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Original Article

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Abstract

The objective of this study was to compare the effects of ascorbic acid and mixed electrolytes on growth performance, ammonia emission and serum antioxidant enzyme activity in broilers during summer period. Two hundred and ten male and female Arbor Acre broilers during grower-finisher period (22-42 days old) were allocated into 3 groups with different additives in drinking water: 1,000 ppm ascorbic acid (T1); 2,000 ppm mixed electrolytes containing only Na^+, K^+ and HCO_3^- (T2) and none (T3 serving as control group). The experiment was conducted in an open broiler house during the summer period between April and May. Results indicated that there were no significant differences in broiler performance among the treatments. The control group (T3) had slightly higher mortality rate compared to the others. There was no significant difference among the groups in ammonia concentration and litter moisture in all weeks. The broilers in T1 (ascorbic acid) had significantly higher glutathione peroxidase (GPx) enzyme and lower MDA levels ($p<0.05$) compared with the other groups. Osmolarity values of the broilers at 4 weeks were low in all groups and the broilers in T2 (mixed electrolytes) had increased serum osmolarity compared with the control ($p<0.05$) at 5 weeks of age. In conclusion, the high ambient temperature in this trial increased the mortality rate in the broilers and exacerbated FCR. The supplementation of both mixed electrolytes and ascorbic acid numerically reduced the mortality rate and slightly increased the weight gain. The electrolytes improved the serum osmolarity of the bird while the ascorbic acid supplement had the benefit of increasing serum GPx enzyme and decreasing serum MDA.

Keywords: ascorbic acid, broilers, electrolytes, glutathione peroxidase, heat stress, malondialdehyde

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Introduction

High ambient temperature and humidity which reduce the performance of commercial poultry production, especially of chickens raised in non-controlled housing environments, are problems commonly encountered in Thailand. High temperatures interfere with broiler comfort and reduce productivity. Chickens respond to heat stress by panting to evaporate metabolic heat increment, increasing respiratory rate and CO_2 output, resulting in alkalosis (increased blood pH) and disruption of the acid-base balance (Toyomizu et al., 2005). The balance may shift towards either alkalosis or acidosis, in which metabolic pathways are diverted to thermo-regulatory adaption rather than growth. Chickens consume increasing quantities of water to compensate for water loss and to dissipate heat, but heat stress reduces water retention due to increasing electrolyte excretion via urine and feces (Belay & Teeter, 1993). Heat stress also reduces levels of potassium and other minerals in chickens, altering the electrolyte balance. Moreover, ammonia generated from the breakdown of nitrogen compounds by microbial activity in the litter can hamper growth performance and health. The effects of ammonia are highly dependent on exposure time, high temperature and litter moisture (Estevez, 2002; Miles et al., 2011).

Heat stress has profound physiological effects on chicken metabolism. It increases lipid peroxidation in broilers (Altanet al., 2000), and under acute heat stress, production of mitochondrial superoxide radical in chicken skeletal muscle (Mujahidet al., 2005).Chronic heat stress may also minimize metabolic antioxidation capacity through a self-propagating scavenging system (Azad et al., 2010). Electrolytes play a significant role in maintaining the acid-base balance, osmotic pressure and electrical potential of cell membranes, and are also essential for intracellular-extracellular homeostasis. Monovalent ions (Na^+, K^+ and Cl^-) are the vital minerals involved in maintaining the acid-base balance of body fluids because they have a higher permeability and greater absorption than divalent ions (Ca^{2+} and Mg^{2+}) (Borges et al., 2004). Plasma Na^+, K^+ and Cl^- levels are affected by heat stress. K^+ and Na^+ concentration decreases as temperature rises, (Borges et al., 2004), while Cl^- increases (Belay and Teeter, 1993). Roussan et al. (2008) reported that different substances had been used to reduce the harmful effects of heat stress on broiler performance, including ascorbic acid, acetylsalicylic acid (ASA), potassium chloride (KCl), and sodium bicarbonate ($NaHCO_3$). Ascorbic acid (vitamin C) has been extensively examined for its beneficial effect on reducing heat stress in broilers for three decades. Ascorbic acid supplementation improved performance of heat challenged broiler chickens and has been associated with a lower plasma level of corticosteroid (Mckee and Harrison, 1995). The inhibitory action of vitamin C on adrenal steroidogenesis is mediated via modulation of steroid hydroxylating enzymes in the adrenal gland (Kitabachi, 1967). Therefore, high dietary intake of vitamin C regulates the elaboration of plasma corticosteroid level during heat stress. Effect of vitamin C on antioxidant enzyme especially glutathione peroxidase and pro-oxidant MDA level, however, is still controversial. Both vitamin C and mixed electrolytes are frequently used additives during summer period in Thailand, although comparative studies of both additives are scarce. This study, therefore, aimed to determine the effects of both additives on serum osmolarity, antioxidant enzyme, blood Heterophil/Lymphocyte ratio, ammonia emission and broiler performance during the hot and humid summer period in Thailand.

Materials and Methods

The protocol for this experiment followed relevant guidelines and regulations on the use of animals for scientific purposes and was certified by the Chulalongkorn University Animal Care and Use Committee (permission No. 12310024).

Animals and Management: There were 210 one-day-old male and female Arbor Acre broilers in this study. The chicks were vaccinated using the Newcastle plus Infectious Bronchitis vaccine (sprayed at the hatchery). They were randomly allotted into 3 groups based on average body weight. Different additives were supplemented in drinking water during 22-42 days of age as follows: Treatment 1 (T1) received 1,000 ppm ascorbic acid (feed grade), Treatment 2 (T2) received 2,000 ppm electrolytes (Selectolyte™ containing potassium sulfate and sodium bicarbonate, Rural Chemical Industries (RCI), Australia) and Treatment 3 (T3) served as the untreated control group. The levels of both ascorbic acid and mixed electrolytes complied with the levels used in the field. Each group comprised 7 replicates of 10 birds each.

The experiment was conducted during the summer period from April to May. Average temperature and relative humidity during the trial were 34.0 ± 0.7 °C and 51.0 ± 3.9%, respectively. All groups were raised under the same condition in an open house at a density of 10 birds /m^2. The floor was covered with a 10cm deep litter of rice hulls. All chicks received clean water and were fed *ad libitum* on commercial broiler feed based on corn-soybean meal as the major ingredient. The proximate analysis of feed is shown in Table 1. This feed was formulated according to the requirements suggested by the National Research Council (1994).

***Determination of ammonia concentrations and litter moisture content*:** Ammonia concentrations were measured using an ammonia detector (BW Technologies, Canada). The measurement was performed at the centre of each replicate at a level of 10 cm above the litter surface. Ammonia levels were determined in the morning (08:00 to 09:00), afternoon (13:00 to 14:00) and evening (16:00 to 17:00) each day for two weeks to check for fluctuations. Thereafter, the measurement was conducted twice a day (08:00 to 09:00 and 15:00 to 16:00) from 22to 42 days old.

Moisture content in the litter samples was determined using hot oven at 105 °C for 24 hr. Triplicate excreta samples of each replicate pen were determined and the percentage of litter moisture

content was calculated as the formula (dry litter weight/wet litter weight × 100).

Determination of growth performance: Growth performance, i.e. body weight (BW) gain, feed conversion ratio (FCR) and mortality rate, was determined in all groups at 3 and 6 weeks of age. BW gain was determined by the difference between weights at the beginning and end of the trial. Feed intake was measured as the difference between feed supplied and remaining feed in each feeder.

Determination of body temperature and blood parameters: At 4, 5 and 6 weeks of age, two chicks per replicate were randomly selected and measured for body temperature using a thermometer inserted into the cloaca**.** Blood was collected from the wing vein or jugular vein using sterile needles. Whole blood samples (using EDTA as anticoagulant) were analyzed for Complete Blood Count (CBC test) including Heterophil/Lymphocyte (H/L) ratio and whole clotted blood was centrifuged to separate serum for determining osmolarity using Osmometer model 3D3 (Advanced Instrument Inc, USA), GPx enzyme activity (Bolcalet al., 2007) and MDA concentrations (Ohkawa et al., 1979).

Table 1 Proximate analysis of commercial diets used in the trial (g/kg unless specify otherwise)

Parameter/Diet	Starter (1-21 d-old)	Grower (22-35 d-old)	Finisher (36-42 d-old)
Gross energy* (MJ/kg)	14.99	15.70	15.67
Crude protein	227.2	201.1	179.4
Crude Fat	44.0	74.1	73.2
Crude Fiber	28.2	29.0	30.0
Ash	61.1	58.8	56.4
Calcium	8.8	9.3	8.6
Phosphorus	7.8	7.4	6.7
Sodium	1.9	1.6	1.5
Potassium	10.3	9.0	7.9
Chloride	2.9	2.7	2.6
dEB**	264.3	223.7	194.0

*Bomb calorimeter; **dEB is calculated by molar concentrations of Na+K–Cl in each diet (Mongin, 1981).

Statistical analyses: All means of experimental treatments were analyzed using one-way ANOVA. When a significant (p<0.05) F statistic was noted, treatment means were separated by Duncan's new multiple range tests using Sigmastat 3.5 program. Data are presented as arithmetic means ± standard error of means (SEM).

Results

The proximate analysis of diets in each period (starter, grower and finisher) is shown in Table 1. The dietary electrolyte balance (dEB) of each diet was calculated as mEq/kg of Na + K – Cl. There were no significant differences in growth performance among all groups of birds at the starter and the grower-finisher period (Table 2). The mortality of chicks during grower-finisher period was highest in control group (T3) (30.0±6.2%) followed by T1 and T2. The body weight gain and FCR was slightly worse in T3 compared to others. During 4-6 weeks, the ammonia concentration and litter moisture content were in the ranges of 4.52-7.31 ppm and 68.83-78.54%, respectively and there was no difference among groups (Table 3). Table 4 showed the changes in hematology, osmolarity and antioxidant enzyme of birds. There was no difference in H/L ratio of chicks in all groups each week, although the H/L was high (1.07-1.31) at week 5 of age. The GPx enzyme was highest at wk 4 ($p < 0.05$) in chicks receiving ascorbic acid (T1) compared to others. In addition, serum MDA was significantly highest in control (T1) group compared to T3. Serum osmolarity of chicks at 5 weeks old was significantly (p <0.05) decreased in T1 chicks compared to T2 and was not different to T1 group.

Discussion

From Table 1, the calculated dEB, especially a commercial finisher diet, was lower (194 mEq/kg) than the level recommended by Johnson & Karunajeewa (1985) at 250 mEq/kg diet. It is common for local corn-soybean meal based diet that the dEB is lower than the recommended level (>250 mEq/kg) to be used in hot and humid condition (Borges et al., 2004; Ahmad & Sarwar, 2006). This can be depicted by the high mortality rate in this study. The H/L ratio, an indicator of stress, was high in all groups of bird during the grower-finisher period particularly at 5 weeks of age. This is consistent with the result of Gross & Siegel (1983), who found that the H/L ratio could be used as a measure of stress in chicken.

The temperature at the grower-finisher period had a negative impact on the growth performance of birds in all groups with the highest mortality found in the control group (T3). During the grower-finisher period, the house temperature (34.0±0.7 °C) and humidity (51.0±3.9%) were both higher than the thermoneutral zone (26.0± 2.0°C) for 4-6 week-old Arbor Acre broilers. Consequently, the broilers encountered heat stress during the grower-finisher period, as confirmed by their high body temperatures (above 42.0°C).

There was no significant difference in ammonia concentration and litter moisture content among treatment groups. However, the average concentration and litter moisture in the control group

were slightly higher than the treatment groups. The ammonia concentrations found in this study were within the recommended limit (<25 ppm) for poultry houses (Carlile, 1984).

There were significant differences in serum osmolarity between the chicks in treatments 2 and 3 at 5 weeks of age. The supplementation of mixed electrolytes maintained the normal level of plasma osmolarity (approximately 315-320 mOsm/L). Belay and Teeter (1993) reported that plasma Na^+, K^+ concentrations decreased significantly during heat stress in broilers. Water intake increases in heat-exposed broilers and then the water consumed enters into the blood circulation, inducing elevated urine production. It is also consistent with the findings of Zhou et al. (1999), who reported that plasma osmolarity was decreased by increased water consumption and this influenced cell function. The improved osmolarity in response to electrolyte supplement had slight positive effect on the performance and mortality. It seems that the broilers receiving mixed electrolytes in water maintained normal serum osmolarity compared with the other groups. This is supported by the overview conducted by Sayed and Scott (2007).

Table 2 Growth performances*of broilers at starterand grower-finisher periods

	Starter (1-21 d-old)			Grower-Finisher (22-42 d-old)		
	T1	T2	T3	T1	T2	T3
Body weight gain (kg/bird)	0.77±0.007	0.77±0.006	0.78±0.007	1.21±0.08	1.21±0.08	1.15±0.07
Feed Intake (kg/bird)	0.99±0.006	0.97±0.009	0.98±0.010	2.94±0.10	2.70±0.16	3.01±0.08
FCR	1.29±0.02	1.26±0.01	1.25±0.01	2.57±0.33	2.33±0.22	2.70±0.18
Mortality (%)	1.43±1.43	0	0	27.5± 9.2	24.3±5.7	30.0±6.2

*Each data entry represents mean±SEM.

Table 3 Ammonia concentrations and litter moisture at 4, 5 and 6 weeks of age

Ammonia concentration* (ppm)	T1	T2	T3
4 weeks	5.31±0.39	5.31±0.29	5.35±0.32
5 weeks	6.86±0.35	7.24±0.44	7.31±0.42
6 weeks	4.52±0.28	4.70±0.32	4.94±0.30
Average	5.56±0.29	5.75±0.31	5.87±0.30
Litter moisture* (%)	T1	T2	T3
4 weeks	77.84±0.77	77.44±1.56	78.54±0.81
5 weeks	76.47±1.48	77.21±1.00	77.75±0.55
6 weeks	68.83±3.20	68.96±3.30	70.19±1.06
Average	74.38±1.16	74.54±1.15	75.64±0.51

*Each data entry represents mean±SEM.

Table 4 Blood Heterophil/Lymphocyte (H/L) ratio, serum osmolarity, GPx and MDA concentrations in broilers at 4, 5 and 6 weeks of age

	T1	T2	T3
4weeks			
Blood H/L ratio	0.47±0.10	0.51±0.10	0.65±0.10
Osmolarity (mOsm/L)	309.7±1.4	309.9±1.4	309.3±1.3
MDA concentrations(µmol/ml)	0.035±0.002	0.044±0.005	0.051±0.009
GPx (unit/mg protein)	2,376.2±253.8^a	1,189.8±293.8^b	1,174.6±346.2^b
Body temperature (°C)	42.5±0.3	42.7±0.2	42.3±0.2
5 weeks			
Blood H/L ratio	1.07±0.14	1.31±0.16	1.18±0.18
Osmolarity (mOsm/L)	316.0±2.0^{ab}	318.9±3.7^a	310.7±2.5^b
MDA concentrations (µmol/ml)	0.063±0.022	0.050±0.004	0.099±0.041
GPx (unit/mg protein)	1,151.6±214.7	1,337.4±237.9	821.2±138.5
Body temperature (°C)	41.8±0.2	42.1±0.2	42.2±0.1
6 weeks			
Blood H/L ratio	0.39±0.14	0.30±0.06	0.63±0.15
Osmolarity (mOsm/L)	321.4±3.2	317.0±2.9	319.3± 2.2
MDA concentrations (µmol/ml)	0.044±0.003^b	0.065±0.005^a	0.075±0.006^a
GPx (unit/mg protein)	4,335.7±720.7	2,986.2±570.9	2,868.6±323.0
Body temperature (°C)	43.0±0.4	43.0±0.3	43.0±0.2

*Means within rows followed by different letters are significantly different at $p<0.05$.

The GPx enzyme of chicks supplemented with ascorbic acid (T1) were significantly higher at 4 weeks of age compared to the other groups. Our result corresponds with the study of Tawfeek et al. (2014),

who found that heat stress birds had the lowest glutathione peroxidase. Ascorbic acid is known to play a vital role in suppression of adrenocortical steroidogenesis, thus ameliorating the negative effects of stress. The reaction of hydroperoxides is catalysed by GPx with lessened glutathione to form glutathione disulphide (GSSG). Hence, elevated GPx enzyme is thought to enhance the steady state of antioxidant system of broilers (Satterlee et al., 1989). High temperature affects many chemical and biochemical reactions resulting in increased reactive oxygen species via accelerated metabolic drive in cells and tissues (Flanagan et al., 1998). Heat stress can be attributable to an increase in MDA production in serum and liver. The MDA concentration in T1was significantly decreased at 6 weeks of age compared with those in T2 and T3.The result is also in agreement with the findings of Sahin et al. (2002), who reported that the addition of either ascorbic acid or vitamin E alone or the addition of both vitamins could decrease serum MDA concentration (p<0.05). The antioxidant action of vitamin C inhibits free radical production by blocking lipid peroxidation (Amakye-Animet al., 2000). However, under extremely hot and humid conditions as in the current study, the antioxidant enzyme levels in the bird receiving ascorbic acid was insufficient to prevent mortality. Oppressive weather conditions can cause not only detrimental changes in the balance of pro- and antioxidant in tissues but also physiological changes because electrolyte balances can affect the mortality of birds.

In conclusion, the heat stress in this trial increased the mortality rate in the broilers and exacerbated FCR. Both ascorbic acid and mixed electrolyte supplements slightly improved the mortality and other performances through distinctive effects. The ascorbic acid supplementation helped to increase the GPx enzyme levels and decreased the MDA levels while the addition of mixed electrolytes maintained the normal osmolarity of serum.

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บทคัดย่อ

การเสริมอิเล็กโทรไลท์และกรดแอสคอร์บิกในไก่เนื้อที่เครียดจากอากาศร้อนในประเทศไทย

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การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลของกรดแอสคอร์บิก(ไวตามินซี) และ อิเล็กโทรไลท์รวมต่อสมรรถภาพการเจริญเติบโต ระดับแอมโมเนียในคอกและระดับเอนไซม์ต้านออกซิเดชั่นในไก่เนื้อที่เลี้ยงในฤดูร้อน ทำการทดลองในไก่เนื้อคละเพศพันธุ์อาเบอร์เอเคอร์ในช่วงอายุ 22-42 วัน แบ่งลูกไก่ออกเป็น 3 กลุ่มตามชนิดของสารเสริมที่ให้ในน้ำดื่ม กลุ่มที่ 1 เสริมกรดแอสคอร์บิก 1,000 พีพีเอ็ม (T1) กลุ่มที่ 2 เสริมอิเล็กโทรไลท์รวมที่ประกอบด้วยโซเดียม โปแตสเซียมและไบคาร์บอเนต 2,000 พีพีเอ็ม (T2) และกลุ่มที่ 3 ไม่เสริมอะไร เป็นกลุ่มควบคุม (T3) ทำการทดลองในโรงเรือนเปิดในระหว่างฤดูร้อนเดือนเมษายนถึงพฤษภาคม ผลการทดลองแสดงให้เห็นว่าสมรรถภาพการเจริญเติบโตของไก่เนื้อทั้ง 3 กลุ่มไม่มีความแตกต่างกัน กลุ่มควบคุมมีอัตราการตายของไก่มากกว่าอีก 2 กลุ่มที่ได้สารเสริมเล็กน้อย ไม่พบความแตกต่างของระดับแอมโมเนียและความชื้นของสิ่งรองนอนตลอดช่วงที่ทดลอง ไก่เนื้อกลุ่มที่ได้รับกรดแอสคอร์บิกมีระดับของเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสสูงขึ้นและระดับ MDA ที่ต่ำลงอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มอื่นๆ ค่าออสโมลาริตี้ของซีรั่มในไก่เนื้ออายุ 4 สัปดาห์มีระดับต่ำในทุกกลุ่ม ไก่เนื้อกลุ่มที่ 2 ซึ่งได้รับอิเล็กโทรไลท์รวมมีระดับออสโมลาริตี้ของซีรั่มสูงขึ้นกว่ากลุ่มควบคุมเมื่อไก่อายุ 5 สัปดาห์ โดยสรุปอุณหภูมิสิ่งแวดล้อมที่สูงในการทดลองนี้เพิ่มอัตราการตายของไก่เนื้อและมีผลเสียต่ออัตราแลกเนื้อ การเสริมทั้งอิเล็กโทรไลท์รวมหรือกรดแอสคอร์บิกช่วยลดอัตราการตายได้บ้างรวมถึงมีผลดีต่อการเพิ่มน้ำหนักเล็กน้อยโดยผ่านกลไกที่ต่างกัน อิเล็กโทรไลท์รวมจะช่วยเพิ่มออสโมลาริตี้ของซีรั่ม ในขณะที่การเสริมกรดแอสคอร์บิกมีผลต่อการเพิ่มเอนไซม์กลูตาไธโอน เปอร์ออกซิเดสและลด MDA ในซีรั่ม

คำสำคัญ: กรดแอสคอร์บิก ไก่เนื้อ อิเล็กโทรไลท์ กลูตาไธโอนเปอร์ออกซิเดส เครียดจากความร้อน มาลอนไดอัลดีไฮด์

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