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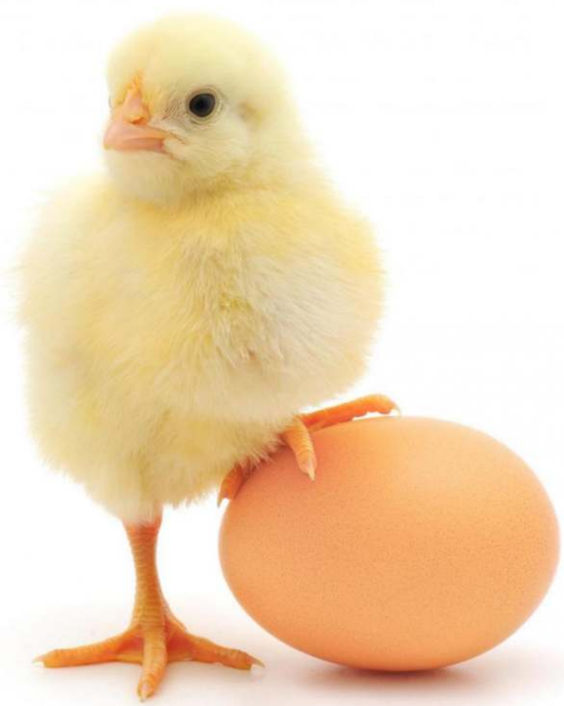

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Effect of Some Water Supplements on the Performance and Immune System of Chronically Heat-Stressed Broiler Chicks

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Abstract: Effects of water supplements, sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl) and acetic acid were evaluated on the performance and immune system of chronically heat-stressed broiler chicks. Two hundred day old broiler chicks were allotted to one of the four groups (n = 50); 1) control [no supplements], 2) $NaHC0_3$, 0.5%, 3) KCl, 0.15% and 4) acetic acid, 1.5 mL/Liter. All chicks were kept in a controlled environmental chamber maintained at 33±2°C from day one to 6 weeks (wks) of age. Significantly higher weight gains coincided with decreased feed conversion ratios for all the supplemented groups as compared to control group at 2, 4 and 6 wks of age. Bursal index, percentage weights of thymus and spleen in relation to body weight and natural agglutinin levels, an indicator of humoral immunity were higher but the heterophil:lymphocyte ratio, an indicator of stress was lower for the supplemented groups as compared to control group. Total aerobic spore formers and *Enterobactraecae* counts in the intestinal swab samples were higher in control group than supplemented groups. Intestinal pH was lower at 2, 4 and 6 weeks of age but water consumption at 5 and 6 wks of age tended to be higher in acetic acid treated group as compared to other groups. Overall, the results indicated significant improvement in the performance and immune response of chronically heat-stressed broiler chicks given the water supplements, acetic acid being slightly superior to $NaHCO_3$ and KCl.

Key words: Heat stress, water supplements, acetic acid, sodium bicarbonate, potassium chloride, immunity, intestinal pH

INTRODUCTION

Poultry health and performance are affected by many interacting environmental and management stressors such as adverse weather conditions (chilling or overheating), overcrowding, caging, debeaking, vaccinations, change in feed and transportation. These stress conditions adversely affect immunity of birds against infectious agents (Radostits *et al*., l994). Heat stress is one of the major constraints that confront poultry production in open house poultry farms, especially in tropical climates. Broiler chicks are homeothermic, maintain their body temperature within a slight range (thermoneutral zone) in which energy needs for thermoregulation are minimum and the net energy for production is maximum (Furlan and Macari, 2002).

At high ambient temperatures, evaporative cooling through panting is important for heat dissipation. This leads to respiratory alkalosis which has been correlated with reduced feed consumption, growth rate and survival (Kutlu, 1996). Heat stress also increases feed conversion ratio but decreases the immune response and ratio of the weight of bursa, thymus and spleen to body weight of the birds (Naseem *et al*., 2005) and increases heterophil:lymphocyte ratio (Borges *et al*., 2004). The negative effects of high ambient temperature on poultry performance can be minimized by electrolytes as they are necessary to maintain physiological functions during hot weather. Several water treatment practices have been proposed to overcome the consequences of heat stress such as allowing broilers to stand in cool water, avoiding drinking hot water or adding water supplements such as sodium bicarbonate ($NaHCO_3$) and potassium chloride (KCl). These practices have been reported to reduce mortalities and/or elevate growth rate. A level of 1.5-2% KCl as a source of potassium was found to maximize gain in 5-8 weeks old broilers (Smith and Teeter, 1987). Heat exposure reduces plasma carbon dioxide and bicarbonate (Balnave and Gorman, 1993) which may affect the blood pH and induce a nutritional requirement for bicarbonate (Teeter *et al*., 1985). Bonsembianate *et al*. (1988) reported that a level of 0.5% sodium bicarbonate stimulates feed and water intake in broilers. To the best of our knowledge there is lack of information on the effects of water supplements on the immune system of heat-stressed broiler chicks in correlation with their overall performance. The main objective of this research was to investigate the effects of water supplements, KCl, $NaHCO_3$ and acetic acid to heat-stressed broiler chicks from hatching to 6 weeks, on their performance and immune system.

MATERIALS AND METHODS

Experimental chicks: A total of two hundred one-day old Hubbard chicks were assigned randomly to one of four treatment groups (n = 50). Control group received no water supplements but the other three groups received one of the following water supplements: $NaHCO_3$ 0.5%, or KCl 0.15%, or acetic acid 1.5 ml/L. Chicks were reared on deep litter, kept under 23 h light: 1 h darkness and stocking density of 10 birds /m^2. Feed and water were provided ad libitum.

Exposure to chronic heat stress and water treatments: Chicks in all groups were kept in controlled environmental chambers with dimensions of 1.80 x 2.40 x 2.34 m. Chamber was provided with thermostatically controlled electric heater and small electric fan for the circulation of air, with two holes of 16 cm diameter for ventilation. All chicks were subjected to temperature of 33±2°C from day one till the end of the experimental period of 6 weeks.

Sample collection and testing: At 2, 4 and 6 wks of age, five chicks from each group were randomly selected, body weight was recorded and then sacrificed. Blood samples were collected in clean and dry centrifuge tubes, all viscera were removed carefully by hand and then carcass weight was recorded.

The data collected included percentages of weights of thymus and spleen in relation to carcass weight (Jin *et al*., 1998), bursal index (Sharma, 1997), heterophil:lymphocyte ratio (H:L) (Gross and Siegel, 1983), serum natural agglutinin (Giambrone *et al*., 1978), intestinal pH (Rahmani and Speer, 2005). Intestinal colonization was evaluated by counting total aerobic spore forming organisms and *Enterobactraecae* in intestinal swabs by the pour plate method (Cruickshank *et al*., 1975). Water consumption was calculated by adding known volume of water to each group and subtracting the remaining before adding again next day.

Statistical analysis: Results are expressed as means ± SEM for each group. Groups were tested for differences by performing the ANOVA and Fisher's least protected significant difference test, using the Statview 4.53 software (Abacus Concepts Inc., Berkeley, CA). Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The three water supplements used in this study, acetic acid, $NaHCO_3$ and KCl have improved weight gains in broiler chicks at 2, 4 and 6 wks of age (Table 1) except KCl treated group at 2 wks of age, which may be due to decreased water consumption at the first week (Table 6) reflecting a period of adaptation. There were no significant differences in feed conversion ratios (data not shown) although the means tended to be lower for supplemented chicks. Acetic acid supplemented group showed higher weight gains, followed by $NaHCO_3$ and then KCl, as compared to control group. High environmental temperatures are known to decrease weight gains in chicks as observed in this study which could be due to several factors including decreased feed consumption, decreased digestion (Har *et al*., 2000) and impaired metabolism (Farrell and Swain, 1977). Suk and Washburn (1995) have shown decreased efficiency of feed utilization with increased environmental temperatures and are of the opinion that decreased feed consumption is closely related to extra heat load accumulated in the course of heat stress. A few earlier studies have reported that alternate management protocols and dietary modifications can alleviate the adverse effects of chronic heat-stress in broiler chicks. Correction of blood acid-base imbalance may be achieved by electrolyte supplementation through drinking water or feed (Naseem *et al*., 2005) suggesting that $NaHCO_3$ and KCl used in this study may have ameliorated heat-stress induced disturbances in acid-base balances.

The improvement in weight gain in KCl-supplemented chicks observed in this study may be due to the fulfillment of potassium requirement, otherwise decreased due to respiratory alkalosis induced by heat stress (Teeter *et al*., 1985). Serum potassium levels decrease during heat stress which may be due to shifting of potassium ions between muscle and extracellular fluid, increased renal excretion, increased potassium ion uptake by erythrocytes and/or skin (Smith and Teeter, 1987; Ait-Boulahsen *et al*., 1989) and a reduced competition between H^+ and K^+ ions for urinary excretion resulting in increased urinary potassium loss (Laiken and Fantesil, 1985). Heat-stressed birds given 1.5% potassium chloride solution exhibited significantly higher serum potassium levels than unsupplemented group (Naseem *et al*., 2005). Weight gains and feed conversion improvements observed in chicks supplemented with 0.5% $NaHCO_3$ in this study are similar to the findings of Balnave and Gorman (1993) and Naseem *et al*. (2005). Keskin and Durgan (1997) and Lopez and Austic (1993) have also reported improved feed conversion with KCl and $NaHCO_3$ supplements. Improved performance observed in acetic acid group may be attributed to decreased intestinal pH (Table 5) which may not be favorable for colonization by bacteria such as *E. coli, Salmonella* and *Clostridium* (Andreatti *et al*., 2000).

At 2, 4 and 6 weeks of age, birds receiving water supplements showed higher bursal index as well as

Table 1: Effect of water supplements on body weights (grams) of heat-stressed chicks

Week	Control	Acetic acid	$NaHCO_3$	KCl
2	304.7±5.2^{c}	380.9±2.4^{a}	375.1±4.9^{b}	255.7±6.9^{d}
4	830.0±2.9^{d}	995.0±5.1^{a}	985.4±5.1^{b}	900.0±2.3^{c}
6	1601.7±27.4^{d}	1950.3±5.3^{a}	1868.6±6.7^{b}	1794.3±5.7^{c}

*Treatment means within a week with different superscript letters are significantly different (p <0.05)

Table 2: Effect of water supplements on bursal index and lymphoid organ body weight ratios of heat-stressed chicks

Lymphoid organ ratio	Week	Control	Acetic acid	$NaHCO_3$	KCl
Bursal index	2	0.16±.01^{c}	0.35±.03^{a}	0.35±.13^{a}	0. 33±.02^{b}
	4	0.33±.03^{d}	0.45±.13^{a}	0.40±.03^{b}	0.36±.02^{c}
	6	0.20±.09^{b}	0.25±.10^{a}	0.25±.09^{a}	0.25±.02^{a}
Thymus (%)	2	0.60±.12^{c}	1.12±.15^{a}	1.15±.12^{a}	1.05±.03^{b}
	4	0.31±.03^{d}	0.59±.24^{a}	0.49±.03^{b}	0.40±.02^{c}
	6	0.21±.03^{d}	0.46±.12^{a}	0.40±.03^{b}	^{c}05.±0.35
Spleen (%)	2	0.13±.01^{c}	0.38±.15^{a}	0.35±.01^{b}	0.34±.03^{b}
	4	0.15±.03^{c}	0.25±.03^{a}	0.25±.03^{a}	0.18±.33^{b}
	6	0.20±.03^{d}	0.26±.03^{a}	0.24±.03^{b}	0.22±.12^{c}

*Treatment means within a week with different superscript letters are significantly different (p<0.05)

Table 3: Effect of water supplements on H:L ratio and natural agglutinin levels of heat-stressed chicks

	Week	Control	Acetic acid	$NaHCO_3$	KCl
H:L ratio	2	0.42±0.03^{a}	0.16±0.02^{c}	0.18±0.03^{c}	0.24±0.00^{b}
	4	0.41±0.01^{a}	0.20±0.03^{c}	0.20±0.01^{c}	0.22±0.33^{b}
	6	0.38±0.04^{a}	0.18±0.12^{d}	0.20±0.12^{c}	0.24±0.15^{b}
Natural agglutinin (Log2)	6	2.31±0.10^{c}	3.11±0.10^{a}	3.11±0.00^{a}	3.01±0.00^{b}

*Treatment means within a week with different superscript letters are significantly different (p<0.05)

increased percentages of thymus and spleen weight ratios as compared to heat-stressed group (Table 2). Our results agree with that of Puvadolpirod and Thaxton (2000) who reported regression of lymphoid organs which is recognized as an important response of chickens to chronic stress. Heat stress has been reported to reduce all organ weights including thymus, bursa and spleen (Bartlett and Smith, 2003). This could be due to reduction in feed intake, thereby providing fewer nutrients for the proper development of these organs. On the other hand, impaired immunity in heat-stressed birds may make them more susceptible to infections such as cryptosporidium or infectious bursal disease. These infections may also adversely affect the development of these lymphoid organs.

The H:L ratio has been used as a reliable indicator of stress in birds (Gross and Siegel, 1983). Heat stress has been shown to increase H:L ratio in broiler chicks (Hester *et al*., 1996). In this study, heat-stressed group showed significantly higher H:L ratio at 2, 4 and 6 weeks of age compared to all supplemented groups (Table 3). This suggests that water supplements may have played a role in ameliorating the heat stress-induced changes in H:L ratio.

Water supplemented chicks showed higher levels of serum natural agglutinin at 6 weeks of age as compared to heat-stressed control chicks that did not receive water supplements (Table 3). These data suggest that heat stress had adverse effect on the level of natural agglutinin of chicks, which in general indicates impairment of their humoral immune response. Results of this study agree with that of Bartlett and Smith (2003) and Siegel (1989) who reported that heat stress adversely affects immune function and impedes with disease resistance.

Water supplements to heat-stressed chickens significantly decreased Total Aerobic Spore Forming Count (TASC) and Total *Enterobactraecae* Count (TEC) at 2, 4 and 6 wks of age (Table 4) compared to the counts observed in unsupplemented chicks subjected to heat stress. Decreased counts observed in chicks receiving supplements suggests improvement of their immunity which is supported by our data on lymphoid organ weight ratios and bursal index as well as data on H:L ratios and natural agglutinin levels.

Drinking water is the most prominent risk factor for the spread of infections in broiler flocks (Kapperud *et al*., 1993 and Gibbens *et al*., 2001). Drinking water acidification is becoming popular although very little is known about the mechanisms involved in the beneficial effects. Acidification of drinking water with organic acids reduced bacterial contamination and had limited effect on the microflora of the intestinal tract and epithelial cells of the intestinal were not damaged (Chaveerach *et al*., 2004). Kadim *et al*. (2008) observed that supplemental ascorbic acid significantly alleviated the heat-related stress effects on the performance of broiler chicks. In this study, acetic acid supplementation significantly decreased intestinal pH (Table 5) measured at 2, 4 and 6 wks of age but increased water consumption (Table 6) throughout the 6 week period as compared to that of control heat-stressed birds not

Table 4: Effect of water supplements on Total Aerobic Spore Forming Count (TASC) and Total *Enterobactraecae* Count (TEC) ($x10^3$/ml) in heat-stressed chicks

	Week	Control	Acetic acid	$NaHCO_3$	KCl
TASC	2	9.9 ± 0.20^a	7.0 ± 0.25^c	8.5 ± 0.22^b	8.5 ± 0.23^b
	4	180 ± 2.89^a	120 ± 2.22^d	125 ± 2.85^c	129 ± 2.15^b
	6	2480 ± 153^a	1601 ± 11^d	1650 ± 133^c	1800 ± 145^b
TEC	2	8.2 ± 0.14^a	6.0 ± 0.12^d	7.0 ± 0.14^c	7.5 ± 0.13^b
	4	135.6 ± 2.33^a	95.0 ± 3.33^d	115.2 ± 3.33^c	120.1 ± 0.33^b
	6	2250 ± 289^a	1601 ± 3^d	1700 ± 22^c	1750 ± 25^b

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$)

Table 5: Effect of water supplements on intestinal pH of heat-stressed Chicks

	pH			
Week	Control	Acetic acid	$NaHCO_3$	KCl
2	6.90 ± 0.01^c	3.99 ± 0.10^d	7.40 ± 0.03^b	7.80 ± 0.10^a
4	7.33 ± 0.07^b	4.17 ± 0.35^c	7.76 ± 0.35^a	7.61 ± 0.12^a
6	7.27 ± 0.02^b	4.67 ± 0.09^c	7.67 ± 0.03^a	7.53 ± 0.09^a

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$)

Table 6: Effect of water supplements on average water consumption by heat-stressed chicks at different weeks

	Water consumption (ml/day/chick)			
Week	Control	Acetic acid	$NaHCO_3$	KCl
1	40.0 ± 4.4^{ab}	43.5 ± 6.2^a	37.1 ± 4.9^{ab}	28.5 ± 4.9^b
2	75.0 ± 4.6^a	73.5 ± 3.7^a	73.5 ± 3.4^a	59.3 ± 4.4^b
3	123.5 ± 7.8^a	133.5 ± 4.5^a	127.1 ± 3.9^a	116.4 ± 5.3^b
4	182.8 ± 8.8^b	220.7 ± 4.9^a	200.7 ± 10.0^{ab}	191.4 ± 9.3^{bc}
5	235.7 ± 2.6^b	256.4 ± 1.8^a	238.5 ± 3.2^b	233.5 ± 2.6^b
6	266.4 ± 4.8^b	288.5 ± 4.3^a	271.4 ± 3.8^b	266.4 ± 4.3^b

*Treatment means within a week with different superscript letters are significantly different ($p<0.05$)

receiving supplements. This may to some extent explain the superior performance of acetic acid supplemented birds. Decreased intestinal pH as well as improved immunity may partly be responsible for significant reduction in TASC and TEC counts in these birds. In conclusion, this study suggested that adding water supplements significantly improved broiler performance under chronic heat stress. Acetic acid treatment showed the best results followed by sodium bicarbonate and then potassium chloride.

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