Estimation of Dietary Sodium Bicarbonate Dose Limit in Broiler under High Ambient Temperatures

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A six-week trial was conducted to estimate the dose limit of sodium bicarbonate (SB) in the diet of broiler chickens exposed to high ambient temperature. The estimation of SB dose limit was based on the growth performance, blood biochemical parameters and histological observation of the renal tissues of chickens. A total of 450 day-old male broilers were randomly distributed to five groups with SB supplemented at levels of 0 (control), 0.5%, 1.0%, 2.0% and 4.0%. The minimum and maximum average ambient temperatures were 26.4°C and 32.5°C, respectively, with relative humidity ranging from 57.2% to 83.8%. Results showed that (1) A linear effect of increasing dietary SB level was observed on body weight gain (BWG) ($P < 0.05$), feed intake (FI) ($P < 0.05$) and feed to gain ratio (F:G) ($P < 0.05$) from day 1 to 21 and day 1 to 42. Supplementation of 4.0% SB decreased BWG ($P < 0.001$) and FI ($P < 0.001$), but increased F:G ($P < 0.001$) from day 1 to 21, and decreased FI ($P < 0.001$) from day 1 to 42. (2) Compared with the control treatment, an SB of 4.0% gave higher incidences of diarrhea ($P < 0.001$), mortality ($P < 0.001$) and ascites related mortality ($P < 0.001$). (3) Rectal temperatures were linearly decreased ($P < 0.05$) with rising SB levels. (4) Blood biochemical analysis indicated that SB higher than 1.0% decreased blood hemoglobin (HGB) ($P < 0.001$), and increased hematocrit (HCT) ($P < 0.001$) and serum malondialdehyde (MDA) concentration ($P < 0.001$) on day 21. (5) Microscopic examination revealed that significant renal haemorrhage appeared when SB exceeded 1.0% and tubular dilation was observed in 4.0% SB treatment. In summary, dietary supplementation of 4.0% SB resulted in poor growth performance and higher mortality. In addition, SB higher than 1.0% affected blood biochemical parameters and caused renal lesions. The current results suggest that the dose limit of dietary SB is less than 1.0% for broilers under high ambient temperatures.

Key words: broiler, dose limit, high ambient temperatures, sodium bicarbonate


Introduction

Extreme thermal condition can decrease growth performance and reproductive efficiency in broilers and other domestic animals (Fuquay, 1981). Poultry production in many regions of the world is adversely affected by heat stress (HS). HS causes an economic loss of $128 million in poultry industries every year (St-Pierre et al., 2003). Broilers are more susceptible to HS because of their body feathers and high metabolic rate (Lin et al., 2006a). Under high ambient temperature, birds increase panting rate for evaporative cooling, which results in carbon dioxide loss and acid-base imbalance (Bottje and Harrison, 1985; Teeter et al., 1985; Teeter and Belay, 1996). Moreover, HS can induce oxidative stress by increasing serum MDA production (Lin et al., 2006b) and activity of anti-oxidative enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Altan et al., 2003).

Sodium bicarbonate (SB) was widely used as a regulatory agent for chickens to alleviate the deleterious effect of HS by maintaining acid-base balance (Hayat et al., 1999; Borges et al., 2003; Borges et al., 2007). Dietary supplementation of 0.5-1.0% SB has been shown to improve growth performance under high ambient temperatures (Teeter et al., 1985; Abou-El-Ella and Ismail, 1999; Borges et al., 2003; Naseem et al., 2005; Tanveer et al., 2005; Ahmad et al., 2006; Ghorbani and Fayazi, 2009). However, overdose of SB had detrimental impacts on birds and may cause poor growth performance (Hayat et al., 1999), a decrease in blood red blood cell (RBC), hemoglobin (HGB) and hematocrit (HCT) (Mubaraki and Julian, 1993) and renal lesion (Mubaraki and Sharkawy, 1999). Scrivner (1946) reported a heavy mortality in 2 to 3-week-old chickens receiving 0.6% SB in their drinking water. Clinical signs of the dead chickens included
subcutaneous oedema and ascites.

Previous research on overdose of SB mainly focused on laying pullets (Davison and Wideman, 1992; Mubarak and Sharkawy, 1999), or broilers supplemented with SB in their drinking water (Davison and Wideman, 1992; Mubarak and Julian, 1993). However, little information is available on the dose limit of SB in broiler chicken’s diets. Since chickens with apparently normal growth may not be in a normal physiological state (Hayat et al., 1999), parameters such as blood metabolites, redox status and renal histopathological examination would be more appropriate than only the analysis of growth performance to determine the dose limit of SB. Thus, a dose limit of 2.0% SB in the diet, analyzed by Hayat et al. (1999), calculated entirely from growth performance, might be overestimated. Therefore, it is indispensable to reevaluate the dose limit of SB in broiler diet based on more comprehensive results. Here, dose limit of dietary SB in broilers under high ambient temperatures was assessed in terms of growth performance, mortality, blood chemical parameters, redox status and histological examination.

Materials and Methods

Bird Management and Diet

The design and conduct of this study were approved by the Institutional Animal Care and Use Committee of China Agricultural University. A total of 450 day-old male broiler chicks (Ross 308) were randomly allocated to five treatments with six pens (2.4 m × 0.6 m × 0.6 m) of 15 chickens each. Levels of SB in diet were 0 (control), 0.5%, 1.0%, 2.0% and 4.0%, respectively.

Diets were formulated based on NY/T33-2004 of China. Feed composition was listed in Table 1. Ingredients of the diet were analyzed for crude protein (CP) (Kjeldahl method), phosphorus (P) (AOAC, 1984), calcium (Ca), and monovalent mineral (Na, K, and Cl) to validate that analyzed and calculated values were similar. Ca, Na and K in feed were determined by a flame atomic-absorption spectrometer (GBC Sens AA Dual, GBC Scientific Equipment, Braeside, Australia); and Cl was analyzed by titration with AgNO3 (Lacroix et al., 1970). The SB was pre-mixed with NaCl and then fully mixed with other feedstuffs of the diet step by step. At the onset of experiment, diet was provided as crumbles.

<table>
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<tr>
<th>Ingredient</th>
<th>Corn</th>
<th>Soybean meal</th>
<th>Soybean oil</th>
<th>Corn gluten meal</th>
<th>Dicalcium phosphate</th>
<th>Limestone</th>
<th>DL-Met</th>
<th>Lys. HCl</th>
<th>Aureomycin</th>
<th>Antioxidants</th>
<th>Choline chloride (50%)</th>
<th>Vitamin premix</th>
<th>Trace mineral premix</th>
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<th>NaHCO3</th>
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1 Diet was calculated to contain the following (g/kg): starter: AME, 2.9 Mcal/kg; CP, 210; Ca, 10; available P, 4.8; Met, 5.0; Met+Cys, 11; finisher: AME, 3.05 Mcal/kg; CP, 190; Ca, 9.0; available P, 3.8; Met, 4.0; Met+Cys, 10;
2 Supplied per kilogram of diet: vitamin A 12500 IU; vitamin D3 2500 IU; vitamin E 30 IU; vitamin K3 2.65 mg; thiamine 2 mg; riboflavin 6 mg; vitamin B12 0.025 mg; biotin, 0.0325 mg; folic acid 1.25 mg; pantothenic acid 12 mg; niacin 50 mg.
3 Supplied per kilogram of diet: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.
4 NaHCO3: feed-grade purity (99%).
and subsequently substituted by whole pellets.

Experiment was started in late June. The room temperature was maintained at 33°C and 31°C from day 1 to 7 and day 8 to 14, respectively. The heating apparatus was removed on day 15 and evaporative cooling pad was shut down throughout the experiment. The maximum and minimum ambient temperatures and relative humidity were measured daily with an electronic thermometer. The light cycle was 24 h light from day 1 to 13 and 23 h light: 1 h dark from the age of day 14. During the experimental period, broilers were highlighted from day 1 to 13 and 23 highlighted: 1 h dark from the age of day 14 and 21, and infectious bursal disease (IBD) vaccinated for Newcastle disease and infectious bronchitis (ND-IB) on day 7 and 21, and infectious bursal disease (IBD) on day 14 and 28.

Growth Performance

On day 21 and 42, body weight gain (BWG), feed intake (FI) and feed to gain ratio (F:G) were calculated and corrected for mortality, if any. Diarrhea and deaths were documented to determine diarrhea incidence and mortality. All dead chickens were subjected to postmortem examinations to determine cause of death. Chickens with ascitic fluid or dead chickens were subjected to postmortem examinations to rectify form mortality, if any. Diarrhea and deaths were documented and calculated the organ indices. Kidney tissues from each treatment were fixed in 10% neutral-buffered formalin, embedded by paraffin technique and stained with haematoxylin-eosin (HE) for histologic examination under a light microscope. Organ Indices and Histological Examination

On day 21 and 42, birds were randomly selected and killed by jugular bleeding after 6 h of feed deprivation. Heart, liver, spleen and bursa were collected and weighed for calculating the organ indices. Kidney tissues from each treatment were fixed in 10% neutral-buffered formalin, embedded by paraffin technique and stained with haematoxylin-eosin (HE) for histological examination under a light microscope.

Blood Collection and Analysis

At the age of day 21 and 42, venous blood samples were collected by brachial vein puncture using two tubes containing anticoagulant EDTA-K$_3$ or no anticoagulation. Red blood cell count (RBC), blood hemoglobin (HGB), hematocrit (HCT) and mean corpuscular volume (MCV) were determined by using an automatic blood analyzer (Sysmex KX-21N, Japan). Blood pO$_2$, pCO$_2$, pH and HCO$_3^-$ were immediately determined by a blood gas analyzer (ABL-800; Radiometer Analytical, Denmark). Serum were immediately harvested by centrifugation of blood samples at 3000 rpm for 15 min. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein (TP) were measured using an automatic biochemistry analyzer (Beckman Coulter Unicel DXC 800, USA). Levels of serum malondialdehyde (MDA), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), blood urea nitrogen (BUN) and uric acid (UA) were determined by employing the colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Statistical Analysis

The data of rectal temperature were analyzed by two-way ANOVA using GLM (Univariate) procedure of SPSS 17.0 for the interactive effects of SB levels and sampling times. Duncan’s multiple-range test was used to separate means that significantly differ at $P<0.05$. The other data were analyzed by one-way ANOVA of SPSS 17.0. The significance of differences among treatment means was evaluated by Least Significant Difference (LSD) post-hoc multiple comparisons test. Linear and quadratic regression analyses were conducted. Significance was $P<0.05$ unless otherwise stated.

Results

Ambient Temperature and Relative Humidity

The minimum and maximum average ambient temperatures (Max T, Min T) and relative humidities (RH) were 26.4°C and 32.5°C and 57.2% and 83.8%, respectively, with a peak of 34.8°C, which indicated the exposure of the birds to high ambient temperatures (Figure 1).

Growth Performance

As shown in Table 2, both linear and quadratic of increasing SB level were found for FI ($P<0.01$), and only linear effect for BWG ($P<0.01$) and F:G ($P<0.01$) during day 1 to 21; Supplementation of 4.0% SB decreased BWG ($P<0.001$) and FI ($P<0.001$), but increased F:G ($P<0.001$) comparing with the control treatment. From day 22 to 42, linear and quadratic response were described for BWG ($P<0.01$), and linear effect for F:G ($P<0.001$) with the rising of SB level; broilers fed with SB had greater BWG ($P<0.001$) and lower F:G ($P<0.001$) than the control group. From day 1 to 42, both linear and quadratic trend were revealed for BWG ($P<0.05$) and linear tendency for F:G ($P<0.001$) with the increasing of SB level; comparing with control treatment, SB supplementation improved BWG ($P<0.001$) and FI ($P<0.01$), and F:G ($P<0.001$) were lower in birds receiving 4.0% SB.

Diarrhea, Mortality and Ascites Related Mortality

There were both linear and quadratic effects of increasing SB for diarrhea incidence, mortality and AS related mortality (Table 3). Broilers fed with 4.0% SB showed the highest incidence ($P<0.001$), mortality ($P<0.001$) and AS mortality ($P<0.001$). Moreover, all died chickens in the 4.0% SB treatment exhibited AS syndrome, such as drooping, expiratory dyspnea, comb cyanosis, hypertrophy and dilation of the heart, and fluid accumulation in pericardium and peritoneal cavity (pictures were not shown).

Rectal Temperature

Irrespective of the SB treatment, rectal temperature was higher ($P<0.001$) in the afternoon than in the morning. Rectal temperature was linearly reduced ($P<0.001$) with the increasing SB level both in the morning and afternoon (Figure 3).

Organ Indices

A linear effect was seen for heart ($P<0.001$) and bursa indices ($P<0.05$) on day 21 (Table 4); SB higher than 1.0% significantly increased heart index ($P<0.001$), and addition of 4.0% SB gave a higher bursa index ($P<0.05$) compared
with the control treatment. On day 42, there was a linear tendency of SB on spleen \((P < 0.01)\) and bursa indices \((P < 0.05)\), and a quadratic trend on heart \((P < 0.001)\) and liver \((P < 0.001)\) indices with the increasing SB level. Heart and liver indices were the lowest in broilers fed with diet supplemented 0.5% SB.

**Blood Test**

On day 21, a linear response was revealed for HGB \((P < 0.001)\), HCT \((P < 0.001)\) and MCV \((P < 0.001)\) with a rising level of SB (Table 5); chickens consumed 2.0% and 4.0% SB showed lower HGB \((P < 0.001)\), and higher HCT \((P < 0.01)\) and MCV \((P < 0.01)\). On day 42, levels of blood RBC, HGB, HCT, MCV were not significant different among treatments.

**Blood Biochemical Parameters**

There were no significant differences in levels of serum ALT, AST, TP, BUN and UA among the treatments (data not shown). On day 21, both linear and quadratic trends were exhibited in serum MDA concentration \((P < 0.01)\) and linear effect was showed in serum GSH-Px activity \((P < 0.05)\) with

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**Table 2. Effect of dietary SB on the performance in broilers**

<table>
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<tr>
<th>SB, %</th>
<th>Day 1 to 21</th>
<th>Day 22 to 42</th>
<th>Day 1 to 42</th>
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<td>BWG (g)</td>
<td>FI (g)</td>
<td>F:G</td>
</tr>
<tr>
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<td>602&lt;sup&gt;a&lt;/sup&gt;</td>
<td>928&lt;sup&gt;b&lt;/sup&gt;</td>
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**Table 3. Effect of dietary SB on diarrhea, mortality and ascites mortality in broilers**

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<th>Diarrhea&lt;sup&gt;1&lt;/sup&gt;</th>
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**Figure 1.** Ambient maximum and minimum temperature and relative humidity from day 14 to 42

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<sup>a-b</sup> Means within column and having superscripts but lacking a common superscript differ \((P < 0.05)\).

<sup>1</sup> Diarrhea chicks were recorded on day 14;<br>
<sup>2</sup> Mortality and ascites related mortality were noted during the whole trial.
the increasing SB level. Comparing with control treatment, serum MDA content ($P<0.001$) was improved in birds fed with SB higher than 0.5%. GSH-Px activity ($P<0.05$) was lower with supplementation of 2.0% and 4.0% SB than that of 0.5% SB (Table 6). However, blood redox status was not affected by dietary SB at the age of day 42.

**Blood Gas Parameters**

There was a tendency of increased blood $\text{HCO}_3^-$ ($P=...
0.081) in broilers exposed to 1.0% SB. Blood pH, pCO2 and pO2 of birds did not differ (P>0.05) in all treatments (data not shown).

Renal Histological Changes

Renal histological examination was presented in Figure 2. Chickens fed with SB higher than 1.0% showed a haem-
orrhage between renal tubules which was determined by the presence of erythrocytes at the age of day 21 and 42. Furthermore, tubular dilation appeared as level of SB reached 2.0% on day 42, indicating that renal lesion was intensified. All the renal histological changes were most obvious in 4.0% SB treatment.

Discussion

HS is a common event that can hamper the growth performance of domestic animals (Fuquay, 1981). According to previous research, dietary supplementation of SB alleviated the detrimental effect of HS by enhancing the assimilation efficiency of nutrients, promoting organ development and maintaining blood acid-base balance (Teeter et al., 1985; Abou-El-Ella and Ismail, 1999; Borges et al., 2003; Naseem et al., 2005; Tanveer et al., 2005; Ahmad et al., 2006; Ghorbani and Fayazi, 2009). However, excessive SB has been shown to decrease growth performance. Our results indicated that indicated 4.0% SB significantly reduced growth performance from day 1 to 21, and resulted in extremely high diarrhea incidence, mortality and AS related mortality. First, we suggest that excessive SB in the diet may decrease feed palatability and feed intake. Second, the higher SB probably quenched the thirst by increasing Na+ intake, because blood osmotic pressure in birds is a thirst-regulating factor (Borges et al., 2003). Chickens would drink excess water because of thirst, which presumably increased feed passage rate and dilution of digestive enzymes that could cause reduced nutrient digestibility and absorption (Ravindran et al., 2008; Ahmad et al., 2009). From day 22 to 42 and day 1 to 42, supplementation of SB in the diet increased BWG in chickens, whereas 4.0% SB showed the highest mortality among all the treatments. To minimize error in measuring BWG, it was corrected for mortality by excluding the dead chickens from each treatment. However, body weight of the chickens that died from ascites was lower than the live ones. Therefore, the BWG in 4.0% SB supplemented group would be over-estimated for its high mortality. This might explain the discrepancy of BWG in broilers between day 1–21 and day 22–42.

SB higher than 1.0% significantly increased the heart and bursa indices on day 21, and supplementation of 0.5–2.0% SB decreased heart and liver indices on day 42. The BWG loss under heat stress probably contributed to the discrepant effects of SB on the heart and bursa indices between day 1–21 and day 22–42 (Bartlett and Smith, 2003). Spleen and bursa indices showed irregular variations, which may be due to organ specificity might be another reason, but no relevant reference was available.

It has been shown that the levels of blood RBC, HCT and HGB were increased in birds exposed to overdose of SB or NaCl (Mubarak and Sharkawy, 1999; Ekanayake et al., 2004). This hematological change was the sub-clinical symptom of pulmonary hypertension or ascites in SB-treated broiler chickens (Ekanayake et al., 2004). Here, we indicated that supplementation of SB higher than 1.0% decreased HGB, and increased HCT and MCV levels on day 21. The decreasing HGB level seemed inconsistent with previous studies. This change in HGB level indicated a compromised oxygen-carrying capacity in blood, which might induce red cells proliferation for higher oxygen-carrying capacity in broilers. Moreover, the physiological alterations of erythrocytes would increase blood viscosity (Burton et al., 1968; Hakim, 1988; Mirsalimi and Julian, 1993), and blood pressure that may stimulate lung vascular remodeling (Yang et al., 2010), and eventually cause AS syndrome.

Heat stress can lead to oxidative stress which is described as the imbalance between oxidative and anti-oxidative status in favor of the oxidation, potentially leading to per-oxidation damage (Sies, 1997). MDA, the product of lipids oxidation, was elevated in chickens supplemented with SB higher than 1.0%, indicating the broilers may be subjected to oxidative injury. SOD, GSH-Px, and CAT were the most important enzymes for preventing oxidative damage (Finkel and Holbrook, 2000). A decrement of serum GSH-Px activity might aggravate the oxidative lesion in chickens supplemented with 2.0% and 4.0% SB compared with that of 0.5% SB. It seems that supplementation of SB higher than 1.0% would disturb redox balance in broilers.

As the most important organ for water and electrolyte equilibrium, the kidney might be more susceptible to dietary SB than other organs. The results of renal histological examination demonstrated that SB higher than 1.0% resulted in renal hemorrage. This histological lesion of the kidney was more severe as dietary SB dose increased. SB higher than 2.0% even caused tubular swelling in birds on day 42. Considering the highest mortality was seen in the 4.0% SB group, death of those birds might be attributed to renal failure. However, it can be inferred that SB higher than 1.0% probably aggravated kidney burden and led to pathological lesions.

In summary, dietary supplementation of 4% SB decreased growth performance, and dramatically increased diarrhea incidence, mortality and AS related mortality. Nevertheless, SB higher than 1.0% decreased blood HGB, increased HCT and serum MDA, and resulted in renal morphological damage. Although dietary addition of 1.0% SB had no negative impact on growth performance of broilers, the biochemical blood parameters as well as renal histological examination were significantly affected. Therefore, we suggest that dose limit of dietary SB is less than 1.0% in broilers under high ambient temperatures.

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