Introduction

Pulmonary hypertension syndrome (PHS), commonly called ascites, is a costly metabolic disease occurring in rapid-growing chickens. Research has shown that dietary sources of energy (Khajali and Fahimi, 2010) and protein (Izadinia et al., 2010) have significant effects on pulmonary hypertensive response in broiler chickens. It has been suggested that rapid-growing broilers may be in the status of metabolic acidosis (Julian, 1993). Metabolic acidosis is associated with pulmonary vasoconstriction and pulmonary hypertension. Experimentally induction of metabolic acidosis initiated by infusing HCl intravenously or respiratory acidosis initiated by inhaling air containing 5% CO₂ resulted in pulmonary hypertension in broiler chickens (Wideman et al., 1999a). Metabolic acidosis induced by oral administration of citric acid also modulated pulmonary hypertension in broilers reared at high altitudes (Hassanpour et al., 2009).

Chloride acidifies the diet whereas bicarbonate alkalinizes the diet. Squires and Julian (2001) replaced dietary calcium carbonate by calcium chloride to increase the level of dietary chloride in a low-land area. Their results indicated that high chloride diets promote the onset of pulmonary hypertension because chloride causes acidosis and vasoconstriction. In this report, we describe an experiment in which sodium bicarbonate was substituted for sodium chloride in diet of broiler chickens reared at high altitude either for entire rearing period (starting from 7-day-old) or half of the period (from 21 days of age). The effect of feeding such diets on growth performance, mixed blood gas values, and pulmonary hypertensive response was studied.

The objectives of the study were to investigate whether the supplementation of sodium bicarbonate instead of sodium chloride to diets of broilers kept on high altitude could result in compensating negative effects on the cardiovascular system.

Materials and Methods

The experiment was conducted in Shahrekord (Iran), an area with the altitude of 2100 m above the sea level. The experimental animals were kept, maintained and treated in accepted standards for the humane treatment of animals. The Institutional Animal Care and Use Committee of Shahrekord University approved all procedures used in this study.

A total number of 180 day-old male broiler chicks (Ross 308) were randomly allotted to three dietary treatments at random. Dietary treatments were: 1) a diet supplemented with sodium chloride, 2) a diet for which dietary sodium chloride replaced by sodium bicarbonate from 7 days of age, and 3) a diet for which dietary sodium chloride replaced by sodium bicarbonate from 21 days of age. Diets had similar levels of AME, crude protein and sodium and formulated to meet nutritional requirements of broiler chicks (NRC, 1994) in the starting and growing stages. Composition of the basal diet to which sodium sources were added is presented in Table 1. The tap water analysis showed only traces of sodium (0.2 mg/l), potassium (0.1 mg/l) and chloride (0.2 mg/l), which probably did not affect the experimental results.

Chicks were housed in a thermostatically-controlled environment and maintained on a 23 h light and 1 h dark lighting regime with free access to feed and water. All chicks were raised on a commercial diet for a week post-hatch. After removing obvious runts, 180 chicks were randomly distributed among 12 floor pens (replicates of 15 birds/m²) according to similar body weight. Four such replicates were then allotted to every of the three treatments at random. The experiment started on 7th d and lasted up to 42 days of age. The temperature was maintained at 25 ± 1, 20 ± 1 and 15 ± 1°C at 7, 14 and 21 days of age, respectively as previously applied (Khajali et al., 2007).

Records of body weight and feed consumption were recorded throughout the trial and feed conversion ratio (FCR) was calculated. Three birds from each pen were chosen for blood collection via brachial vein for measuring hematocrit (BMC 24 Microhaematocrit centrifuge, Pars Azma Co., Isfahan, Iran), differential leukocyte count (Gross and Siegel, 1983), Pulse oximetry (HbO₂), and mixed blood gas analysis on 42 days of age. Blood samples for measuring mixed blood gas values were obtained anaer-
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Table 1. Composition of the basal diet in different feeding stages (g/kg)

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>Starter diet (7–21d)</th>
<th>Grower diet (21–42d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>573</td>
<td>657</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>345</td>
<td>295</td>
</tr>
<tr>
<td>Fish meal</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>Soya oil</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Salt</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Trace mineral premix</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>ME (Mj/kg)</td>
<td>12.55</td>
<td>12.55</td>
</tr>
<tr>
<td>CP (g/kg)</td>
<td>217</td>
<td>188</td>
</tr>
<tr>
<td>Met + Cys (g/kg)</td>
<td>9.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Lys (g/kg)</td>
<td>12.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Na (g/kg)</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Cl (g/kg)</td>
<td>***</td>
<td>****</td>
</tr>
<tr>
<td>Na + K + Cl (meq/kg)</td>
<td>*****</td>
<td>*****</td>
</tr>
</tbody>
</table>

* Salt in treatment 1 and 2 was 0.35% sodium chloride, and 0.5% sodium bicarbonate, respectively.
** Salt in treatment 1, 2, and 3 was 0.30% sodium chloride, 0.44% sodium bicarbonate, and 0.44% sodium bicarbonate, respectively.
*** Dietary chloride content in treatment 1 and 2 was 0.25 and 0.07%, respectively.
 **** Dietary chloride content in treatment 1, 2 and 3 was 0.21, 0.05 and 0.05%, respectively.
***** Dietary electrolyte balance in treatment 1 and 2 was 303 and 361, respectively.
****** Dietary electrolyte balance in treatment 1, 2, and 3 was 270, 321, and 321, respectively.

Results

Body weight gain, feed intake and FCR during the starting and growing stages are shown in Table 2. There was significant difference among the treatments in both feeding stages. Feeding the low-chloride diets resulted in significantly (P < 0.05) lower weight gain and feed intake and impaired FCR when compared to the control (NaCl group).

Table 2 depicts the physiological variables as affected by dietary chloride and bicarbonate. Feeding the low-chlorine diets significantly (P < 0.05) elevated pH, PO2, HbO2, and plasma bicarbonate and potassium concentrations. On the other hand, H/L ratio and RV/TV were significantly reduced as low-chloride diets were fed to broilers. Hematocrit and plasma sodium concentration were not affected by substitution of bicarbonate for chloride. The ratio of H/L tended to decrease by replacing Cl- for HCO3– so that the difference between treatments 1 and 2 was significant (P < 0.05).

Discussion

Many works have examined the effects of the addition of salts via the drinking water or feed on the performance of broilers under heat stress conditions. For instance, Dai and Bessei (2009) have recently indicated that addition of KCl and NaCl in drinking water increased water intake and water to feed ratio and improved broiler performance under tropical condition. However, limited works examined the effects of salts, particularly NaCl or NaHCO3 with reference to pulmonary hypertension in broiler chickens (Shlosberg et al., 1998; Squires and Julian, 2001). As depicted in Table 1, replacing Cl- with HCO3– by means of complete substitution of NaHCO3 for NaCl resulted in a higher dietary electrolyte balance (DEB). DEB was 303 and 361 meq/kg in the starter diets, and 270 and 321 meq/kg in the grower diets, for the NaCl and NaHCO3 groups, respectively.

In the present study, broiler growth performance was generally low. The poor performance was because the chicks were raised in an extremely hypobaric (high altitude) condition. It is estimated that each 500 m increase in altitude above sea level reduces oxygen availability by about 1% from 20.95% at sea level (Julian, 2007). Khajali and Dastar (2005) reported that body weight gain of broiler
Table 3. Venous blood gas analysis of treatments measured at 42 days of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>pO2 (mmHg)</th>
<th>pCO2 (mmHg)</th>
<th>HCO3− (mMol/L)</th>
<th>HbO2 (%)</th>
<th>H/L</th>
<th>Hematocrit</th>
<th>RV/TV</th>
<th>Na (mM/L)</th>
<th>K (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>7.42</td>
<td>35.5a</td>
<td>42.9</td>
<td>27.0b</td>
<td>68.1b</td>
<td>0.934</td>
<td>40.7</td>
<td>0.272</td>
<td>83.3</td>
<td>3.11b</td>
</tr>
<tr>
<td>Bicarbonate1</td>
<td>7.59a</td>
<td>38.5ab</td>
<td>46.9</td>
<td>44.2a</td>
<td>84.8a</td>
<td>0.63b</td>
<td>42.5</td>
<td>0.201</td>
<td>96.7</td>
<td>3.73a</td>
</tr>
<tr>
<td>Bicarbonate21</td>
<td>7.59a</td>
<td>39.5a</td>
<td>43.6</td>
<td>40.8a</td>
<td>84.6a</td>
<td>0.751</td>
<td>41.2</td>
<td>0.213</td>
<td>94.5</td>
<td>3.64ab</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>1.79</td>
<td>1.98</td>
<td>1.76</td>
<td>2.41</td>
<td>0.09</td>
<td>0.93</td>
<td>0.01</td>
<td>0.92</td>
<td>0.18</td>
</tr>
</tbody>
</table>

a, b Means within a column not sharing a common superscript are significantly different (P < 0.05).

HbO2: saturation of hemoglobin with oxygen
H/L: heterophils to lymphocytes ratio
R/V: Right ventricle to total ventricles weight ratio
Number of birds tested = 12 except for RV/TV

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Significant elevation of the blood pH and plasma bicarbonate level due to feeding low-chloride diet is attributed to increased intake of bicarbonate. High blood pH itself, increases the affinity of hemoglobin for oxygen (Isshack et al., 1986) and results in enhanced PO2 and HbO2 as observed in the experiment. Increase in the blood pH can cause dilatation of the pulmonary vasculature which reduces the right ventricular workload (Wideman et al., 1999a). Consequently, the pulmonary hypertension will be attenuated by decrease the right ventricular weight ratio (RV/TV) as happened in the present study.

The values of PO2 and hematocrit values reported herein are respectively lower and higher than those reported at low altitudes. Blood haematocrit is a sensitive indicator of physiological change attributable to atmospheric oxygen. Bekker et al. (2003) suggested that 19.6% atmospheric O2 is the minimal allowable level for housing birds to avoid cardiac changes related to pulmonary hypertension. The availability of atmospheric oxygen is estimated to be 17% in this study and this is the reason that hematocrit values are very high. Although an increasing trend can be seen in hematocrit of birds fed low-chloride diets, the difference among the treatments were not significant. Nevertheless, it can be concluded that long-term effect of higher hematocrit in the chickens fed low-chloride diets is higher resistance in pulmonary blood vessels and pulmonary hypertension and this is another explanation for significantly higher RV/TV in birds fed low-chloride diets.

When broilers were given low-chloride/high bicarbonate diets, no significant change was seen in plasma sodium concentration. This is because dietary sodium content of experimental diets was similar (Table 1). Interestingly, plasma level of potassium was increased by feeding low-chloride/high bicarbonate diets. This may imply the cooperative relationship between plasma potassium and bicarbonate which is known as chloride shift. Chloride shift refers to the exchange of bicarbonate and chloride across the membrane of red blood cells in which potassium salt of bicarbonate is actively involved (Brandali et al., 1981).

The ratio of H/L was significantly reduced when the low-chloride diets fed to birds from day old age. This may be speculated by reduced stress on the birds fed low-chloride/high bicarbonate diets presumably due to lower RV/TV. The H/L ratio is commonly used as an indicator of stress in birds (Gross and Siegel, 1983). The H/L values reported herein are much higher than normal values which can be found in broilers and this could be attributable to severely hypoxic condition (i.e. high altitude) of the present study.

In conclusion, substitution of bicarbonate for chloride resulted in increased PO2 and HbO2 and decreased pulmonary hypertension as indicated by lower RV/TV. Feeding low-chloride/high bicarbonate diets either for 21 or 42 days impaired broiler growth performance.

Acknowledgements

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Summary

An experiment was conducted in order to examine the effects of low-chloride and high-bicarbonate diets on growth performance, blood parameters, and pulmonary hypertensive response in broiler chickens. One hundred and eighty day-old male chicks were used in a completely randomized design with 3 dietary treatments including 4 replicates of 15 chicks as follow: 1) a diet supplemented with sodium chloride, 2) a diet for which dietary sodium chloride replaced with sodium bicarbonate from day old age, and 3) a diet for which dietary sodium chloride replaced with sodium bicarbonate from 21 days of age.

The results indicated that weight gain, feed intake and feed conversion ratio were significantly (P < 0.05) impaired when chloride was totally replaced by bicarbonate. Feeding low-chloride diets from day old age significantly (P < 0.05) increased blood pH, partial pressure of oxygen (PO2), the saturation rate of hemoglobin with oxygen (HbO2), and plasma bicarbonate and potassium concentrations though it significantly (P < 0.05) decreased hetero-
oophil to lymphocyte ratio (H/L) and right ventricle to total ventricles weight ratio (RV/TV). In conclusion, substitution of bicarbonate for chloride resulted in increased PO2 and HbO2 and decreased pulmonary hypertension as indicated by lower RV/TV.

**Zusammenfassung**

Einfluss von Rationen mit niedrigem Chlorid- und hohem Bikarbonatgehalt auf Wachstum, Blutparameter und Auftreten von Aszites bei in großer Höhe über dem Meeresspiegel gehaltenen Broilern


Das vollständige Ersetzen von Natriumchlorid durch Natriumbikarbonat beeinträchtigte signifikant (P < 0,05) die Gewichtszunahme, die Futteraufnahme und die Futterverwertung. Die Fütterung der Rationen mit geringem Chloridgehalt vom ersten Tag an führte zu einer signifikanten (P < 0,05) Zunahme des pH-Wertes, des Sauерstoffpartialdrucks (PO2), der Sättigung des Hämoglobins mit Sauерstoff (HbO2) und der Konzentrationen an Bikarbonat sowie Kalium im Blutplasma. Gleichzeitig wurden sowohl das Verhältnis von Heterophilen zu Lymphozyten (H/L) und das Verhältnis des Gewichtes der rechten Herzkammern zum Gesamtgewicht der Kammern (RV/TV) signifikant vermindert (P < 0,05). Es wurde der Schluss gezogen, dass das Ersetzen von Chlorid durch Bikarbonat in den Futterrationen PO2 und HbPO2 deutlich erhöhen und gleichzeitig den Bluthochdruck, gemessen als RV/TV, vermindern kann.

**Stichworte**

Broiler, electrolytes, chloride, bicarbonate, performance, ascites

**Key words**

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**References**


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