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South African Journal of Animal Science 2019, 49 (No. 3)

Effect of betaine and ascorbic acid in drinking water on growth performance and blood biomarkers in meat ducks exposed to heat stress

J-S. Shin, K-H. Um*, H-J. Park, Y-S. Choi, H-S. Lee & B-S. Park[#]

College of Animal Life Science, Kangwon National University, Chuncheon, Gangwondo, 24419
Republic of Korea

(Received 21 February 2019; Accepted 17 March 2019; First published online 11 May 2019)

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Abstract

The aim of this study was to compare the effect of drinking water that contains various levels of ascorbic acid (AA) and betaine (BT) on growth performance and blood biomarkers in meat ducks exposed to heat stress. Six hundred and forty one-day-old Cherry Valley ducks were randomly assigned to one of seven treatment groups: i) control group, drinking water without ascorbic acid or betaine, ii) AA100, drinking water containing ascorbic acid at 100 milligrams per litre, iii) AA200, drinking water containing ascorbic acid at 200 milligrams per litre, iv) AA300, drinking water containing ascorbic acid at 300 milligrams per litre, v) BT400, drinking water containing betaine at 400 milligrams per litre, vi) BT800, drinking water containing betaine at 800 milligrams per litre, and vii) BT1200, drinking water containing betaine at 1200 milligrams per litre. The ducks were exposed to heat stress (11:00 to 17:00, 33 °C to 43 °C, relative humidity 70%, artificially controlled-environment houses) from the 22nd to 42nd days. Ducks from treatment groups AA300 and BT1200 displayed significantly increased bodyweight gains compared with those in the control group. Blood levels of glucose, and activities of aspartate aminotransferase and alanine aminotransferase in all treatment groups were significantly lower than those observed in the control group. Red blood cell count, platelet number, electrolytes and blood gas concentrations were significantly higher in all treatment groups compared with those in the control group. Blood pH levels in all treatment groups were lower than those in the control group. In conclusion, drinking water containing ascorbic acid or betaine improved the growth performance via biomarker homeostasis in blood of meat ducks exposed to heat stress.

Keywords: body weight gain, blood lipid, red blood cell, electrolytes; blood gas, blood pH

[#] Corresponding authors: bspark@kangwon.ac and kracopop9969@naver.com

Introduction

Heat stress during summer jeopardizes animal well-being and decreases their productivity by activating physical stress mechanisms (Gaughan *et al.*, 2013; Sharma *et al.*, 2013). Providing broiler chickens under heat stress with feed that contains ascorbic acid may improve energy metabolism regulation, collagen biosynthesis, heart health and blood flow by relaxing blood vessels to reduce the stress (Boyera *et al.*, 1998; Yoon *et al.*, 2013). The addition of 200 mg to 250 mg ascorbic acid/kg feed or drinking water of broiler chickens under heat stress may also improve their growth performance (Sahin *et al.*, 2003; Kadim *et al.*, 2008). However, such studies have not been performed in ducks. Betaine, a trimethyl derivative of glycine, is an amphipathic compound that has three hydrophobic methyl groups and a hydrophilic carboxyl group. It acts as an *in vivo* donor of choline, methionine and methyl groups (Del Vesco *et al.*, 2014). Betaine is involved in protein synthesis and energy metabolism. It can improve the growth performance of animals by mitigating damage caused by heat stress (Ratriyanto *et al.*, 2009). Betaine improves growth performance by regulating the osmotic pressure in animals under heat stress to prevent dehydration, maintain water content in cells, save energy and enhance nutrient bioavailability (Mahmoudnia & Madani, 2012). It has been reported that the bodyweight of broiler chickens under heat stress is increased by adding 800 mg to 1000 mg betaine/kg feed or 0.05% to 0.2% betaine to drinking water (Ratriyanto *et al.*, 2009). Providing feed that contains 1.5% betaine to duck layers during summer has been reported to improve egg production (Awad *et al.*, 2014).

In previous studies, the authors verified that providing meat ducks that are exposed to heat stress during summer with feed that contains betaine can help them maintain their blood profile homeostasis and relieve their stress by increasing short chain fatty acids in the caecal contents, thus improving their productivity (Bang *et al.*, 2015; Hwangbo *et al.*, 2015). This study was conducted to investigate the effect of supplemental drinking water with betaine or ascorbic acid on growth performance and blood biomarkers in meat ducks under heat stress.

Materials and Methods

Animal experimental procedures conformed to the scientific and ethical regulations provided in EEC Directive of 1986; 86/609/EEC. They were approved by the Institutional Animal Care and Use Committee of Kangwon University (KW-141027-1). A total of 640 Cherry Valley (*Anas platyrhynchos*) meat ducks was obtained from a hatchery in Yangpyeong, South Korea. The experiment followed a completely randomized design (7 treatments, 6 replications per treatment, 15 ducks per 5.2 m² pen). The seven treatment groups consisted of the control group, which was provided with drinking water without ascorbic acid (AA) and betaine (BT), and treatment groups that were provided with drinking water that contained 100 mg (AA100), 200 mg (AA200), or 300 mg (AA300) ascorbic acid per litre drinking water, or 400 mg (BT400), 800 mg (BT800), 1 200 mg (BT1200) betaine per litre drinking water, respectively. The composition of the experimental diets is shown in Table 1, which were formulated as per NRC (1994) specifications.

Table 1 Formula and nutrient composition of experimental diet (as-fed basis)

Ingredients (g/kg)	Starter, 1 - 21 days	Finisher, 22 - 42 days
Maize	503.0	588.2
Soybean meal	335.0	180.0
Wheat	50.0	90.0
Tallow	40.0	50.0
Whole soybean	-	4.10
Fish meal	30.0	10.0
Limestone	10.4	9.8
Dicalcium phosphate	8.3	7.0
Common salt	2.0	.5
Mineral premix ^a	5.5	5.0
Vitamin premix ^b	8.0	6.0
L-Lysine	3.1	4.5
DL-Methionine	3.9	3.5
Threonine	-	0.11
Choline chloride (50%)	0.8	1.0
Tryptophan	-	0.4
Nutrient composition		
Metabolizable energy (MJ/kg)	13.4	13.3
Crude protein	229.9	185.1
Calcium	8.7	8.3
Available P	5.0	4.6
Lysine	14.1	11.2
Methionine	6.5	5.8

^a Mineral premix supplied per kilogram of diet: Mn: 110 000 mg; Zn: 100 000 mg; Fe: 40 000 mg; Se: 300 mg; Cu: 5 000 mg; I: 1 250 mg; Co: 250 mg.

^b Vitamin premix supplied per kilogram of diet: vitamin A: 10 000 000 IU; vitamin D₃: 5 000 000 IU; vitamin E: 20 000 IU; vitamin K: 3 000 mg; vitamin B₁: 2 000 mg; vitamin B₂: 6 000 mg; vitamin B₆: 3 000 mg; vitamin B₁₂: 16 000 mg; pantothenic acid: 13 000 mg; folic acid: 1 500 mg; niacin: 50 000 mg; biotin: 100 mg

Ascorbic acid (ascorbic acid 100%, Northeast Pharmaceutical Group Co., Ltd, China) solutions were prepared by dissolving 100 mg, 200 mg and 300 mg powder in 1000 mL water. Betaine (betaine 97% coated, Beta-Key, Excentials, Netherlands) solutions were prepared by dissolving 400 mg, 800 mg and 1200 mg powder in 1000 mL water. Meat ducks were reared by conventional methods in a normal environment (22 °C to 26 °C) during the starter and growing phases (days 1 to 21). Drinking water that contained ascorbic acid or betaine was provided during a period of artificial heat stress that corresponded to the finishing phase (days 22 to 42). After applying heat stress (11:00 to 17:00, 33 °C to 43 °C, automatic control, relative humidity 70%) on 21 days of the finishing period, the normal environment was maintained.

All animals were reared with continuous lighting and ad libitum feeding during the entire rearing period. During the experimental period, bodyweight gain, feed intake and feed conversion ratio (feed intake/body weight gain) were investigated as productivity measurement variables.

All ducks were fasted for 10 hours from midnight the day before the last experimental day. Three ducks with similar bodyweights were randomly selected from each of the pens and anesthetized with a 1.5% mixture of halothane in oxygen. Approximately 5 mL of blood was collected via cardiac puncture in plain tubes (Greiner Co. Ltd, Australia) using a syringe and 23 gauge needles. Serum was separated from blood by centrifugation at 4 °C and 3000 rpm for 20 min. Blood levels of glucose, triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) as well as aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) enzymes are measured as activity using an automated analysis instrument (Hitachi 917, Japan) and diagnostic kits (Sigma Chemical Co, St. Louis, MO, USA). For haematological indices, total red blood cell (RBC) counts, haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), platelet crit (PCT) and mean platelet volume (MPV) were measured using an automated blood cell counter (Forcyte, Oxford Science, USA). Additionally, blood electrolytes, such as sodium, potassium and chloride (VetScan i-STAT 1 handheld analyser, Abaxis, USA), and blood gas (RAPIDChem 744/754 blood gas analysers, Siemens, USA) were measured.

Statistical analyses of all data were performed using Statistical Package for Social Sciences (SPSS) Windows 21.0 (SPSS Inc., Chicago, IL, USA). After conducting a one-way analysis of variance (ANOVA) for mean values for each treatment group, Duncan's multiple range test was performed to confirm significant differences at 95% confidence level ($P < 0.05$).

Results and Discussion

The bodyweight gain, feed intake and feed conversion ratio of ducks in the treatment groups were significantly better than those of the control group under heat stress during the 21 days ($P < 0.05$) (Table 2). The body weight of ducks in the AA300 and BT1200 groups was higher at 22 - 42 days than that of the control group by 272 g (9.4%) and 258 g (8.9%), respectively. Additionally, the bodyweight gain of ducks in the AA300 group was higher than those in the AA100 and AA200 groups ($P < 0.05$). Similarly, the bodyweight gain of ducks in the BT1200 group was higher than that of ducks in the BT400 and BT800 groups ($P < 0.05$). The feed intake of ducks in the AA300 and BT1200 groups was 282 g (4.9%) and 267 g (4.6%), respectively, which were significantly higher than that observed in the control group (5 802 g, $P < 0.05$). Feed conversion ratio of ducks was not different between treatment groups. This result is consistent with previous studies that found that the supplementation of ascorbic acid or betaine in drinking water to broiler chickens under heat stress improved their growth performance (Kadim *et al.*, 2008; Ratriyanto *et al.*, 2009).

The blood HDL-C, total cholesterol, triglycerides, LDL-C, blood sugar levels, and liver function-related enzymes, AST and ALT of meat ducks fed under heat stress are shown in Table 3. Except for blood HDL-C levels, the blood levels in ducks in the various AA and BT treatment groups were significantly lower than those of the control group ($P < 0.05$). The levels of low blood biomarkers, except for HDL-C in the AA and BT groups, can be attributed to the higher bioenergy metabolism in the AA and BT groups compared with the control (Park *et al.*, 2013; Yoon *et al.*, 2013). The provision of ascorbic acid to broiler chickens that are exposed to a high-temperature environment can relieve heat stress through constantly maintaining the of ALT and AST (McKee *et al.*, 1997). Because the provision of drinking water that contained ascorbic acid or betaine decreased blood lipid and sugar levels in ducks exposed to heat stress, that might indicate that blood lipid level is reduced owing to a lower threshold of 3-hydroxy-3-methylglutaryl-coenzyme A, a cholesterol biosynthesis enzyme, and the degradation of bile acid, which inhibits cholesterol resynthesis in the liver (Yakhkeshi *et al.*, 2012). This result suggests that providing drinking water that contains ascorbic acid or betaine may help meat ducks to relieve heat stress by enhancing blood lipid and energy metabolism.

Table 2 Effect of drinking water with betaine and ascorbic acid on growth performance in ducks under heat stress

	Control	Ascorbic acid (mg/L)			Betaine (mg/L)			SEM
		100	200	300	400	800	1200	
Bodyweight g								
Initial	48	49	49	49	49	48	48	2.061
1 - 21 days	1 330	1 326	1 352	1 336	1 325	1 343	1 343	72.06
22 - 42 days	2 886 ^c	3 025 ^b	3 058 ^b	3 158 ^a	3 034 ^b	3 061 ^b	3 144 ^a	125.8
Feed intake g								
1 - 21 days	1 849	1 830	1 860	1 859	1 843	1 852	1 853	81.57
22 - 2 days	3 990 ^b	4 173 ^a	4 158 ^a	4 225 ^a	4 183 ^a	4 156 ^a	4 210 ^a	155.7
1 - 42 days	5 802 ^b	6 002 ^a	6 018 ^a	6 084 ^a	6 024 ^a	6 008 ^a	6 069 ^a	218.2
Feed conversion ratio (feed intake/body weight gain)								
1 - 21 days	1.39	1.38	1.38	1.39	1.39	1.38	1.38	0.071
22 - 42 days	2.01	1.98	1.97	1.92	1.98	1.96	1.93	0.106

^{a,b,c} Means with no common superscript within the same row are significantly different ($P < 0.05$)

Table 3 Effect of drinking water with betaine and ascorbic acid on blood lipid profile, glucose, aspartate aminotransferase and alanine aminotransferase activities in ducks under heat stress

	Control	Ascorbic acid (mg/L)			Betaine (mg/L)			SEM
		100	200	300	400	800	1200	
TC (mg/dL)	303.6 ^a	265.3 ^c	272.3 ^b	246.2 ^d	258.4 ^c	261.9 ^c	253.2 ^c	3.856
HDL-C (mg/dL)	75.39 ^e	88.95 ^d	89.45 ^d	120.0 ^a	93.60 ^c	95.68 ^c	102.9 ^b	2.797
TG (mg/dL)	141.5 ^a	105.7 ^b	100.7 ^c	79.15 ^d	112.3 ^b	115.2 ^b	83.18 ^d	4.370
LDL-C (mg/dL)	172.3 ^a	129.8 ^c	148.3 ^b	93.39 ^d	132.6 ^c	148.3 ^b	127.7 ^c	5.148
Glucose (mg/dL)	190.7 ^a	182.7 ^b	172.8 ^d	160.7 ^f	164.6 ^e	177.7 ^c	162.9 ^f	2.061
AST (IU/L)	86.00 ^a	45.33 ^d	44.33 ^{de}	35.23 ^e	51.33 ^b	42.67 ^e	48.33 ^c	3.550
ALT (IU/L)	47.67 ^a	41.33 ^c	37.83 ^d	28.67 ^f	44.17 ^b	33.67 ^e	35.83 ^e	1.253

^{a,b,c,d,e,f} Means with no common superscripts within the same row are significantly different ($P < 0.05$). TC: total cholesterol, HDL-C: high density lipoprotein, TG: triglyceride, LDL-C: low density lipoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase

Red blood cell and platelet count profiles were greater in the treatment groups compared with the control group ($P < 0.05$) (Table 4). The highest RBC and platelet counts were found in AA300 and BT1200 ($P < 0.05$). This result indicates that drinking water containing ascorbic acid or betaine may relieve ducks' heat stress by preventing a decrease in haematological indices. It has been reported that the haematocrit level is lowered in animals that are exposed to heat stress owing to red blood cell damage, decreased red blood cell production, or decreased size of the red blood cells (Hilman *et al.*, 1985). While a decrease in red blood cell count and haemoglobin in humans and animals causes iron deficiency anaemia under normal environmental conditions, heat stress may cause blood dilution owing to increased water intake, even without the change in plasma volume that is involved in extracellular water evaporation, ultimately resulting in decreases in red blood cell and haemoglobin levels (Türkyilmaz, 2008).

Table 4 Effect of drinking water with betaine and ascorbic acid on red blood cell and platelet profile in ducks under heat stress

	Control	Ascorbic acid (mg/L)			Betaine (mg/L)			SEM
		100	200	300	400	800	1200	
Red blood cell								
HCT (%)	31.02 ^e	37.61 ^d	43.53 ^b	47.37 ^a	41.31 ^c	43.40 ^b	46.81 ^a	1.044
RBC (M/ μ L)	3.51 ^d	4.57 ^c	4.43 ^c	6.47 ^a	4.02 ^c	4.14 ^c	5.47 ^b	0.183
Hb (g/dL)	12.18 ^f	16.66 ^e	18.50 ^d	22.42 ^b	17.92 ^d	21.21 ^c	25.42 ^a	0.795
MCV (fL)	127.8 ^e	135.7 ^d	147.3 ^b	149.5 ^{ab}	140.1 ^c	141.3 ^c	150.9 ^a	1.721
MCHC (g/dL)	37.26 ^f	43.25 ^e	52.19 ^b	57.26 ^a	49.51 ^{cd}	51.16 ^{bc}	55.25 ^a	1.407
RDW (%)	4.31 ^e	5.48 ^d	6.35 ^c	8.02 ^a	6.05 ^c	6.71 ^c	7.77 ^b	0.257
Platelets								
PLT (K/ μ L)	24.28 ^g	35.69 ^f	38.25 ^e	47.33 ^b	42.06 ^d	44.37 ^c	50.13 ^a	1.594
PCT (%)	0.03 ^e	0.05 ^c	0.06 ^{abc}	0.06 ^{ab}	0.05 ^{bc}	0.05 ^{bc}	0.07 ^a	0.001
MPV (fL)	8.33 ^f	10.22 ^e	14.20 ^c	18.31 ^a	14.41 ^c	14.37 ^c	1666 ^b	0.645

^{a,b,c,d,e,f,g} Means with no common superscript within the same row are significantly different ($P < 0.05$)

HCT: haematocrit, Hb: haemoglobin, MCV: mean corpuscular volume,

MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, PLT: platelet count,

PCT: platelet crit, MPV: mean platelet volume

Blood electrolyte concentrations in treatment groups were significantly greater than those in the control group ($P < 0.05$) (Table 5). In particular, sodium (Na^+), potassium (K^+), and chloride (Cl^-) concentrations in the AA300 group were higher than the control group. This result indicates that providing drinking water that contains ascorbic acid or betaine to meat ducks under heat stress may relieve their stress by maintaining constant blood electrolyte concentrations, thus controlling osmotic pressure. Blood Na^+ , K^+ , and Cl^- concentrations are decreased in poultry and buffalo owing to heat stress (Kumar *et al.*, 2010). It has also been reported that the sodium-potassium ATP pump is important for the *in vivo* energy metabolism and the maintenance of intracellular water balance, while osmotic pressure control through betaine may enhance cell proliferation (Zulkifli *et al.*, 2004). The decrease in blood Cl^- in the heat stress control group might be because more Cl^- ions are required to accelerate the acidification of blood to normalize blood pH following the development of respiratory alkalosis, since heat stress increases blood pH (Borges *et al.*, 2007).

Table 5 Effect of drinking water with betaine and ascorbic acid on blood electrolytes (mmol/L) in ducks under heat stress

	Control	Ascorbic acid (mg/L)			Betaine (mg/L)			SEM
		100	200	300	400	800	1200	
Sodium	140.6 ^d	148.1 ^c	150.6 ^b	152.6 ^a	147.2 ^c	150.1 ^b	150.2 ^b	7.070
Potassium	3.80 ^d	4.57 ^b	5.01 ^a	5.11 ^a	4.50 ^{bc}	4.37 ^c	4.55 ^b	0.093
Chloride	107.2 ^b	113.4 ^a	114.2 ^a	115.2 ^a	113.4 ^a	115.2 ^a	114.6 ^a	5.610

^{a,b,c,d} Means with no common superscript within the same row are significantly different ($P < 0.05$)

Blood pH values in the treatment groups were lower than those in the control group, particularly in AA300, which had the lowest blood pH ($P < 0.05$). On the other hand, blood gas concentrations in treatment groups were significantly greater than those in the control group ($P < 0.05$, Table 6). This result indicates that

providing drinking water that contains ascorbic acid or betaine may relieve heat stress by maintaining constant blood gas concentrations through the biological functions of betaine (Park & Kim, 2016). Although heat stress can decrease blood pCO₂ (partial pressure of carbon dioxide) in broiler chickens, maintaining pCO₂ at an appropriate level during heat stress is necessary since poultry remove CO₂ continuously by panting through the respiratory system to reduce their body temperature by evaporating water from their lungs (Hilman *et al.*, 1985; Zulkifli *et al.*, 2004; Mahmoudnia & Madani, 2012).

Table 6 Effect of drinking water with betaine and ascorbic acid (milligrams per litre) on blood pH and blood gas concentration in duck under heat stress

	Control	Ascorbic acid (mg/L)			Betaine (mg/L)			SEM
		100	200	300	400	800	1200	
pH	7.43 ^a	7.41 ^{ab}	7.40 ^b	7.35 ^c	7.40 ^b	7.40 ^b	7.39 ^b	0.410
PCO ₂ (mm Hg)	41.72 ^d	45.78 ^c	49.01 ^a	49.27 ^a	48.21 ^{ab}	47.49 ^{bc}	47.07 ^{bc}	1.723
HCO ₃ (mmol/L)	21.23 ^b	26.01 ^a	27.12 ^a	26.67 ^a	25.66 ^a	27.21 ^a	26.60 ^a	0.502
AnGap (mmol/L)	14.73 ^c	17.32 ^b	18.90 ^a	18.87 ^a	17.02 ^{ab}	17.88 ^b	18.47 ^a	0.379
TCO ₂ (mmol/L)	21.50 ^d	27.65 ^b	29.40 ^a	29.37 ^a	29.01 ^a	26.55 ^c	28.27 ^a	0.711

^{a,b,c,d} Means with no common superscript within the same row are significantly different ($P < 0.05$)

Conclusions

The results of this study indicate that providing drinking water that contains ascorbic acid at 300 milligrams per litre or betaine at 1200 milligrams per litre to meat ducks that are exposed to heat stress in summer can relieve heat stress and improve productivity by controlling blood biomarkers, haematological indices, blood electrolytes and blood pH.

Acknowledgements

This study was funded by Agenda for the 2015 Rural Development Administration (PJ010088), Republic of Korea.

Authors' Contributions

K-HS, K-H and B-SP designed the study, and H-JP, Y-SC and H-SL performed the biochemical determination, and KHU, BSP wrote the paper with input from all authors.

Conflict of Interest Declaration

The authors have no conflicts of interest to declare.

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