ASSESSMENT OF ACUTE PHASE RESPONSE IN TURKEYS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI* OR HAEMORRHAGIC ENTERITIS VIRUS

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**Abstract**

Acute phase responses in turkeys infected with a pathogenic strain of *E. coli* or haemorrhagic enteritis virus (HEV) were evaluated on the basis of levels and dynamics of selected acute phase proteins. Two groups of 20 6-week-old turkeys each were infected through their abdominal air sacs with $4 \times 10^9$ CFU/ml of O78:K80:H9 *E. coli* serotype or injected intravenously with $10^3.6$ EID$_{50}$ of the HEV. Another group of 20 birds formed an uninfected control group. Blood samples were taken from all the birds prior to infection and 6, 12, 24, 48, 72, 96, 120, 168, and 240 hrs after the infection. Serum levels of ceruloplasmin, haptoglobin, albumin, TIBC, UIBC and total iron were determined and blood fibrinogen level was assayed. The obtained results revealed a higher response enhancement in the case of experimental colibacillosis than in the case of HEV infection. This was manifested with more significant changes in the levels of ceruloplasmin, haptoglobin, fibrinogen, albumin and TIBC.

**Key words:** turkeys, *E. coli*, HEV, acute phase proteins, acute phase response.

Acute phase reaction is an early and complex, although not specific, reaction of an organism to various stimuli such as: infection, injuries, tumour growth, or tissue necrosis (14). One of the characteristic changes of this phase is intensification or retardation of specific blood plasma protein synthesis in the liver. These proteins are described as acute phase proteins (APPs). In birds, similar to mammals, APPs synthesis involves pro-inflammatory cytokines such as: IL-1, IL-6 and MGF which also function as the main mediators of acute phase system response. The cyto-kines are released by activated macrophages, lympho-cytes, and heterophils (13). It was shown that in mam-mals, higher acute phase response is induced more by bacteria and their components than by viruses (7). In case of virus infections, which induce acute phase response via γ-interferon, a higher response takes place when the infection is accompanied with tissue necrosis or when bacterial complications occur during which additional cytokine release takes place (12). Compa-rible levels of α$_1$-glycoprotein were found in birds infected with ILT, IB and IBD viruses and in birds infected with *E. coli* (11, 22). In addition, the transferine level grew after infection with reticuloendotheliosis (32) similar to the case of natural *Staphylococcus aureus* infection (3) or when *E. coli* liposaccharides were administered (8). However, a secondary bacterial factor in these viral infections (i.e. bacteria inducing APPs synthesis) cannot be excluded from considerations.

The objective of the study was a comparison of acute phase response in turkeys due to infection with *E. coli* or haemorrhagic enteritis virus (HEV), based on the concentration of the selected serum APPs assay.

**Material and Methods**

This study involved 60 white BUT 9 type broad-breasted turkeys, bred in isolated vivariums of the Department of Poultry Diseases. The experimental birds were sensitive to *E. coli* and HEV infections. Two groups of twenty 49-day-old birds were either infected through their abdominal air sacs with $4 \times 10^9$ CFU/ml of O78:K80:H9 *E. coli* strain or injected intravenously with $10^3.6$ EID$_{50}$ of HEV material prepared following the methodology of Koncicki (16). The third group of 20 birds formed an uninfected control group. Blood samples were taken from all the birds, from the wing vein or tibial vein prior to and 6, 12, 24, 48, 72, 96, 20, 168, and 240 h after the infection (h p. i.). During the experiment, a clinical examination was performed and the dead or slaughtered birds after the experiment were
subject to pathological examination. At 72 h p. i., 3 HEV infected turkeys were slaughtered to determine the spleen index and to examine the spleen for presence of the virus. For this purpose, a Domermuth micro method AGP test was performed (6). Subsequently, the birds that died of *E. coli* infection were subject to bacteriological examination with the use of a differential McConkey medium. Inoculations were based on heart, liver and spleen materials.

The levels of caeruloplasmin, haptoglobin, albumin, total iron and unsaturated iron binding capacity (UIBC) were assayed in the serum, whereas fibrinogen was assayed in the blood. Caeruloplasmin was determined with the use of Ravn (27) p-phenylenidiamine (Sigma Chemical) based enzymatic method. The level of haptoglobin was determined with the use of spectrophotometry method of Owen, Better and Hoban (24) adapted for birds with guaiacol (Fluka Chemica) (19). An albumin assay was performed with the use of bromocresol green based spectrophotometry - ChF Reagent®. To determine total iron binding capacity (TIBC), serum total iron and unsaturated iron binding capacity were assayed with a ferrozine-based colorimeter (ChF Reagent® Iron/TIBC Ferrozine kit). TIBC was calculated from the formula: TIBC = serum total iron + unsaturated iron binding capacity. Fibrinogen was assayed according to the Clauss coagulation method.

The obtained results were analysed statistically using a two factor variance analysis to determine the average, standard deviation and significance (P ≤ 0.05).

**Results**

The *E. coli* infected turkeys were apathetic, dejected and they lost appetite starting from 6 h p. i.; later on they had diarrhea and dyspnoea. The symptoms gradually faded at 72 h p. i. Some of the tested birds stumbled and were stricken with inflammatory edema of the wing and hock joint. Some of the birds died 24 h p. i. (1 specimen), 48 h p. i. (1 specimen) and 168 h p. i. (1 specimen). In HEV infected turkeys there were no clinical symptoms. The *E. coli* infected birds that died or were slaughtered demonstrated fibrinous inflammation of the air sac, pericardial sac and liver capsule and catarrhal inflammation of intestinal mucosa and fibrin deposits between the intestines. Some birds showed enlargement of joint capsules and serofibrinous exudate in wing and leg joints. *E. coli* was found during bacteriological examinations of the intestines taken from the above birds. However, in HEV infected turkeys, enlargement and mottle of the spleen and a raised spleen index of up to 2.75±0.48 were found. An AGP test indicated the presence of the HEV in the spleen. The levels of caeruloplasmin, haptoglobin and fibrinogen are shown in Table 1. In the serum of *E. coli* infected turkeys, significant increase in caeruloplasmin content was found 6 h p. i. and it remained until the end of the observation. This content was significantly higher than that in HEV infected birds and birds from the control group. In the HEV infected group, the increase in caeruloplasmin content was found at 24, 48, 72 and 120 h p. i., however, when compared with the control group it was significant only after 120 h p. i. *E. coli* infected turkeys showed a significant decline in haptoglobin level at 6, 48 and 72 h p. i. The haptoglobin concentration also declined in HEV infected turkeys, but not until 48 h p. i. and 240 h p. i. However, a significant increase in haptoglobin level was observed in 96 and 120 h p. i. only in *E. coli* infected birds and it was significant when compared to the remaining groups of turkeys. The level of fibrinogen in *E. coli* infected birds increased after 24 h p. i. until the end of the observation and it was significantly higher when compared with the fibrinogen level in the HEV infected group. In comparison to the control group, a significant difference was found in 12, 24, 48, 120 and 240 h p. i.

The levels of albumin and figures for iron transformation are shown in Table 2. As the table shows, the level of serum albumin was significantly lowered from 12 to 96 h p. i. in turkeys infected with *E. coli*. The level of albumin did not decline in HEV infected bird serum, although in 24 and 168 h p. i., when compared to the control group, a significantly lower level of albumin was detected. TIBC rose markedly in the *E. coli* infected group from 72 to 168 h p. i., but it was significant when compared with respective levels in HEV infected birds only after 72 h p. i. TIBC increase in *E. coli* infected turkeys was found to be accompanied with a total serum iron decline from 6 to 96 h p. i., which also took place from 24 h to 72 h p. i. in serum of the two remaining groups.

The total serum iron level was significantly lower in *E. coli* infected turkeys at 6, 12, 96, 168 and 240 h p. i. when compared with HEV infected and the control group. A significant increase in UIBC was also found in *E. coli* infected turkeys and it was visibly higher 6, 12 and 72 h p. i. when compared with the control group and after 6, 72 and 240 h p. i. when compared with the HEV infected group.

**Discussion**

The changes in mammalian serum APPs level are used for detecting pathological changes. However, in birds, APPs levels are analysed to monitor disease development (4). Most of the published studies have concentrated on analysis of one APP and this study attempts to analyse acute response phase, based on several selected APPs.

The growth of caeruloplasmin in chicken serum after infecting the birds with *E. coli* or administering *E. coli* liposaccharides, was shown by Butler and Curtis (2, 5) and by Piercy (25). The results of this study are convergent with the results obtained by Piercy (25) and the differences might have occurred due to different species of experimental birds.
### Table 1
Levels of serum caeruloplasmin (Cp), haptoglobin (Hp) and blood fibrinogen (Fib) (n=17, X±SD)

<table>
<thead>
<tr>
<th>Parameters/ Groups</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>168</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp (mg/dl)</td>
<td>3.75±0.65</td>
<td>7.65±1.54</td>
<td>7.65±1.26</td>
<td>9.14±1.55</td>
<td>8.78±2.06</td>
<td>8.78±2.19</td>
<td>13.58±5.66</td>
<td>15.53±5.09</td>
<td>12.18±5.53</td>
<td>17.4±5.58</td>
</tr>
<tr>
<td>Hp (mg/dl)</td>
<td>3.74±0.12</td>
<td>3.92±0.16</td>
<td>4.02±0.3</td>
<td>5.32±0.46</td>
<td>5.45±0.49</td>
<td>4.71±0.71</td>
<td>4.71±0.53</td>
<td>4.38±0.67</td>
<td>2.68±0.28</td>
<td>3.88±0.33</td>
</tr>
<tr>
<td>Fib (mg%)</td>
<td>1.57±0.12</td>
<td>1.60±0.38</td>
<td>0.85±1.28</td>
<td>1.94±1.65</td>
<td>1.65±1.23</td>
<td>0.98±1.83</td>
<td>1.83±0.57</td>
<td>1.83±0.57</td>
<td>3.85±0.57</td>
<td>1.83±0.57</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>3.03±1.39</td>
<td>4.40±1.39</td>
<td>3.64±1.39</td>
<td>5.21±1.39</td>
<td>4.85±1.54</td>
<td>3.48±1.54</td>
<td>4.54±1.54</td>
<td>2.98±1.54</td>
<td>2.11±1.54</td>
<td>2.10±1.54</td>
</tr>
</tbody>
</table>

### Table 2
Levels of albumin (Alb), total iron binding capacity (TIBC), total iron (Fe) unsaturated iron binding capacity (UIBC) in serum (n=17, X±SD)

<table>
<thead>
<tr>
<th>Parameters/ Groups</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>168</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alb (g/dl)</td>
<td>1.62±0.21</td>
<td>1.37±0.43</td>
<td>1.23±0.21</td>
<td>1.27±0.17</td>
<td>1.20±0.17</td>
<td>1.18±0.11</td>
<td>1.14±0.14</td>
<td>1.33±0.20</td>
<td>1.58±0.26</td>
<td>1.64±0.10</td>
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<tr>
<td>TIBC (µg/dl)</td>
<td>292.2±0.54</td>
<td>310.4±0.44</td>
<td>319.0±0.30</td>
<td>327.8±0.17</td>
<td>340.0±0.21</td>
<td>342.6±0.14</td>
<td>407.5±0.22</td>
<td>397.0±0.22</td>
<td>383.0±0.22</td>
<td>323.0±0.22</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>212.1±0.76</td>
<td>84.1±0.76</td>
<td>78.8±0.76</td>
<td>82.2±0.76</td>
<td>103.0±0.76</td>
<td>90.6±9.71</td>
<td>98.6±8.71</td>
<td>111.2±12.5</td>
<td>125.5±13.8</td>
<td>138.5±13.8</td>
</tr>
<tr>
<td>UIBC (µg/dl)</td>
<td>146.2±0.56</td>
<td>218.0±0.56</td>
<td>214.1±0.56</td>
<td>291.0±0.56</td>
<td>250.5±0.56</td>
<td>375.1±0.56</td>
<td>370.0±0.56</td>
<td>241.5±0.56</td>
<td>237.0±0.56</td>
<td>265.0±0.56</td>
</tr>
</tbody>
</table>

For explanations, see the bottom of Table 1.
Similar results for growth of caeruloplasmin in HEV infected turkeys that were fed lipid peroxides additive premixes were found by Koncicki et al. (15). However, in both cases the studied level was significantly lower than in turkeys infected with E. coli. In this study, similar to the studies done by Hawkey and Hart (9), a significant growth of blood fibrinogen level in E. coli infected turkeys was observed. However, in HEV infected turkey blood and in the control group blood samples, fluctuations of blood fibrinogen were observed. The available bibliography on this protein interaction lacks data on virus-infected birds. There is also no bibliographical data on acute phase response in haptoglobin level, resulting from an infectious factor. Musquera et al. (20, 21) found only haptoglobin growth in chickens under local inflammation conditions.

In contrast, in this study, there was found a significant enhancement of synthesis and an increase in haptoglobin level in E. coli infected turkeys but slight changes within the level in HEV infected birds. In the case of E. coli infected birds, a more explicit decline in albumin level than in HEV infected or uninfected birds was found. This corresponds with the results obtained by other authors (10, 28).

Hypoferremia, under conditions of acute response to bacterial infection, takes place in turkeys, similar to mammals (18). This is indicated by the growth of TIBC and UIBC levels and a decline in total iron in the serum of E. coli infected birds. Similar results were obtained in bird studies done by other authors (3, 8, 25). In the group of HEV infected and uninfected turkeys, only a slight fluctuation of TIBC and UIBC and decline in serum Fe were observed. The results of this study are close to Zimbera's results (33, 34) for retrovirus-infected turkeys.

The results of our study show a higher acute response in the case of experimental colibacillosis than in the case of HEV virus infection.

This is closely related to the course and intensification of an inflammatory process during E. coli infection. During experimental colibacillosis of the respiratory system, activation of heterophils takes place. The heterophils which are chemotopic (31), release cytokines, including IL-6 pro-inflammatory cytokine (26). After phagocytizing the bacteria, heterophils are deteriorated and their remains are metabolized by macrophages. Stimulated macrophages release IL-1, IL-6, a factor similar to TNF-α, macro-phage stimulating factor and a granulocyte colony stimulating factor (17). These cytokines act locally in the spot of inflammation and systemically during acute phase response stimulate synthesis and change in APPs levels. The HEV APPs synthesis activation mechanism differs from the above discussed. As was shown by Shuresh and Sharma (29), the first week of HEV infection features a significant decline in the number of B lymphocytes and an increase in the number of T lymphocytes. Activated lymphocytes produce TNF and INF-γ, which subsequently stimulate macrophage cytokine production, including IL-6 and TNF. Those cytokines participate in activating hepatocyte APPs synthesis. As the study results show, the process is less intensive in the case of HEV infection.

In analysing the kinetics of changes in protein level, it should be emphasized that the changes were faster in the case of bacterial infection (caeruloplasmin - 6 h p. i., fibrinogen -24 h p. i.) than in viral infection (caeruloplasmin - 24 h p. i., fibrinogen - 48 h p. i.). There was also found a transient decline in serum fibrinogen and haptoglobin content during the first stages of infection. This phenomenon is related to the higher vascular permeability and higher catabolism of some "positive" APPs during the first stages of an inflammatory reaction (14). In addition, some APPs levels were subject to fluctuation in uninfected specimens, which might have resulted from a stress condition due to the immobilization of experimental birds and blood sampling. It is known that glucocorticoids released from the adrenal glands stimulate APPs synthesis. They probably affect hepatocytes, causing post-translation changes and higher mRNA transcription rate (1). This stimulating effect does not concern all APPs and may be related to gene activation which is dependent on glucocorticoids (23).

At the same time, glucocorticoids are ascribed to the function of "downward" regulation of acute phase response through stabilizing the macrophage membranes and indirectly inhibit cytokine release (7, 30). Thus, when interpreting APPs level, gluco-corticoids that affect synthesis and APPs fluctuation should be taken into account. The serum APPs level changes observed in our study suggest that acute response in turkeys is stronger in the case of E. coli infection than in the case of HEV inflammation.

References