Effect of Dietary T-2 Fusariotoxin Concentrations on the Health and Production of White Pekin Duck Broilers

P. Rafai,*1 H. Pettersson,† Á. Bata,‡ Z. Papp,* R. Glávits,# S. Tuboly§, A. Ványi,* and P. Soós§

*Department of Animal Hygiene, Szent István University, Faculty of Veterinary Science, István u. 2. H-1078 Budapest, Hungary; †Department of Animal Nutrition and Management, Swedish University of Agricultural Science, S-75007 Uppsala, Sweden; ‡Dr. Bata Ltd., H-2364 Öcsa, Hungary; #Central Veterinary Institute, Táboronok u. 2. H-1149 Budapest, Hungary; and §Department of Epizootology, Szent István University, Faculty of Veterinary Science, Hungária krt. 23–25., H-1143 Budapest, Hungary

ABSTRACT The effects of different dietary levels of T-2 toxin on production, biological, immunological, and pathological parameters of growing white Pekin ducks were studied to establish the “no effect” dietary concentration of, and “no effect” exposure time to, pure T-2 toxin. Day-old white Pekin ducks were randomly allotted to nine groups of 10 ducks each. One group served as a control, and no mycotoxin was added to its feed. The feeds of the experimental groups were supplemented with 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, and 4.0 mg purified T-2 toxin/kg, respectively, from Day 1 until Day 49 of the experiment. Dermatotoxic oral lesions developed in most experimental ducks within 2 d after the start of feeding T-2 toxin-contaminated feeds. The gradual disappearance of macroscopic signs indicated the development of tolerance in groups treated with the lower T-2 toxin content. No repair was found in the 3 and 4 mg/kg groups. Dietary concentrations of T-2 toxin below 0.4 mg/kg had no effect on the average weekly weight gain in the first 6 wk, but a severe decrease was found in the last week of the experiment. The 0.6 mg/kg dietary T-2 toxin had no effect on weight gain in the first 3 wk. At Week 4 and later, the weekly weight gain was significantly reduced, and the final live weight of this group was also significantly lower than that of the control. Dietary T-2 concentrations of 1 mg/kg and greater uniformly depressed growth rate. Only the 3 and 4 mg/kg groups refused feed during the first week. From Week 3 on, the feed intakes of the 0.6 to 4 mg/kg groups were usually less than that of the control group, indicating feed refusal. Serum and plasma chemical values and hematological parameters failed to show dose-dependent effects. The blastogenic response of lymphocytes to nonspecific and specific mitogens was distinctly impaired by the T-2 toxin at all levels in the feed. In the 3 and 4 mg/kg groups, the histological examination revealed lymphocyte depletion in the spleen and bursa of Fabricius.

(Key words: T-2 toxin, weight gain, immunity, dermatonecrosis, duck)

INTRODUCTION Trichothecene toxins have powerful cytotoxic and cytostatic capacities (Schiefer and Beasley, 1989), hinder protein synthesis (Rosenstein and Lafarge-Frayssinet, 1983), and damage the nervous system (Feuerstein et al., 1989), the parenchymatic organs (Schiefer and Beasley, 1989), and the immune system (Taylor et al., 1989) in a range of animal species. In addition, trichothecenes damage ovarian function in pigs (e.g., Rafai, 1999) and geese (Ványi et al., 1994) with resultant decreased egg production, reduced hatchability, and lower viability of goslings and turkey poults (Fazekas et al., 1993). These dose-dependent effects on livestock, geese, and domestic fowl are well documented; however, very few data, if any, are available in the relevant literature on ducks.

From the early works of Schlosberg et al. (1984, 1986), it is known that Muscovy ducklings (Cairina muschata) are exceptionally sensitive to the dermatonecrotic effects of T-2 toxin and diacetoxyscirpenol, another related trichothecene. This extreme sensitivity renders them useful as bioassay animals to detect mycotoxins (trichothecenes and aflatoxins) in contaminated grains. The findings of Shlosberg et al. (1984, 1986) were reinforced by the studies made by Sályi and Glávits (1995) on Muscovy ducklings that were fed two levels of T-2 toxins in feed (0.75 and 1.5 mg/kg, respectively) between 4 and 26 d of age. The effects of extremely high doses of T-2

Received for publication December 20, 1999.
Accepted for publication May 8, 2000.
1To whom correspondence should be addressed: prafai@univet.hu.

Abbreviation Key: ConA = concanavalin A; FCR = feed conversion rate; PHA = phytohemagglutinin; PHG = purified horse globulin antigen.
toxin (20 and 30 mg/kg feed) were also studied on Mallard ducklings (Hayes and Wobeser, 1983). No dose-dependent effects of low dietary concentrations of T-2 toxin on ducks have been reported, despite the fact that field cases of intoxication of growing ducks with T-2 toxin have been suspected occasionally, and agreement has been found between clinical-pathological findings and the T-2 toxin content of the feed (Sályi and Glávits, 1995).

Most countries regulate the maximum tolerable levels of mycotoxins in food by law or codex standards for the maximum protection of consumers. Apart from aflatoxins, and in some countries ochratoxin-A, such regulations are nonexistent for feeds or feed ingredients. Instead, advisory limit concentrations or guidelines have been established by relevant scientific bodies and applied in several countries. Due to the paucity of relevant data, it is customary to apply the advisory limit concentrations that are established for domestic hens to ducks. The proven difference in sensitivity to T-2 toxin of the domestic hen and duck has prompted a study of the effect of different dietary levels of T-2 toxin on the production, biological, and pathological parameters in growing White Pekin ducks. The present study has also attempted to establish the “no effect” dietary concentration of, and “no effect” exposition time to, pure T-2 toxin for growing White Pekin ducks.

MATERIALS AND METHODS

After preliminary phenotypic selection for vigor, 90 day-old White Pekin ducklings were individually wing banded and randomly allotted to nine groups. The groups were housed separately in climatic chambers that provided optimum climatic conditions throughout the experiment. Wood shavings were used for bedding. Until 10 d of age, the feed was offered ad libitum from cardboard trays. Afterwards, cylindrical self-feeders were used. Water was supplied from conical, and later, valve-operated waterers. Known quantities of prestarter, starter, and grower feeds were measured into the feeders at 0700 h each morning, and what remained was collected and weighed the next morning prior to distribution of fresh feed. This procedure allowed for calculation of daily feed consumption, feed conversion rate (FCR), daily toxin intake, and the proportion of feed refused by each group. Individual body weights were taken once a week.

Prestarter, starter, and grower feeds were checked for the presence of eight mycotoxins (zearalenone, T-2 toxin, HT-2 toxin, nivalenol, deoxynivalenol, diacetoxyscirpenol, fusarenon-X, and ochratoxin-A). The feeds used throughout the experiment were either free of these mycotoxins, or contained them only in trace concentrations (less than 50 µg/kg).

One group served as control, and no mycotoxin was added to its feeds. The feeds of the experimental groups were supplemented with 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, and 4.0 mg/kg purified T-2 toxin, respectively, from Days 1 through 49 of the experiment. These groups are referred to as 0.2 to 4.0 mg/kg groups.

The T-2 toxin was prepared by fermentation of Fusarium sporotrichoides strain NRRL 3299 on liquid medium. The mycelium was extracted by ethylacetate; the extract was then evaporated and purified by liquid-liquid extraction on silica gel column chromatography. The eluent was then concentrated and crystallized in a vacuum. The purity of the effective substance was measured by gas and liquid chromatography and was found to be greater than 90%. The T-2 toxin concentrations of the feeds were regularly checked by the gas-liquid chromatography method as described by Bata et al. (1983, 1984a,b, 1986).

The clinical status of the ducks was observed daily. At 35 and 39 d of age, the ducks were stunned by ether and then exsanguinated using an electric knife. The method of killing was in full accordance with the principles of the Hungarian Animal Welfare Act adopted by the Hungarian Parliament (Law: XXVIII/98). Blood samples were directly collected for determination of erythrocyte count, hemoglobin concentration, packed cell volume, mean cell volume of erythrocytes, lymphocyte count, and count of the heterophils using a particle counter.

The blastogenic responses of lymphocytes to the specific antigen (PHG) and to non-specific mitogens [phytohemagglutinin (PHA) and concanavalin-A (ConA)] were studied as follows: Lymphocytes were separated by Ficoll-paque, then cultured in RPMI-1640 medium at a density of 10^6/mL (Misheh and Shigi, 1980) in Greiner’s plates with 24 holes. The culturing media contained 10% fetal calf serum and additives (penicillin and streptomycin; glutamin and amphotericin-B). Twenty micrograms of milliliter PHG, 100 µL PHA-P5 (10 µg/ml), and 100 µL ConA3 (5 µg/ml) were added to parallel cultures of lymphocytes (900 µL). After 72 h of incubation at 37 C under 5% CO2, the cells were lysed by the addition of 1 mL 0.1% Triton X-106 and by gental vibration for 45 min. The rate of proliferation was estimated spectrofluorometrically in a spectrofluorometer at 365 nm excitation and 450 nm emission of the cellular DNA stained with 5 µg/ml 4,6-diamino-2-phenylindodihidrochloride dissolved in 2 mL phosphate buffer saline for 15 min (Nagatha et al., 1987). Serum and plasma samples were also separated from the blood and used for determination of glucose, triglycerides, cholesterol, alanine amino transferase, aspartate amino transferase, bilirubine, total protein, urea-N, uric acid, Ca, and alkaline phosphastase.
concentrations and activity. These clinical blood parameters were measured spectrophotometrically using analytical Chem-14 test rotors in an Analyst instrument according to the methods suggested by the manufacturer and reported by Hedman et al. (1995).

Ducks that died during the experiment and those bled at the conclusion of the experiment were autopsied. Body weights and weights of the liver, kidneys, heart, and bursa of Fabricius of the autopsied birds were recorded and used for further calculations. Body weight, condition, and feather development were also studied. The mucosa of the oral cavity (palate and tongue) and commissures of the mouth were examined and graded using a 0 to +++ scale, according to the following definitions: 0 indicates no macroscopic lesions; + indicates one or more small, demarked, pale-colored areas on the surface of the epithelial layer (incipient dermatonecrosis); ++ indicates more and larger, demarked, or confluent, initially butter-yellowish, then later, especially in the central part of the affected areas, brownish and thickened areas (extensive dermatonecrosis); and +++ indicates diffuse dermatonecrosis that affected the complete depth of the mucosa, occasionally with hemorrhages and ulcers (diffuse, deep dermatonecrosis).

Samples taken from the liver, kidneys, heart, spleen, bursa of Fabricius, thymus, ileocecal Peyer’s patches, and bone marrow were also studied histologically. Data were analyzed by one-way ANOVA (SPSS computing package) and by fitting response curves (Microsoft Excel).

RESULTS AND DISCUSSION

Dermatotoxic oral lesions developed in almost all experimental ducks within 2 d after start of feeding T-2 toxin-contaminated feeds. The macroscopic signs gradually disappeared, and a tolerance seemed to develop in the groups that consumed feeds contaminated with less than 2 mg/kg T-2 toxin. This effect may explain our findings on ducks that died compared with those sacrificed at the end of the experiment. Tables 1 and 2 summarize our findings on oral dermatotoxicity in ducks that died during the experiment and those sacrificed at the end of the trial.

The dermatotoxic effect of T-2 toxin is well documented, not only in the Muscovy (Schlosberg et al., 1984, 1986; Sályi and Glávits, 1995), but also in Mallard ducklings (Neiger et al., 1994) and many other species, including geese (Palyusik et al., 1968), chickens (Chi et al., 1978; Bitai et al., 1979, 1981; Kubena et al., 1993), turkeys (Richards et al., 1978), and pigs (Pang et al., 1987a; Harvey et al., 1990; Rafai et al., 1995a). In day-old Muscovy ducklings, dermatonecrosis is seen within 16 or more hours after consumption of T-2-contaminated feeds (Shlosberg et al., 1986). Severity and time course show dose dependency. Healing is also rapid following removal of the toxin. According to the observations of Sályi and Glávits (1995), T-2-dependent alterations seen on the palatal mucosa, the tongue, and the mucocutaneous junction of the commissure of the mouth show rapid repair after conclusion of feeding T-2-contaminated feed.

Body weight gains are given in Table 3. Weight gain for the whole treatment period decreased by 18, 8, 34, 9, 18, 19, 53, and 47% in the 0.2, 0.4, 0.6, 0.8, 1, 2, 3, and 4 mg/kg groups, respectively. The weight depression of the 0.2, 1, and 2 mg/kg groups was comparable with that reported by Kubena et al. (1997) in chickens treated with 5 mg/kg dietary T-2 toxin. The less-toxic nivalenol also reduced the body weight gain of growing chickens by 11% when diets were fed containing 6 and 12 mg/kg (Hedman et al., 1995).

From a practical point of view, it is remarkable that the 0.2 and 0.4 mg/kg dietary T-2 toxin concentrations impaired growth rate only after 6 wk of treatment. In contrast, diets contaminated with 0.6 mg/kg T-2 toxin decreased the weight gain in Week 4 and later during the experiment. Higher (≥ 1 mg/kg) dietary concentrations of T-2 toxin, which seldom occur naturally, depressed the growth rate shortly after the start of feeding. This finding is in agreement with that of the short-term experiment of Neiger et al. (1994), who demonstrated that 2 mg/kg dietary T-2 toxin depressed the growth rate of Mallard ducklings significantly. As expected, very high dietary doses of T-2 toxin (20 and 30 mg/kg) significantly suppressed the weight gain of young Mallard ducks (Hayes and Wobeser, 1983).

In the first few days of the experiment, the experimental ducks showed transient anorexia, which quickly disappeared by the second half of Week 1 in the groups treated with lower doses of T-2 toxin (data are not shown). No major reductions in feed intake were seen in the 0.2 to 2 mg/kg groups in the first week. However, the two higher T-2 concentrations (3 and 4 mg/kg) evoked distinct reductions in feed intake. In the later phases of the experiment, feed consumption of the experimental groups tended to lag behind those of the controls. From Week 3 onward, feed intakes of the 0.6 to 4 mg/kg groups were generally lower than that of the control group, which indicated feed refusal. The FCR of the groups showed no dose-dependent, consistent changes with weeks of the treatment. On average, in the 49 d of treatment, the control ducks consumed 3.31 kg feed/kg of live weight. Average FCR for the 0.2, 0.4, 0.6, and 0.8 mg/kg groups was 3.96, 3.86, 3.14, and 3.54 kg/kg, respectively. Ducks that consumed feeds contaminated with 1 and 2 mg/kg T-2 toxin had 8.31 and 5.06 kg/kg FCR, respectively, indicating an impaired growth rate. The 4.98 and 3.26 kg/kg FCR, respectively, in the 3 and 4 mg/kg groups indicate both depressed feed intake and weight gain.

Impaired growth rate of groups treated with higher dietary concentrations of T-2 toxin was not accompanied

---

8SPSS Inc. Headquarters, 233 S. Wacker Drive, Chicago, IL 60606.
by changes of the serum chemistries investigated in the present experiment. The blood values of the control ducks fell within the range of normal values reported for the domestic duck (Harrison and Harrison, 1986), White Pekin duck (Pedersoli, 1989), Mallard duck (Driver, 1981; Hayes and Wobeser, 1983), and American Black duck (Mulley, 1979; Franson, 1982). Although serum chemistry values of the experimental ducks showed some fluctuation around the relevant control values, neither dose-dependent effects nor major differences from the foregoing reference values were established. This result supports the findings on nivalenol-treated broiler chickens (Hedman et al., 1995). It is remarkable that there was no indication of hepatotoxicity, which is in agreement with the results of Hayes and Wobeser (1983). Correlation analysis between serum chemical values and dietary concentrations of T-2 toxin also failed to reveal dose-dependent changes.

The mean count and mean corpuscular volume of erythrocytes of the control ducks showed excellent agreement with those reported by Hebert et al. (1989) on the basis of examination of five male and five female adult White Pekin ducks. Likewise, the packed cell volume and hemoglobin contents of the controls fell within the physiological range reported for domestic ducks (Harrison and Harrison, 1986). The counts of lymphocytes and heterophils in the controls were somewhat lower than those reported for American Black ducks (Mulley, 1979) and Mallard ducks (Driver, 1981). Although there were minor differences between the control and experimental ducks with respect to the foregoing parameters, the statistical analyses revealed no consistent change with the treatments. This observation is in agreement with the observations of others. Recently, Boston et al. (1996) reported no effect of 5.8 mg/kg dietary deoxynivalenol on the packed cell volume in Mallard ducks. Dietary T-2 toxin treatment also failed to induce hematopoietic suppression in chickens (e.g., Wyatt et al., 1973; Chi et al., 1977, 1978), pigs (e.g., Weaver et al., 1978), sheep (e.g., Friend et al., 1983), or cattle (e.g., Weaver et al., 1980) in terms of erythrocyte count, hematocrit values, and hemoglobin concentrations. According to Hayes and Wobeser (1983), however, very high dietary concentrations of T-2 toxin (20 and 30 mg/kg) significantly reduced hemoglobin concentration and packed cell volume in young Mallard ducks at the end of a 3-wk trial.

The effect of dietary T-2 toxin treatment on the blastogenic response of peripheral blood lymphocytes to PHG and nonspecific mitogens is summarized in Figure 1. Data show that after 35 d of treatment, even the lowest level of dietary T-2 toxin (0.2 mg/kg) tested caused depression in an important element of cellular immunity. The inhibitory effect of T-2 toxin was not dose-dependent and was of nearly the same order after stimulation with specific antigen and nonspecific mitogens in most groups. More variation between groups and less inhibition were, however, found after stimulation with PHA.

Antigen- or nonspecific mitogen-induced proliferation of peripheral blood lymphocytes is a good indicator

### Table 1. Oral lesions in ducks that died during the experiment

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Age, d</th>
<th>Tongue</th>
<th>Palate</th>
<th>Commissures of mouth</th>
<th>Bottom of oral cavity</th>
<th>Pharynx</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/kg</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>++</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>+++</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>16</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

1. No macroscopic oral lesions were found in ducks that died in the control group or in the 0.2, 0.6, and 0.8 mg/kg treatment groups.

2. Definition of scoring: 0 = no macroscopic lesions; + = one or more small, demarked, pale-colored areas on the surface of the epithelial layer (incipient dermatonecrosis); ++ = more and larger, demarked, or confluent, initially butter-yellowish, then later, especially in the central part of the affected areas, brownish and thickened areas (extensive dermatonecrosis); +++ = diffuse dermatonecrosis that affected the complete depth of the mucosa, occasionally with hemorrhages and ulcers (diffuse, deep dermatonecrosis).

### Table 2. Oral lesions in ducks sacrificed at the end of the experiment

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Percentage of ducks showing oral lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No lesion</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>50</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>—</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>—</td>
</tr>
</tbody>
</table>

1. No macroscopic oral lesions were found in ducks in the control ducks or in ducks of the 0.2 to 1.0 mg/kg treatment groups.

2. Definition of scoring: 0 = no macroscopic lesions; + = one or more small, demarked, pale-colored areas on the surface of the epithelial layer (incipient dermatonecrosis); ++ = more and larger, demarked, or confluent, initially butter-yellowish, then later, especially in the central part of the affected areas, brownish and thickened areas (extensive dermatonecrosis); +++ = diffuse dermatonecrosis that affected the complete depth of the mucosa, occasionally with hemorrhages and ulcers (diffuse, deep dermatonecrosis).
of the initiation of an immune response, which ultimately determines host resistance to disease. Antigen-specific blastogenic assays and lymphocyte proliferation tests provoked by nonspecific mitogens have been used extensively to assess the immuno-compentence of poultry (Heidrich et al., 1987; Glávits et al., 1990; Honda et al., 1994; Chaffer et al., 1997; Baxi and Oberoi, 1999; Vickery et al., 1999).

The adverse effects of mycotoxins, such as trichothecenes, on the immunological competence of poultry have also been known for some time, both from practice and from experimentation (see Corrier, 1991; Richards et al., 1991; Reddy, 1995; Rotter et al., 1996). Thus, it is surprising that there are few reports about effects of mycotoxins on the blastogenic response of avian lymphocytes (be it peripheral, thymic, or splenic). To our knowledge, only the effects of aflatoxin B1 (Cheng et al., 1996) and ochratoxin-A (Elissade et al., 1994) have been studied in ducks and broiler chicks, respectively, using lymphocyte proliferation tests.

The in vitro inhibitory effect of trichothecenes on the blastogenic response of lymphocytes from species other than poultry and water fowl has been well documented. Peripheral human, bovine, pig, and murine lymphocytes have mainly been studied with T-2 toxin and, in some cases, other trichothecenes as well (Cooray, 1984; Taylor et al., 1987; Tomar et al. 1988; Visconti et al., 1991; Thuvander et al., 1999).

When compared, T-2 toxin and other Type A trichothecenes inhibited the mitogen response of peripheral blood lymphocytes at lower concentrations than Type B trichothecenes.

The effects of T-2 toxin in vivo on the mitogen-stimulated blastogenic response of lymphocytes after toxin exposure to mice, pigs, sheep, and calves (Lafargue-Frassinet et al., 1979; Friend et al., 1983; Mann et al., 1984; Pang et al., 1987b; Taylor et al., 1987; Pang et al., 1988; Rafai et al., 1995b) have been studied. Inhibitory and stimulatory effects, depending on animal, doses, and mitogens, have been found. In pigs, the blastogenic responses of lymphocytes to homologous antigens, PHA and ConA, were decreased to different extents by diets containing T-2 toxin (Rafai et al., 1995b). The lowest tested and effective dose was 0.5 mg T-2 toxin/kg feed. In acute studies, the exposure of pigs to T-2 toxin, both topically and by inhalation, significantly reduced the blastogenic response to PHA and ConA of peripheral (Pang et al., 1987b) and pulmonary (Pang et al., 1988) lymphocytes.

Type B trichothecenes also have less effect than T-2 toxin in vivo. Overnes et al. (1997) and Hedman et al. (1997) demonstrated that neither deoxynivalenol nor nivalenol exposure depressed mitogen-induced blastogenesis in young pigs.

The impaired blastogenic response of lymphocytes was, however, not accompanied by the same dose-dependent effect on the primary and secondary lymphoid organs. No significant dose-dependent differences between the relative weights of the bursa of Fabricius and the spleen were found (Table 4). Neiger et al. (1994), in their 9-d experiment with Mallard ducklings, found that 2 mg/kg dietary T-2 toxin significantly decreased the absolute weight of the bursa of Fabricius. However, in their case, the final body weights of the experimentally treated ducks were also significantly inferior to those of the controls. In the present study, the calculation with

![FIGURE 1. The blastogenic response of lymphocytes to specific and non-specific mitogens; ConA = concanavalin A; PHA = phytohemagglutinin; PHG = purified horse globulin antigen.](https://academic.oup.com/ps/article-abstract/79/11/1548/1488713/1552/RAFAI-ET-AL)
TABLE 4. Relative weights 1 of bursa of Fabricius and the spleen: macroscopic and histological
findings of the primary and secondary lymphoid organs of ducks 2

<table>
<thead>
<tr>
<th>Dietary T-2 concentrations, mg/kg</th>
<th>Treatment groups</th>
<th>Bursa of Fabricius: lymphocyte depletion</th>
<th>Thymus: lymphocyte depletion</th>
<th>Spleen: lymphocyte depletion</th>
<th>Peyer’s patches: lymphocyte depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1</td>
<td>0.13 ± 0.03</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td>0.69 ± 0.04</td>
<td>0.09 ± 0.02</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>0.09 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.6</td>
<td>4</td>
<td>0.09 ± 0.03</td>
<td>0.09 ± 0.02</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.8</td>
<td>5</td>
<td>0.13 ± 0.04</td>
<td>0.10 ± 0.04</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

1The organ weights were calculated for the metabolic body size of ducks (g/bwt 0.75).
2Figures involve data of all (lost + sacrificed) ducks and indicate the proportion (% of ducks).

EFFECT OF T-2 TOXIN ON PEKIN DUCKS

Metabolic body weight minimized the effect of live weight differences. When the relative weights of the spleen and bursa of Fabricius of control and experimental Mallard ducklings treated with 20 or 30 mg/kg dietary T-2 toxin for 14 and 21 d were compared (Hayes and Wobeser, 1983), no significant effect was found on the relative weights. Kubena et al. (1997) also failed to demonstrate atrophic effects of another trichothecene (deoxynivalenol) in broiler chicks. Indeed, 15 mg/kg dietary deoxynivalenol significantly increased the weight of the bursa of Fabricius relative to body weight on Day 21 of the experiment. Hedman et al. (1995) could find atrophy of neither bursa nor spleen in chickens after feeding up to 12 mg/kg of nivalenol for 20 d.

Only the two highest doses brought about lymphocyte depletion in the lymphoid organs studied (see Table 4). Lymphoid necrosis and depletion of lymphocytes from the spleen, bursa of Fabricius, cecal tonsils, and thymus were reported for chickens fed diets containing ≥50 (Hoerr et al., 1982a) or 1.5, 2.0, 2.5, or 3.0 mg/kg T-2 toxin (Hoerr et al., 1982b).

Altogether, 17 ducks died during the experiment. One control and two experimental ducks (from the 0.2 and 0.6 mg/kg groups) died on Days 38, 46, and 43 of the experiment, respectively. These apparently suffocated, as the autopsy revealed (hemostatic hemorrhage and edema in the lungs). The deaths of another three ducks (two from the 0.6 mg/kg group on Days 40 and 46, and one from the 0.8 mg/kg group on Day 40) were thought to be related to the toxin treatment (autopsy finding: catarrhal enteritis). The mortality in the 3 and 4 mg/kg groups was considerable, with five (on Days 9, 12, 15, 30, and 40) and 6 (on Days 16, 16, 17, 21, 27, and 33) ducks dead, respectively. At autopsy, retarded growth, wasting, and disturbed feathering were found along with necrosis, cruppous-dipteroid inflammation in the oral cavity, and mucosal erosion in the gizzard (Table 5). Histological examination of the affected areas revealed severe vacuolar degeneration in the epithelial cells, hyper- and parakeratosis, acanthosis, and infiltration of lymphocytes, neutrophils, and macrophages in the coreum.

Data of the present investigation show that dietary T-2 toxin can affect the health, performance, and an important element of cellular immune response of growing White Pekin ducks.

Same T-2 toxin has been found in 5, 6, and 36% of barley, wheat, and oat samples, respectively, analyzed in European surveys between 1979 and 1992 (Pettersson, 1995). Concentrations above 1 mg/kg were rare in the samples, but HT-2 toxin normally was found with T-2 toxin in equal or higher concentrations. Unpublished data indicated that 75% of 61 different duck feeds, sent by feed manufacturers to our laboratory in Budapest for analysis, was contaminated with T-2 toxin (range: 0.05 to 0.39 mg/kg; average of contaminated samples: 0.196 mg/kg). In the light of the present investigation and the analytical data referred to above, the chance for field occurrence of intoxication with T-2 toxins alone seems,
however, to be rather low in Hungary. Our experimental data show that only long-term feeding of ≥0.4 mg/kg dietary T-2 toxin may impair the production of growing ducks. In large-scale production systems, in which rations for ducks change at least three times (prestarter, starter, and grower feeds), and several shipments of feed are used during the 49 d of rearing, long-lasting (6 to 7 wk) feeding of diets contaminated with ≥0.4 mg/kg T-2 toxin seems unlikely. However, in the case of backyard farms, where one shipment of feed may last for several weeks, and the low concentrations of T-2 toxin may be combined with other trichothecenes, the chance of intoxication with low doses of T-2 toxin can not be excluded from consideration.

ACKNOWLEDGMENT

This research was supported by the National Committee of Technical Development (Budapest, Hungary). The authors appreciate the assistance of the Committee.

REFERENCES


TABLE 5. Macroscopic and histological findings of ducks that died in the experiment (findings found in % of ducks that died in the experiment)

<table>
<thead>
<tr>
<th>Macroscopic findings</th>
<th>T-2 toxin 3 (mg/kg)</th>
<th>T-2 toxin 4 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retarded growth</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Wasting</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Disturbed feathering</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Necrosis in the oral cavity</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cruprous-diphtheroid inflammation in the oral cavity</td>
<td>40</td>
<td>83</td>
</tr>
<tr>
<td>Mucosal erosion in gizzard</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Atrophy of thymus and bursa of Fabricius</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Oral cavity: coagulating necrosis of epithelial layer and cruprous-diphtheroid inflammation of mucosa</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Thymus: lymphocyte depletion</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bursa of Fabricius: lymphocyte depletion</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Spleen: lymphocyte depletion</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Peyer’s patches: lymphocyte depletion</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>


