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EFFECT OF ASCORBIC ACID AND SODIUM BICARBONATE ON FUNCTIONAL TRAITS AND ANTIOXIDANT ENZYMES IN BLOOD OF BROILERS UNDER HEAT STRESS

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ABSTRACT

In this study, the response of broilers that kept under heat stress to vitamin C and sodium bicarbonate supplements were examined in an experiment using 80 male Ross 308 chicks. Chickens are randomly transported in standard cages in 4 to 42 days in heat stress conditions (temperature 35 ° C). In this study, four ration treatments were applied include:1) basal ration, 2) basal ration + 250 mg/kg vitamin C, 3) basal ration + 0.25% sodium bicarbonate and 4) basal ration + 250 mg/kg vitamin C + 0.25% sodium bicarbonate. The rations were fed to chickens in period of 1 to 42 days. The experiment consisted of 4 treatments and 4 replicates per treatment and 5 chicks per cage and a total of 80 male chicks were used. Testing was conducted in a completely randomized design. The results showed that the addition of sodium bicarbonate and vitamin C in the ration insignificant effect on FCR, weight gain and feed intake. Yield and carcass components investigations were also noted that these characteristics were not significantly affected by the treatments. The results showed that concentrations of antioxidant enzymes such as SOD and CT and MDA significantly affected by vitamin C and sodium bicarbonate. SOD and CT significantly increased and MDA concentration was significantly reduced by vitamin C and sodium bicarbonate ration concentration in the treatment d. The SRBC titration results showed no significant difference between treatments. In overall, we conclude that concurrent use of vitamin C and sodium bicarbonate that increases blood antioxidant standards and reduces oxidant criteria.

Keywords: Sodium Bicarbonate, Broiler, Heat Stress, Vitamin C

INTRODUCTION

Aviculture is growing rapidly in tropical regions of the world.Large parts of the continents of Asia, Africa and South America that have a significant share of the population in the world are located in this weather conditions.Achieving adequate production in the tropics weather is difficult because in such environment, thermal stresses occur in poultry that increase the negative effects such as reduced feed intake, weight loss, reduction in carcass quality, increased mortality, increased feed conversion ratio and in overall leads the decrease of the yeild of poultry (1, 10 and 11).Researchers have suggested strategies to reduce the harmful effects of heat stress.Addition of electrolyte supplements such as sodium bicarbonate and potassium chloride in drinking water or feed will cause growth improvement of broilers under heat stress conditions (6).

Electrolytes, particularly sodium bicarbonate 0.5% or ammonium chloride 0.3% to 1% or sodium zeolite can reduce alkalosis resulting from heat stress (6).Sodium bicarbonate stimulates food and water consumption at high environmental temperatures .With the addition of these compounds in the ration of broiler chicks under heat stress, weight gain increase up to 0.9% (6).Borges et al (2007) have shown that the addition of sodium bicarbonate 0.5% and 1% levels in rations of broilers under heat stress that were exposed to temperatures of 34-36 and 39-41resulted in improved feed intake, weight gain and feed conversion ratio.Vitamin C (ascorbic acid) can outweigh the negative effects of heat stress conditions, especially in conjunction with vaccination and strengthen the immune system and the defense of broilers under heat stress (13, 18).

Vitamin C is one of the materials that their usage have been considered and studied to cope with heat stress conditions in poultry.Heat stress causes changes in physiological and endocrine secretion that may lead to a minimum efficiency of the immune system .In these conditions, Vitamin C prohibits the activity of 21-hydroxylase and 11-beta-hydroxylase (key enzymes in biochemical pathways of Kurt Kyvstrvn) (8). As a result, the decrease in the secretion of Kurt Kyvstrvns by vitamin C, inhibits the negative effects of heat stress on performance and immune system (21).Vitamin C can increase the immune response of broilers and can have a stimulatory effect on the phagocytic activity of leukocytes or on retico endothelial system performance and also the formation of antibodies (20).

Cier et al (1992) in Palestine examined the effect of ascorbic acid (vitamin C) on the production of broiler chickens in summer so

that the birds of the age of 27 days were kept at room temperature 44.5 ° C and high humidity.The use of vitamin C in the ration increased body weight in male broilers while this effect was lower in females but FCR did not improve under the influence of vitamin C.

Heat stress reduces blood antioxidant capacity and increase susceptibility to oxidative damage (16). Research has shown that the total antioxidant capacity of blood and the body affected by a series of enzymatic and non-enzymatic mechanisms of compounds containing hydrophilic (uric acid, bilirubin, glutathione and vitamin C), hydrophobic compounds (especially vitamin E) and catalase, superoxide dismutase and malondialdehyde (19).

This study aimed to evaluate the effect of adding vitamin C and sodium bicarbonate on yield, antioxidant enzymes and immune system of broilers under heat stress.

MATERIALS AND METHODS

The test was conducted for forty-one days in the field of educational Shelter of Mahdasht Islamic Azad University in the warm season. Experiment was performed with 4 treatments and 4 replications of 16 cages (experimental unit), where each replication consists of 5 commercial strains Ras- 308 male broiler chickens.Each of the 4 treatments was identified with codes 1, 2, 3 and 4. Thus the treatments included

Treatment no.1: basal ration (control group)

Treatment no.2: basal ration with 0.25% sodium bicarbonate

Treatment no.3: basal ration with 250 mg/kg vitamin C

Treatment no.4: basal ration with 0.25 % sodium bicarbonate and 250 mg/kg vitamin C

Rations were set based on corn and soybean feeding and the advice of breeding and feeding of broiler chickens Ras- 308 by UFFDA software .In this experiment to establish minimum temperature (i.e., 32 ° C for at least 7 hours per day from 10 am to 4 pm), heat source was used in the low temperature condition of the indoor and for temperature control some thermometers were placed at the height of 45 cm from the floor.For the measurement of feed intake, weight gain and feed conversion rate the following formulas were used:

Number of days in each period ×Daily feed intake in each period =feed intake in each period.

Daily gain w

$$\textbf{Feed conversion ratio} = \frac{\textit{average feed consumption in each period}}{\textbf{average increase in weight during the period}}$$

At the end of the period, the yield of carcass of slaughtered chicken and their parts (thighs, chest, heart rate, liver, visceral fat) were examined. Also to investigate the effects of heat stress on antioxidant enzymes (superoxide dismutase and catalase) and malondialdehyde, blood samples were taken samplingrepetition. .A week later, i.e.the age of 39 days, for SRBC titration, blood sampling of the wing of marked birdswere performed and was sent to the veterinary laboratory for SRBC titration andafter the results obtaining, the data were analyzed by SPSS software.

Table 1: food used in Treatments used in the beginning, growth and finishing stages of growth of broiler

Chemical composition (%)	Beginning (1-11 days)					Growth (12-22 days)			T
	Treatment 1	Treatment 2	Treatment 3	Treatment 4		Treatment 1	Treatment 2	Treatment 3	
Corn	54.98	54.98	54.98	54.98		54.18	54.18	54.18	
Soybean meal	32.70	32.70	32.70	32.70		31.60	31.60	31.60	
Fishmeal	5.00	5.00	5.00	5.00		4.90	4.90	4.90	
Soybean oil	3.20	3.20	3.20	3.20		5.00	5.00	5.00	
Dicalcium phosphate	1.84	1.84	1.84	1.84		1.7	1.7	1.7	
Calcium carbonate	0.79	0.79	0.79	0.79		0.65	0.65	0.65	
DL-methionine	0.34	0.34	0.34	0.34		0.28	0.28	0.28	
L - lysine hydrochloride	0.27	0.27	0.27	0.27		0.13	0.13	0.13	
Vitamin Supplements	0.25	0.25	0.25	0.25		0.25	0.25	0.25	
Mineral supplements	0.25	0.25	0.25	0.25		0.25	0.25	0.25	
Salt	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	
Vitamin C (mg / kg)	0	0	250	250		0	0	250	
Sodium bicarbonate	0	0.25	0	0.250		0	0.25	0	

Table 2: Chemical compounds in Treatments used in the beginning, growth and finishing stages of growth of broiler

Nutrients	Beginning (1-11 days)				Growth (12-22 days)				Finishing (23-42 days)							
Treatment	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 1	Treatment 2	Treatment 3	Treatment 4		Treatment 1	Treatme					
Digestible energy (گرم) (Kcal / kg)	3040	3040	3040	3040	3192	3192	3192	3192		3044						
(%) Crude protein	22	22	22	22	19	19	19	19		22						
%) Digestible methionine	0.61	0.61	0.61	0.61	0.49	0.49	0.49	0.49		0.61						
ble methionine + cysteine (%) مضم (%)	0.91	0.91	0.91	0.91	0.76	0.76	0.76	0.76		0.91						
(%) Lysine	0.20	0.20	0.20	0.20	0.95	0.95	0.95	0.95		0.20						
(%) Threonine	0.76	0.76	0.76	0.76	0	0	0	0	0	0						
(%) Tryptophan	0.21	0.21	0.21	0.21	0.19	0.19	0.19	0.19		0.21						
Digestible arginine	0.27	0.27	0.27	0.27	1.14	1.14	1.14	1.14		1.27						
Digestible isoleucine	0.79	0.79	0.79	0.79	0.70	0.70	0.70	0.70		0.79						
%) Digestible valine	0.88	0.88	0.88	0.88	0.79	0.79	0.79	0.79		0.88						
%) Calcium	1.00	1.00	1.00	1.00	0.85	0.85	0.85	0.85		1.00						
(%) Phosphorus	0.5	0.5	0.5	0.5	0.41	0.41	0.41	0.41		0.50						
) Sodium absorption	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16						
ı-anion difference (meq / kg)	210	233	210	233	197	220	197	220		210	233					
(%) Chlorine	0/28	0/18	0/28	0/18	0/24	0/16	0/24	0/16		0/28	0/18					

RESULT AND DISCUSSION

Comparison of the results of functional traits in each of the treatment is summarized in Table 3.The results of this test showed no significant differences between the different treatments of Vitamin C and sodium bicarbonate in feed intake and weight gain ($P>0.05$).

Most used feed in the beginning period and the total period was related to the combined treatment (0.25 % sodium bicarbonate and 250 mg/kg vitamin C), while in the growth period vitamin C had the highest rate while there was no significant difference between body weight gains. Ibrahim and Mobarak (2002) used vitamin C in the daily drinking water for 10 weeks (at doses of 250 and 500 mg /L).They found that 500 mg / Lof vitamin C, during the summer, leads to a significant increase ($P<0.01$) in body weight but did not differ significantly from the 250 mg value.

The combined use of sodium bicarbonate and vitamin C in the beginning period had the highest increase in weight but in the growth and finishing periodsand the whole period, the highest weight gain was observed in the control group containing basal ration (Table 3).

Table 3 - Comparison of the averages of functional traits of broilers under heat stress in different treatments and in the beginning period (1-11), growth (11-22), finishing period (22-42) and the whole period (1-42)

Treatment	Food consumption (g)				Weight gain(g)				CV			
	beginning	growth	finishing	whole	beginning	growth	finishing	whole	beginning	growth	finishing	whole
Control	312	907	2500	3720	253	706	1485	2445	1.33	b1.30	1.79	1.57
Sodium bicarbonate (0.25 %)	319	1044	2375	3739	247	636	1473	2356	1.29	a1.64	1.67	1.61
Vitamin C (250 mg/kg)	294	917	2500	3711	234	620	1335	2189	1.26	Ab1.49	1.88	1.69
Sodium bicarbonate + Vitamin C (0.25 %+250 mg/kg)	345	948	2500	3793	260	643	1335	2257	1.32	Ab1.48	1.80	1.68
SEM	8.40	31.20	31.20	46.90	4.93	19.07	72.61	87.82	0.01	0.05	0.07	0.05

Hayat et al (1999) and Lin et al (2006) reported that the addition of sodium bicarbonate to feed or drinking water increase food intake of broiler chicks under heat stress. In this condition, the acid-base balance of the blood changes and sodium bicarbonate stimulates feed and water consumption by providing carbonate ions and modifies the negative effects of heat stress. In this study, sodium bicarbonate and with vitamin C increase the feed consumption. Nasim (2005) and Nassiri Moghaddam et al (2005) observed no significant effect of 0.35 % sodium bicarbonate in broiler feed on feed conversion ratio.

In the case of feed conversion ratio, the addition of sodium bicarbonate and vitamin C increases the conversion yield and this increase was statistically significant only in the period of growth ,while in the initial period, the finishing period, and the whole period there was no significant difference between treatments .So that the best feed conversion ratio was related to the control treatment and the highest conversion rate was related to vitamin C. Fuentes et al (1998) suggested that sodium bicarbonate stimulation of water consumption removes excess heat and helps in maintenance of electrolyte balance in bird and rations containing sodium bicarbonate will lead to FCR improvement by increasing growth.

Carcass yield and its components (thigh, chest, heart rate, liver, visceral fat) were investigated (Table 4).In general, none of the treatments did not affect carcass components statistically ($P>0.05$). The comparison of average of yield of carcass shows the highest amount was related to vitamin C with sodium bicarbonate treatments while sodium bicarbonate treatment had the lowest amount.Zeinali et al. (2009) reported that levels of sodium bicarbonate and its interaction with selenium

had not significant effect on carcass yield and carcass composition.

The blood antioxidant enzymes i.e. superoxide dismutase (SOD) ,catalase (CT) and oxidation index of blood i.e. malondialdehyde (MDA) in broiler chickens were calculated in the growth period.In this experiment, it was found that in the whole period, the amount of antioxidant enzymes were significantly different in different treatments (P>0.05) (Table 4).

Broilers with fed rations containing a combination of vitamin C and sodium bicarbonate showed the highest concentration of superoxide dismutase in blood which significantly had difference with the other treatments and the lowest concentration of the enzyme was related to the sodium bicarbonate treatment.

Also the amount of superoxide dismutase (SOD) in the group treated with vitamin C were significantly more than sodium bicarbonate treatment while sodium bicarbonate treatment was not significantly different from control. In the thermal stress condition, superoxide dismutase is an important enzyme for dealing with membrane lipid peroxidation and increases the production of free radicals such as O2 and HO in the blood of chickens (16). Superoxide dismutase (SOD), glutathione peroxidase and catalase have important role in antioxidant defense in chicks that have heat stress condition (5).

By observation and analysis of the results, it was found that the highest concentrations of catalase (CT) was related to the treatments with vitamin C and sodium bicarbonate while the lowest amount of the enzyme was related to the control.CT enzyme concentration of vitamin C treatment and sodium bicarbonate treatment was not significantly different. In general, the highest concentrations of SOD and CT were related to vitamin C and sodium bicarbonate treatment. The results suggests that simultaneous use of ascorbic acid and sodium bicarbonate had a positive effect on increasing the amount of antioxidant enzymes and the least amount was related to the control group. The obtained results are consistent with the results of Shakeri (2000).

Comparison of averages of malondialdehyde (MDA) showed that four experimental rations were significantly different and from the highest to the lowest amount are: control, sodium bicarbonate, vitamin C and vitamin C treatments with sodium bicarbonate. As shown in Table 5, the enzymes SOD and CT increase with using a combination of vitamin C and sodium bicarbonate which represents an increase of the antioxidant capacity of the blood, resulting in a decrease in a rate of oxidation of blood (MDA) and hence in can be

concluded that in treatment no.4, broilers cope with heat stress well and increase of vitamin C and sodium bicarbonate significantly modify the effect of heat stress.

Comparison of the results antibody titration against SRBC among different treatments (Table 5) did not show significant differences (P>0.05). However, among different treatments, greatest average was related to treatment no.3 (250 mg/kg vitamin C) and lowest value was observed in treatmentno.1 (basal ration).

As a result, it can be concluded that addition of vitamin C and sodium bicarbonate increases the sensitivity of the immune system and with the arrival of sheep red blood cells in the chicks body, a significant reaction was occurred in the chicks of vitamin C and sodium bicarbonate treatment compared to the control (no vitamin C and sodium bicarbonate).No significant difference between treatments could be due to the low amount injection of sheep red blood cells (SRBC) into the body. Shakeri (2000) and Taghiloo (2010) also showed the addition of vitamin C has no significant effect on the immune system during sheep red blood cells injection (SRBC titration).

Table 4 - Comparison of antioxidant enzymes and blood oxidation index in blood of the broilers at 39th day of age

Treatment	SOD (Unit / ml)	CT (Unit / ml)	MDA (micro Mole/ml)
Control	**[c]2.39**	**4.06**	**232.82**
Sodium bicarbonate (0.25 %)	**2.28**	**5.99**	**[b]161.12**
Vitamin C (250 mg/kg)	**4.54**	**5.79**	**[c]103.85**
Sodium bicarbonate + Vitamin C (0.25 %+250 mg/kg)	**7.25**	**8.91**	**60.87**
SEM	**0.39**	**0.34**	**13.02**

- In each column, the non-similar number have significant different with each other (p<0.05).

Table 5 - Results of anti-SRBC antibody titration at 32th days of age

Treatment	SRBC (ml)
Control	3.50
Sodium bicarbonate (0.25 %)	23.62
Vitamin C (250 mg/kg)	73.62
Sodium bicarbonate + Vitamin C (0.25 %+250 mg/kg)	31.83

- In each column, the non-similar number have significant different with each other ($p<0.05$).

CONCLUSIONS

The results of these tests showed that the use of vitamin C and sodium bicarbonate, increased ability to cope with heat stress effectively individually and in combination in the ration of broilers under heat stress. Although functional traits such as the consumption of grain per capita, weight gain and feed conversion ratio was not significantly increased in chickens ($P>0.05$), the use of vitamin C and sodium bicarbonate significantly increased the antioxidant capacity of blood in the chick ($P>0.05$). This increase was observed in all treatments compared to the control. The results of antibody titration against SRBC showed no significant difference between treatments ($P>0.05$). However, greater amounts of antibodies to SRBC were synthesized in the body of broilers under heat stress in ration of vitamin C and sodium bicarbonate.

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