*., 2009). Therefore, it can be concluded that chronic heat stress does not affect lipid peroxidation to the same extent as that obtained under acute heat stress conditions. Unfortunately, the effects of ASA, KCl or $NaHCO_3$ on the oxidative stability of skeletal muscle of broilers are not available in the literature.

Conclusion

The heat-stressed broilers responded positively to continuous supplementation of ascorbic acid, ASA, KCl, and $NaHCO_3$ throughout the periods of heat stress as evidenced by live BW, daily gain, total feed intake, FCR, and mortality rate. According to the result of this study supplemental of Ascorbic acid, ASA, KCl, and $NaHCO_3$ are recommended in heat-stressed chicks.

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