This study was conducted to investigate the effects of deoxynivalenol (DON) contamination of broiler diet on productive performance and the index of stress in broiler chicken, Heterophil:Lymphocyte ratio (H/L ratio) and other physiological manifestations of stress in poultry. Furthermore, the effects of microbial feed additive (Mycofix Plus) to counteract the adverse effects of DON were also investigated. Thirty two male 1-d old broiler chicks (Ross 308) were divided into four groups (8 birds per group). Each group was fed with one of the following dietary treatments; 1) basal diet, 2) diet contaminated with 10 mg DON/kg feed, 3) diet contaminated with 10 mg DON/kg feed and supplemented with Mycofix plus, 4) diet supplemented with Mycofix® Plus. The live body weight, body weight gain and feed to gain ratio were measured for each group weekly. At 5 wk old, 6 birds from each group were slaughtered and blood was collected into heparinized vials. Complete white blood cell counts and differential leukocyte counts were performed and the heterophil to lymphocyte ratio (H/L ratio) was calculated.

Contamination of broiler diet with 10 mg DON/kg diet increased the feed conversion rate (feed to gain ratio) and decreased the weekly body gain at 3, 4 and 5 wk compared to controls. Interestingly, addition of Mycofix counteracts the adverse effect of DON on the feed to gain ratio and body weight gain and it returns to the normal value as control birds. Moreover, addition of mycofix in the absence of DON decreased the feed: gain ratio and increased the weekly body gain in broiler chickens. Contamination of broiler diet with 10 mg of DON resulted in an increase (P < 0.05) in the heterophils (H) counts and (H/L ratio) compared with controls. In contrast, the lymphocyte (L) counts were decreased (P < 0.05). However, addition of Mycofix to diet in the presence of DON counteracts the toxic effects of DON. From these results, we can conclude that contamination of broiler diet with DON increases the stress responses and decreases the feed intake, live body weight and body weight gain of broilers. Moreover, addition of Mycofix® Plus to diet contaminated with DON counteracts the adverse effects on productive performance and the physiological stress responses produced by DON.

INTRODUCTION

Contamination of broiler diet with mycotoxins exhibit a variety of biological effects in animals such as: liver and kidney toxicity, central nervous system abnormalities, estrogenic responses and others. Mycotoxins exert their effects through reduction of nutrients absorption [1] and suppression of the immune system [2, 3].

Trichotheccenes such as DON and T-2 toxin reduce immunity by inhibiting protein synthesis and thus cell proliferation. Some mycotoxins are cytotoxic to lymphocytes in vitro. However, there is no information available regarding the effect of contamination of broiler diet with deoxynivalenol (DON) on physiological stress response. Physiological manifestations of stress in poultry include changes in the number of circulating leukocytes in particular a pronounced heterophilia and lymphocytopenia which is a reliable indicator of stress [4-6].

In addition Gross and Siegel [7] compared plasma corticosterone concentration and H/L ratio responses to various stressors and concluded that the H/L ratio is a better indicator of stress in poultry. In the present study, the heterophil: lymphocyte ratio (H/L ratio) was used as an index of stress in chicken as [8].

Therefore, the present study was conducted to investigate the effect of contamination of broiler diet with 10 mg deoxynivalenol mycotoxin on physiological manifestations of stress in broilers. The effect of feed additive (Mycofix plus, BIOMIN GmbH Industriestrasse 21, 3130 Herzogenburg, Austria) addition to broiler diet in the presence and absence of deoxynivalenol mycotoxin contamination on the heterophil: lymphocyte ratio was also investigated.
MATERIALS AND METHODS

Birds and Housing

Thirty two one day male broiler chicks (Ross 308) were obtained from a commercial hatchery and divided randomly into four groups (8 birds). The birds were housed in battery cages (4 birds per cage, 2 replicates for each group).

Diets

The control group was fed starter and grower diets based on corn, soya HP, soya oil, and a premix with vitamins, minerals, amino acids (Lysine, Methionin, and Threonine), salt, and monocalcium phosphate. Birds of each group were fed with one of the following dietary treatments; 1) basal diet, 2) diet contaminated with 10 mg DON/kg feed, 3) diet contaminated with 10 mg DON/kg feed and supplemented with a microbial feed additive, Mycofix plus (2.5 kg/ton of diet), 4) diet supplemented with Mycofix plus (2.5 kg/ton of diet).

The chicks were fed with the normal starter diets from days 1 to 13 and low protein grower diet from day 14 – 35. The feed additives were delivered by Biomin® GmbH, Herzogenburg, Austria. The birds had free access to water and feed.

Growth performance

Birds of each group were individually weighed weekly and the body weight gain was calculated. The feed intake for each group was measured weekly and the feed:gain ratios were calculated.

Measurement of stress index

At 5 wk old, 6 birds from each group were bled and blood samples were collected in heparinised tubes and sent to the central laboratory of the veterinary university of Vienna for analysis. Blood films were air dried (unfixed) and stained in concentrated May-Grunwald stain for 6 min, 1:1 May-Grunwald stain-distilled water for 1.5 min and 1:9 Geisma stain for 15 min [9]. To determine the counts of heterophil and lymphocyte, 100 cells per film were examined by light microscopy. All blood counts including granulocytes (Heterophil, Basophil and Eosinophil) and non-granulocytes (Lymphocyte and monocyte) were examined by the same investigator. The results are presented as the percentage of each cell occurring in each film. The H/L ratio was examined by dividing the number of heterophils by the number of lymphocytes [7].

Statistical analysis

Statistic SPSS program version 17.0 was used for data analysis. Kolmogorov Smirnov test was used to test the normal distribution of the data. Results are given as means ± SEM. Analysis of variance (ANOVA) was performed between the four groups followed by Duncan test to find the significance between dietary treatments. Statements of statistical significance were based on P ≤ 0.05.

RESULTS

Growth performance

Contamination of broiler diet with 10 mg DON/ kg diet decreased the live body weight (996.25 ± 29.71) compared to controls (1205.75 ± 29.71) at 5 wk (P=0.001). Interestingly, addition of Mycofix counteracts the adverse effect of DON on the live body weight of birds (1224.88 ± 29.71) and it returns to the normal value as control birds (1205.75 ± 29.71). Furthermore, addition of Mycofix Plus in the absence of DON increased the live body weight (1293.38 ± 29.71) at 5 wk compared controls (1205.75 ± 29.71).

At 5 wk, the weekly body weight gain was low for DON fed group at 3, 4 and 5 wk compared to controls (P < 0.05, Table 1). Mycofix addition to the contaminated diet could also counteract the adverse effect of DON on the weekly body weight gain and it returns to the normal value as control birds. However, addition of mycofix in the absence of DON significantly increased the weekly body gain of broiler chicken. Moreover, the feed conversion rate (feed to gain ratio) was higher for DON group at 3, 4 and 5 wk (3.01, 3.38 and 2.37 respectively) than control group (2.90, 2.97 and 1.76 respectively).

Additionally, Mycofix could counteract the adverse effect of DON on FCR and it returns to the normal value (2.49, 3.11 and 1.91 at 2, 3 and 4 wk respectively) as control birds (2.90, 2.97 and 1.76 respectively). However, addition of mycofix in the absence of DON decreased the FCR at week 3 than control and had a similar feed: gain ratio at week 4 and 5 (2.95 and 1.79) as control birds (2.97 and 1.76).
Table 1: The effect of addition of microbial feed additive to broiler diet contaminated with Deoxynivalenol mycotoxin on the body gain (g) of broiler chicken

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Age (week)</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol (10 mg/ kg diet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol Plus Mycofix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2.5 kg/ ton diet) (n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycofix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1 155.50</td>
<td>255.75</td>
<td>3.45</td>
<td>0.466</td>
</tr>
<tr>
<td>W2 148.00</td>
<td>237.88</td>
<td>7.62</td>
<td>0.744</td>
</tr>
<tr>
<td>W3 171.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.90</td>
<td>0.078</td>
</tr>
<tr>
<td>W4 184.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.10</td>
<td>0.078</td>
</tr>
<tr>
<td>W5 386.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>241.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.15</td>
<td>0.044</td>
</tr>
</tbody>
</table>

<sup>a, b, ab</sup>Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 8/treatment).

Stress responses and Heterophil:Lymphocyte ratio

Contamination of broiler diet with 10 mg of DON resulted in an increase (P < 0.05, Table 2) in the heterophils (H) counts (60.33 ± 4.70) compared with controls (44.14 ± 4.24). However, addition of Mycofix to diet in the presence of DON counteracts the effect of DON on H counts (55.50 ± 4.23).

In contrast, Lymphocyte (L) counts were decreased (P < 0.05) due to contamination of diet with DON (29.67 ± 3.38) compared with controls (43.29 ± 2.96). However, supplementation of diet with Mycofix in the presence of DON increase (P < 0.05) the L counts (34.33 ± 1.78) compared to DON group (29.66 ± 3.38).

Moreover, the heterophil to lymphocyte ratio (H/L ratio) was increased (P < 0.05, Table 2) for birds fed diet contaminated with DON (2.37 ± 0.51) compared with controls (1.07 ± 0.15). Interestingly, Mycofix supplementation to diet contaminated with DON decrease (P < 0.05) the H/L ratio (1.64 ± 0.13) compared with DON group (2.37 ± 0.51).

Table 2: The effect of addition of microbial feed additive to broiler diet contaminated with Deoxynivalenol mycotoxin on differential leucocytic counts and index (Heterophil: Lymphocyte ratio) in broiler chicken

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>DON</th>
<th>DON Plus Mycofix</th>
<th>Mycofix</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H %</td>
<td>44.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>43.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.62</td>
<td>0.044</td>
</tr>
<tr>
<td>L %</td>
<td>43.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.62</td>
<td>0.020</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>7.14</td>
<td>6.33</td>
<td>7.33</td>
<td>9.67</td>
<td>0.95</td>
<td>0.674</td>
</tr>
<tr>
<td>Basophil %</td>
<td>4.43</td>
<td>3.50</td>
<td>2.93</td>
<td>5.17</td>
<td>0.55</td>
<td>0.502</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>0.29</td>
<td>0.17</td>
<td>0.67</td>
<td>0.50</td>
<td>0.15</td>
<td>0.697</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>1.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19</td>
<td>0.050</td>
</tr>
</tbody>
</table>

<sup>a, b, ab</sup>Means within the same row with different superscripts are significantly different (One way ANOVA followed by Duncan test, n = 6/treatment).

**DISCUSSION**

*Fusarium* species produce a vast array of mycotoxins, many of which are economically important in regard to animal production. Because of mycotoxins can increase incidence of disease and reduce production efficiency. The current study demonstrated clear effects of DON on physiological stress response and immunological functions of broilers.

Most experimental studies [10-16] with poultry show a highly variable effect of DON on performance indicating that zootechnical traits might not be a sensitive indicator of toxicity of this *Fusarium* toxin. However, in the current experiment, feed refusal, reduced weight gain and feed conversion were adversely affected (P < 0.05) by inclusion of 10 mg/kg DON in broiler diets. This finding may be ascribed to the inhibition of protein synthesis by this toxin. We may hypothesize that when the birds are fed with low protein diets, the toxic effect of DON will be more evident. However, the supplementation of Mycofix was useful in counteracting toxic effects of DON on performance.

Concerning the haematological variables, we observed decreases in total leucocyte count and lymphocyte counts. This can be attributed to the fact that the immune system is very sensitive to DON [17, 18].

To our knowledge, this is the first report demonstrating the effect of 10 mg/ kg purified DON on physiological stress response. Physiological manifestations of stress in poultry include changes in the number of circulating leukocytes in particular a pronounced heterophilia and lymphocytopenia which is a reliable indicator of stress [4-6].
In the present study, the feeding of DON contaminated diets resulted in an increase (P < 0.05) in the heterophils (H) counts and (H/L ratio) compared with controls. These responses may indicate predictable physiological changes to the presence of mycotoxin. In contrast, the lymphocyte (L) counts were decreased (P < 0.05). However, addition of Mycofix to diet in the presence of DON counteracts the toxic effects of DON. These results indicate that dietary supplementation of Mycofix relatively increased the L counts and relatively decreased the H counts which could help to overcome the stresses of broilers due to exposure for mycotoxin, suggesting that supplementation of broiler diet with Mycofix can modulate the physiological stress responses.

CONCLUSION

In conclusion, it can be concluded that the contamination of broiler diet with DON increases the stress physiological responses in broiler due to the adverse effect on nutrients absorption. Moreover, addition of Mycofix to diet counteracts the physiological stress manifestations produced by DON.

REFERENCES