
EXPERIMENTAL AFLA- AND OCHRATOXICOSIS
IN CHICKENS VACCINATED WITH CU FOWL
CHOLER A VACCINE

II. INFLUENCE ON HUMORAL RESPONSE AND RESISTANCE TO
CHALLENGE WITH VIRULENT P. MULTOCIDA ORGANISM.

REFAI, M.K.; S.M. GERGIS; A.A. EL-SANOUSI; S. ABDEL
HAMID AND M. HASHAD

SUMMARY After vaccination with the living CU P. multocida vac-
cine in the groups of chickens intoxicated with aflatoxin and/or ochratoxin
A, the immune response was greatly altered and depressed at 30 and 40
days of age. At 50 days, the effect of ochratoxin was nonsignificant. The
relative weight of bursa of Fabricius was not significantly altered in all
intoxicated groups till 40 days of age. The thymus showed significant
decrease in relative weight in birds intoxicated with both aflatoxin Bl and och-
ratoxin A either singly or combined at 20, 30 and 40 but not at 50 days of
age. These mycotoxins have also been found to adversely influence the
weight of spleen if given only in a combined but not in a single form to
chickens especially at 20 and 30 days of age. Both toxins singly or com-
bined had the ability to suppress the resistance of birds to challenge infec-
tion with virulent P. multocida. Toxins given singly had more immuno-
suppressive effect than in combined from.

INTRODUCTION

Like other animal species, poultry are liable to many environmental
multifactorial stressors (microbial and non microbial). The defence
mechanisms of poultry against these stressors share to a large extent in
the well-being of such animals. Mycotoxins are one of these stressors
which are found to impair the immune mechanisms of birds (Thaxton
and Hamilton, 1971).

Mycotoxins may not only alter the host resistance to different pathogens
but also lead to what is called vaccination failure (reviewed by Shalaby,
1989). This is considered one of the great problems facing poultry indus-
try in Egypt. The vaccination failure has been found to occur even if the

Received: 20. 12. 1992
Experimental afla and ochratoxicosis

Bird consumed doses below those associated with clinically apparent mycotoxicosis, thus hindering correct diagnosis (Smith and Hamilton, 1970).

The aim of this investigation was to study the capability of such mycotoxins either singly or combined to alter the chicken humoral immune response to Cu fowl cholera vaccine, beside evaluating the resistance of intoxicated vaccinated chickens to challenge infection with the virulent P. multocida organism. The probable adverse effect of aflatoxin B₁ and ochratoxin A on the organs of the immune system namely bursa, thymus and spleen was also studied.

MATERIAL AND METHODS

Experimental chickens: Three hundred one day old male L.S.L chickens were used in this study. They were raised on a ration pretested for absence of mycotoxins.

Aflatoxin B₁ and Ochratoxin A: These were obtained in a pure form from Fluka company (Buchs, Switzerland), Lot No. 05032 and 74711 respectively. Aflatoxin final concentration in the ration was 0.75 ppm (Kubena et al., 1988).

Fowl Cholera Vaccine: The living attenuated fowl cholera (Cu strain) vaccine was obtained from Shering Corporation (Omaha Nebraska USA, Lot No. 81156).

Indirect Haemagglutination Test: The test has been used according to the procedures described by Carter and Rappay (1962) for measurement of anti-Pasteurella multocida antibodies in the sera of vaccinated chickens.

Challenge Test (Heddleston and Robers, 1967): Chickens of all groups (see experimental design in Refai et al., 1993- this volume) were challenged by injection of the virulent P. multocida serovar 5:A (100 lds₅₀ per chick).

Relative Weight of Lymphoid Organs (Giambrone, 1989): The alive
body weights of the birds were determined, then after killing the birds, the lymphoid organs (bursa, thymus and spleen) were carefully obtained and weighed. The organ relative weight was determined according to the following equation:

\[
\text{Weight of the organ} = \frac{\text{Organ Relative Weight}}{\text{Total alive body weight}} \times 1000
\]

**Experimental Design:** The different groups of chickens dealt with in this work have been treated with mycotoxins and vaccinated as fully described by Refai et al. (1993, this volume).

**RESULTS AND DISCUSSION**

Looking to the results documented in table (1) one can notice the capability of both aflatoxin B₁ and ochratoxin A either singly or combined to exert their actual immunosuppressive effect which was reflected on the level of circulating anti-P. multocida antibodies in vaccinated birds especially at 30 and 40 days of age. This adverse effect persisted with a statistical significant depression at 50 days of age only in birds given aflatoxin B₁ and in those who received both toxins simultaneously, whereas Ochratoxin A alone did not show significant difference if compared with the control non intoxicated vaccinated birds.

### Table (1):

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Age of birds</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>2b</th>
<th>3a</th>
<th>3b</th>
<th>4a</th>
<th>4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>10.22±1.3</td>
<td>10.02±0.0</td>
<td>11.32±2.1</td>
<td>7.9 ± 5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 days</td>
<td>11.28±2.5</td>
<td>9.7 ± 5.6</td>
<td>5.0 ± 4.8</td>
<td>10.0 ± 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>4.25±3.10</td>
<td>0.25 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>25.0 ± 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 days</td>
<td>10.25±0.0</td>
<td>10.02±5.0</td>
<td>0.35±2.0</td>
<td>15.0 ± 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 days</td>
<td>7.3 ± 0.0</td>
<td>10.02±5.0</td>
<td>0.35±2.0</td>
<td>15.0 ± 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The levels of antibodies in the serum samples of chickens were determined by passive hemagglutination test (PHA) using formalized sheep RBCs which were sensitised with Pasteurella multocida capsular antigen.

1. See Table (1).
2. The tabulated data represent the mean titres of anti-P. multocida antibodies in serum samples of chickens in the different groups.
Experimental afla and ochratoxicosis

The persisting continuous effect of aflatoxin B₁ could be explained on the basis that, degradation of immunoglobulins may be due to the specific activity of lysozymes (Tung et al., 1970).

The absence of observable adverse effect of ochratoxin A on the level of antibodies against P multocida at 50 days of age may be due to the higher resistance of younger chickens to the toxic effect of this mycotoxin than in younger ones (Huff et al., 1974).

The above mentioned results were found to agree fully with those documented by Campbell et al. (1983), who reported that aflatoxin B₁ and ochratoxin A could impair the immune response against P. multocida and other immunogens.

Due to their adverse effects on the functional activities of immune system, aflatoxin B₁ and ochratoxin A have been found to depress the protective capability of vaccinated chickens against challenge with the virulent strain of P. multocida. As shown in table (2) the protection level decreased markedly to 66.7%, 66.7% and 73.4% in birds toxicated with aflatoxin B₁, ochratoxin A and both in combination respectively, compared with the normal protection level of 80% in non-toxicated vaccinated chickens. Similar findings have been previously obtained by Pier and Heddleston (1971) and Pier (1974).

<table>
<thead>
<tr>
<th>Table (2)</th>
</tr>
</thead>
</table>

Results of protection test of chickens treated with mycotoxins and/or vaccinated against challenge with virulent Pasteurella multocida serovar 2: A strain   

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of challenged chickens</th>
<th>No. of survived chickens</th>
<th>Mortality percentage</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>7</td>
<td>46.6</td>
<td>53.3</td>
</tr>
<tr>
<td>1b</td>
<td>15</td>
<td>13</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>2a</td>
<td>15</td>
<td>1</td>
<td>92.3</td>
<td>7.7</td>
</tr>
<tr>
<td>2b</td>
<td>15</td>
<td>11</td>
<td>26.6</td>
<td>72.4</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>33.3</td>
<td>66.7</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>10</td>
<td>33.3</td>
<td>66.7</td>
</tr>
</tbody>
</table>

* The birds were challenged by i.m. inoculation dose of 3×10⁶ cells of P. multocida strain at the 45th day of age (i.e., 21 days post vaccination).

* See Table (3).

** The % of mortality was calculated as follows:

\[
\text{Mortality} = \frac{\text{No. of died birds}}{\text{No. of challenged birds}} \times 100
\]
It could be seen from table 4, 5 and 6 that the relative weight of bursa of Fabricius was not significantly altered in all types of treatment with mycotoxins till 40 days of age. The thymus showed a significant decrease when both aflatoxin B₁ and ochratoxin A were given either singly or combined at 20, 30 and 40 but at 50 days of age. It has also been found that these mycotoxins work to adversely influence the weight of spleen if given to chickens in a combined form especially at 20 and 30 days of age, but singly they failed to exert such effect, except a transient significant decrease at 40 days in ochratoxicated birds. These findings are supported by previous report Giambrone et al. (1978) and Campbell et al. (1983).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>2.125 ± 0.34</td>
<td>2.350 ± 0.21</td>
<td>3.930 ± 0.00</td>
<td>3.730 ± 0.30</td>
<td>3.750 ± 0.32</td>
</tr>
<tr>
<td>20 days</td>
<td>3.335 ± 0.37</td>
<td>3.350 ± 0.32</td>
<td>3.720 ± 0.35</td>
<td>4.550 ± 0.38</td>
<td>4.150 ± 0.40</td>
</tr>
<tr>
<td>30 days</td>
<td>3.200 ± 0.35</td>
<td>4.000 ± 0.40</td>
<td>3.875 ± 0.30</td>
<td>6.450 ± 0.40</td>
<td>6.250 ± 0.35</td>
</tr>
<tr>
<td>40 days</td>
<td>4.130 ± 0.40</td>
<td>5.750 ± 0.45</td>
<td>3.600 ± 0.33</td>
<td>8.500 ± 0.45</td>
<td>6.500 ± 0.45</td>
</tr>
<tr>
<td>50 days</td>
<td>5.640 ± 0.53</td>
<td>6.450 ± 0.56</td>
<td>5.800 ± 0.35</td>
<td>8.750 ± 0.50</td>
<td>8.050 ± 0.50</td>
</tr>
</tbody>
</table>

* The bursae were obtained after the chickens were previously alive weighed and then their relative weights were estimated (see below).

# Table (1): The relative weights of bursa of Fabricius in chickens treated with mycotoxins and/or vaccinated with P. multocida.

** See table (1).

The relative weights of the organ =
Weights of the obtained organ / Weight of alive bird.

The relative weights of the organ =
Weights of the obtained organ / Weight of alive bird.

The relative weights of the organ =
Weights of the obtained organ / Weight of alive bird.
Experimental asla and ochratoxicosis

Table (4)

Relative weights of the thymus in chickens treated with mycotoxins and/or vaccinated with P. multocida:

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Age of Bird</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>2b</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>3.1±0.44</td>
<td>4.66±0.67</td>
<td>4.4 ±1.5</td>
<td>3.8±0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 days</td>
<td>4.16±0.66</td>
<td>6.90±0.14</td>
<td>5.03±0.16</td>
<td>5.69±0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>5.1 ±0.3</td>
<td>6.35±0.15</td>
<td>5.9 ±0.15</td>
<td>4.3 ±0.0</td>
<td>4.4 ±0.1</td>
<td>4.1±0.05</td>
<td>8.3±0.35</td>
</tr>
<tr>
<td>40 days</td>
<td>6.6±0.42</td>
<td>7.34±0.48</td>
<td>6.6±0.49</td>
<td>5.1 ±0.49</td>
<td>8.3±0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 days</td>
<td>7.7±0.80</td>
<td>8.16±0.28</td>
<td>6.99±0.5</td>
<td>7.34±0.35</td>
<td>8.3±0.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Thymus was obtained and dealt with like bursa of Fabricius (Table 3).

** The tabulated figures represent the mean, relative weights of the thymus ± SD estimated according to the equation presented under Table (3).

Table (5)

The relative weights of spleen in chickens given mycotoxins as well as vaccinated with P. multocida

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Age of Bird</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>2b</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>1.12±0.28</td>
<td>1.42±0.57</td>
<td>1.40±0.34</td>
<td>1.89±0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 days</td>
<td>1.42±0.32</td>
<td>6.60±0.99</td>
<td>0.69±0.09</td>
<td>0.89±0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>1.76±0.80</td>
<td>1.42±0.35</td>
<td>1.35±0.30</td>
<td>1.46±0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 days</td>
<td>1.9 ±0.36</td>
<td>1.32±0.54</td>
<td>1.32±0.32</td>
<td>1.32±0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 days</td>
<td>2.7 ±0.35</td>
<td>2.32±0.54</td>
<td>2.17±0.31</td>
<td>2.47±0.18</td>
<td>2.34±0.47</td>
<td>2.66±0.36</td>
<td></td>
</tr>
</tbody>
</table>

* As mentioned before the spleen of birds in the different groups were taken and weighed.

** The calculated mean relative weights of spleen ± SD (see Table 3).
REFERENCES


