LEVEL OF ELECTROLYTES AND PERCENTAGE OF T-LYMPHOCYTE SUBPOPULATIONS IN BLOOD OF BROILER CHICKENS FED MIXTURES WITH DIFFERENT CONTENTS OF SODIUM CHLORIDE

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Abstract

The objective of this study was to evaluate the effect of a varied content of sodium in diets for broiler chickens on serum levels of macroelements and on the percentage of CD4+, CD8+, and CD4+CD8+ T-lymphocyte subpopulations in blood. The study was conducted on 432 one-day-old cockerels representing six experimental groups (eight replications, each with nine birds). The chickens were administered for 5 weeks feed mixtures in which the content of Na was differentiated by the addition of NaCl in the following amounts (%): group 1 – 0, group 2 – 0.127, group 3 – 0.254, group 4 – 0.382, group 5 – 0.509, and group 6 – 0.636. Potassium content and electrolyte balance (expressed as the total molar mass of Na+ and K+ minus molar mass of Cl–) were kept at a similar level. On day 35 of age, their body weight was measured and eight birds from each treatment were selected from blood analysis. Serum concentrations of Na, K, Cl, Ca, P, and Mg and the percentage of CD4+, CD8+, and CD4+CD8+ T-lymphocyte subpopulations were measured. The administration of the NaCl-free feed mixture caused a decrease in the levels of Na and chlorides and an increase in concentrations of K, Ca, and Mg, as compared to the cockerels from groups fed the mixtures with an addition of Na. Differentiated addition of NaCl to the feed mixtures had no significant effect on the percentage of the analysed subpopulations of CD4+, CD8+, and CD4+CD8+ T-lymphocytes. Sodium deficiency in the diet was found to affect a decrease in the body weight of the chickens examined.

Key words: chickens, sodium, feed additive, electrolyte balance, T-lymphocyte subpopulations.

Intensive advances in the fattening of broiler chickens is feasible owing to genetic studies that assure their rapid growth, but also owing to systematic improvements in feeding that enable covering the nutritional needs of these birds. One of the factors that determine the rapid growth of broiler chickens is the sufficient content of electrolytes in a diet, in particular sodium (2). The beneficial effect of an increased sodium content in diet on the rate of growth and feed conversion ratio in poultry was demonstrated in experiments conducted in the 1990s (7, 11). They resulted in changes in sodium content in a grower type mixture from 0.15% (17) to 0.20% (18). Further studies demonstrated the key role of Na+, K+, and Cl– ions in maintaining the acid-base balance, as well as the pH of blood and tissues, especially under conditions of thermal stress (2, 8, 9). Good results of broiler chicken fattening were achieved when the birds were administered feed mixtures with sodium content ranging from 0.20% to 0.30% (16, 20, 26). The increased dietary content of sodium has also been found to increase water intake by the birds, which results in higher moisture content of faeces and litter (14, 16). This, in turn, increases the risk of the incidence of various poultry diseases, particularly of foot pad dermatitis (4, 15). A similar effect may occur as a consequence of the synergistic effects of sodium and chlorine in a diet (15). This is of significance due to the fact that the most common sodium additive in feed mixtures is sodium chloride. It is estimated that chickens’ demand for chlorine is covered when the mixtures they are fed contain from 0.12% to 0.20% of NaCl (18). An increasing dietary content of chlorine is accompanied by increasing incidence and severity of hypertrophy of the capital tibial epiphysis (14). In addition, it is a common knowledge that the lack of electrolyte balance in feed and body may result in the
occurrence of various pathological states in birds, including: disorders of bone formation, e.g. tibial dyschondroplasia (10), degeneration of skeletal muscles (22), or disorders of immune processes (21). Pimentel and Cook (21) demonstrated that the serological response of chickens immunised with sheep red blood cells (SRBC) and fed a diet with NaCl addition ranging from 0% to 0.75% increased with a higher NaCl content in the diet. In addition, these authors showed that the humoral immunological response of the chickens was inhibited when the sodium content in the diet was lower than 0.14% and chlorine content was lower than 0.17%. When the concentration of chlorine in diets for chickens was increased from 0.17% to 0.27%, at sodium content accounting for 0.04%, the serological response was also inhibited in the SRBC-immunised chickens. The data reported above point to the need for more comprehensive identification of physiological effects of varied contents of electrolytes in diets for chickens, especially in the context of electrolyte balance in blood and their effect on non-specific cellular immunity, being of great significance to the health status of birds.

On account of the above, a study was undertaken to evaluate the effect of varied content of NaCl in feed mixtures for broiler chickens on serum levels of macroelements, cellular response of the immune system manifested by the percentage of CD4+, CD8+ and CD4 ‘CD8’ T-lymphocyte subpopulations in blood, and body weight gains of the birds.

Material and Methods

The study was conducted with 432 one-day-old meat type Ross 308 cockerels, divided into six experimental groups (eight replication groups, each with nine birds). The chickens were kept in a 3-floor battery, each with cage having an area of 0.5 m². The birds had free access to water and feed mixture for 35 days and were reared following the binding technology by the Ross Company (1).

The chickens were fed feed mixtures in which Na content was differentiated with the addition of NaCl (Table 1) and K content was kept at a constant level. NaCl was added to basal feed mixtures in the form of 1% premix prepared under laboratory conditions and thoroughly mixed. The basal feed mixtures contained: maize, post-extraction soybean meal, wheat, soybean oils, amino acids (lysine, methionine, and threonine), chalk, monobasic calcium phosphate, and a premix of trace elements and vitamins. Their nutritive value was consistent with recommendations stipulated by the producer of breeding material (1). Contents of Na, K, and Cl in the feed mixtures were determined at the National Laboratory for Feedingstuffs in Lublin, using a GBC atomic absorption spectrometer by Avanta (Na and K) and the biamperometric method (Cl).

At the termination of the study, all birds were weighed and 48 broilers were chosen for biochemical blood analyses (eight birds from each group taking into account the recorded average body weight). The blood was then sampled from their wing vein to determine serum levels of Na, K, Cl, Ca, P, and Mg. Analyses were carried out with a multi-function biochemical analyser COBAS INTEGRA 400 PLUS (Roche, Swiss). Concentrations of Na, K, and Cl – with indirect potentiometry.

In order to determine the percentage of T-lymphocyte subpopulations, blood (2 ml) was sampled from the wing vein of five birds from each group to haematological test tubes with EDTA-K2 anticoagulant, and diluted (1:1) with a PBS buffer with the addition of 1% FCS. The prepared blood samples were then overlaided on 3 ml of Histopaque – 1.077 gradient (Sigma-Aldrich, Germany) and centrifuged in 15-ml FALCON test tubes (BD) at 400 g at room temperature for 30 min. After centrifugation, the formed buffy coat of mononuclear cells was gently collected to sterile test tubes, rinsed twice with a PBS buffer with the addition of 1% FCS and suspended in 1 ml of PBS. The number of cells was counted with the chamber method. From the resultant suspension, 10⁶ cells were transferred to cytometric test tubes, to which 2 µl of antibodies against surface domains of CD4 (mouse anti-chicken CD4-PE clone CT-4, SouthernBiotech cat. No. 8210-09) and CD8 (mouse anti-chicken CD8a-FITC clone 3-298, SouthernBiotech cat. No. 8405-02) receptors of T-lymphocytes were added. The samples were incubated for 30 min in the dark. Afterwards, the cells were rinsed twice with PBS, centrifuged at 250 g for 7 min at 4°C, and the resultant pellets were suspended in 400 µl of PBS. The samples prepared this way were examined by means of an Epics XL flow cytometer (Beckman Coulter, USA). The immunophenotypic analysis of the cells was conducted with specialist CXP software (Beckman Coulter, USA).

Numerical results of the analyses were elaborated statistically using a one-way analysis of variance in the orthogonal and non-orthogonal systems. The significance of differences was determined with Duncan’s test. All calculations were performed with Statistica 6.0 software.

Results

Results achieved for the percentage content of Na, K, and Cl and the electrolyte balance in the experimental diets affected by the NaCl addition are presented in Table 1. They demonstrate that the increasing addition of NaCl to the experimental feed mixtures resulted in statistically higher body weight gain, which was due to the higher percentage of Na and Cl in the mixtures introduced.

Administration of the feed mixture without the NaCl addition caused a significant (P<0.001) decrease in the contents of Na and chlorides and an increase in the contents of K, Ca, and Mg as compared to the groups fed a grower-type mixture with an additional source of Na (Table 2). In the other groups, irrespective of the level of NaCl addition, the contents of Na, K, Ca, and chlorides were similar. The lowest NaCl addition caused
an increase in serum levels of P and Mg, compared to
the groups administered the feed mixtures with a higher
addition of NaCl.

The results presented in Table 3 show that
administration of experimental feed mixtures had no
significant effect on the percentage of CD4⁺, CD8⁺, and
CD4⁺CD8⁺ T-lymphocyte subpopulations.

**Table 1**
Addition of NaCl, contents of Na, K, and Cl in diet, diet electrolyte balance (mEq/kg), and final body weight of broiler chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>NaCl addition (%)</th>
<th>Content in diet (%)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>2</td>
<td>0.127</td>
<td>0.07</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>0.254</td>
<td>0.12</td>
<td>0.89</td>
</tr>
<tr>
<td>4</td>
<td>0.382</td>
<td>0.17</td>
<td>0.88</td>
</tr>
<tr>
<td>5</td>
<td>0.509</td>
<td>0.22</td>
<td>0.89</td>
</tr>
<tr>
<td>6</td>
<td>0.636</td>
<td>0.26</td>
<td>0.88</td>
</tr>
</tbody>
</table>

DEB – diet electrolyte balance; ABC – P≤0.001.

**Table 2**
Contents of selected macroelements in blood serum of broiler chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Na (%)</th>
<th>Na (mmol/dl)</th>
<th>K (mmol/dl)</th>
<th>Chlorides (mmol/dl)</th>
<th>Ca (mg/dl)</th>
<th>P (mg/dl)</th>
<th>Mg (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>136±2.5</td>
<td>8.46±1.23</td>
<td>103±1.2</td>
<td>12.1±0.69</td>
<td>7.04±0.73</td>
<td>3.09±0.36</td>
</tr>
<tr>
<td>2</td>
<td>0.07</td>
<td>151±3.2</td>
<td>5.72±0.83</td>
<td>115±0.8</td>
<td>11.1±0.47</td>
<td>7.29±0.75</td>
<td>2.56±0.25</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>150±2.2</td>
<td>5.32±0.79</td>
<td>116±0.8</td>
<td>10.5±0.62</td>
<td>6.41±0.55</td>
<td>2.31±0.14</td>
</tr>
<tr>
<td>4</td>
<td>0.17</td>
<td>150±2.9</td>
<td>5.21±0.47</td>
<td>114±0.5</td>
<td>11.2±0.32</td>
<td>6.78±0.28</td>
<td>2.41±0.16</td>
</tr>
<tr>
<td>5</td>
<td>0.22</td>
<td>149±2.0</td>
<td>5.45±0.57</td>
<td>113±0.6</td>
<td>10.6±0.27</td>
<td>6.52±0.49</td>
<td>2.30±0.19</td>
</tr>
<tr>
<td>6</td>
<td>0.26</td>
<td>148±1.3</td>
<td>5.54±0.66</td>
<td>112±0.7</td>
<td>10.5±0.73</td>
<td>6.12±0.60</td>
<td>2.24±0.10</td>
</tr>
<tr>
<td>SEM</td>
<td>0.876</td>
<td>0.211</td>
<td>0.749</td>
<td>0.118</td>
<td>0.107</td>
<td>0.055</td>
<td></td>
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<tr>
<td>P</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
<td>0.010</td>
<td>&gt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

abc – P≤0.005.

**Table 3**
Percentage of CD4⁺, CD8⁺, and CD4⁺CD8⁺ T-lymphocyte subpopulations in blood of broiler chickens fed diets with varied addition of NaCl

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>CD4⁺CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.55±2.75</td>
<td>4.95±1.26</td>
<td>0.29±0.31</td>
</tr>
<tr>
<td>2</td>
<td>11.65±3.46</td>
<td>6.92±1.56</td>
<td>0.95±0.77</td>
</tr>
<tr>
<td>3</td>
<td>10.86±1.44</td>
<td>6.76±1.37</td>
<td>0.48±0.10</td>
</tr>
<tr>
<td>4</td>
<td>9.62±2.94</td>
<td>6.49±2.95</td>
<td>1.00±1.03</td>
</tr>
<tr>
<td>5</td>
<td>10.96±3.03</td>
<td>7.72±2.76</td>
<td>0.61±0.65</td>
</tr>
<tr>
<td>6</td>
<td>10.57±3.16</td>
<td>6.44±1.36</td>
<td>0.64±0.68</td>
</tr>
<tr>
<td>SEM</td>
<td>0.496</td>
<td>0.367</td>
<td>0.119</td>
</tr>
<tr>
<td>P</td>
<td>0.842</td>
<td>0.425</td>
<td>0.534</td>
</tr>
</tbody>
</table>
Discussion

The balance of cations, especially of Ca, Mg, Na, and K, and anions (PO₄, SO₄, and Cl) in feedstuff and tissues of birds is a prerequisite of body homeostasis. Hence, while determining the electrolyte balance in feed and body of poultry, consideration is given to the content of the major cation, namely Na, as well as K cation and Cl anion (3, 25). Disturbances in homeostasis, e.g. as a result of metabolic acidosis, may lead to disorders in the mineralisation process, especially of the bones of the limbs (9), while an excess of Cl in a feed mixture may induce negative effects on the absorption and metabolism of vitamin D. The lack of electrolyte balance in feed and in the body may, additionally, lead to disorders of immune mechanisms (21).

In this experiment, feeding the broiler chickens a feed mixture without a NaCl addition resulted in statistically significant differences in concentrations of the elements examined in blood serum, compared to the chickens administered feed mixtures with the addition of NaCl (Table 2). Concentrations of Mg, Ca, and K were higher in the chickens fed the Na-free feed mixture, whereas those of Cl and Na were lower in the chickens administered feed mixtures with the addition of NaCl, irrespective of its dose.

The lower concentration of Na (136 mmol/dl) and the high concentration of K (8.46 mmol/dl) in blood serum of the birds administered a feed mixture without Na addition differed substantially from reference values for Na and K (24), by 10% and 60%, respectively. This could be a significant factor inhibiting the growth of the chickens in this group (Table 2) (6). In the other groups administered feed mixtures with the addition of 0.127-0.636% NaCl, the determined serum contents of the analysed elements approximated the reference values (24). In experiments conducted by other authors with a sufficient or even relatively high Na content in feed mixtures (from 0.15% to 0.30%), no differences were observed in serum levels of Na nor K (15, 16, 19). In a study by Johnson and Karunajeewa (7), where in 12 diets the Na content ranged from 0.23% to 0.73%, Cl content from 0.26% to 0.99%, and electrolyte balance from 327 to 700 mEq/kg, the serum contents of Ca, inorganic P, Mg, Na, K, and Cl of chickens did not depend on the administered feed. This points to the high stability of blood electrolytes, which may, however, be disturbed by a considerable deficiency of one or a few components, e.g. Na (5), as in the case of the discussed experiment, in the group receiving a grower type feed mixture without NaCl addition. The observed disturbances in blood electrolytes resulted in the inhibition of body weight gains of the broiler chickens examined (Table 2).

The available literature lacks data on the effect of NaCl content in feed mixtures on the immune system in general, and especially on the non-specific parameters of the cellular immunity. Only Piementel and Cook (21), when investigating the impact of Na and Cl in diet on the humoral immune response, have demonstrated that in SRBC-immunised chickens the response was inhibited by a deficiency or imbalance of those macroelements in the diet. Taking into account the fact that SRBC is a thymus-dependent antigen, it may be speculated that the serological response to this antigen is determined by T-lymphocytes (12). Therefore, the determination of varied addition of NaCl to feed mixtures on the percentage of CD4⁺, CD8⁺, and CD4⁺CD8⁺ T-lymphocytes subpopulation was advisable from the practical point of view. It is of great significance taking into account that the immune systems of birds bred in the intensive system are affected by a number of contagious and non-contagious factors that may induce immunosuppression, and thus increase the birds’ susceptibility to infections with various pathogens, diminish the efficacy of immunoprophylaxis, and decrease production indices (cit. 23). The obtained results (Table 3) did not confirm these speculations, as the content of NaCl in the feed mixtures did not affect the percentage of subpopulations of the T-lymphocytes examined. Although the achieved results seem promising, elucidation of complex issues linked with the functioning of the immune system of the broiler chickens administered feed mixtures with various additions of NaCl requires further study.

In summary, it may be concluded that the addition of NaCl to feed mixtures, covering the demand for Na cation and Cl anion, known to be the most significant for body homeostasis, exerts a significant effect on the level of electrolytes in the blood of chickens and on their body weight gains. In turn, the varied contents of NaCl in the feed mixtures administered were found not to affect the percentage of CD4⁺, CD8⁺, and CD4⁺CD8⁺ T-lymphocytes subpopulations.

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References
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