Individual and Combined Effects of Fumonisin B1 and Moniliformin on Clinicopathological and Cell-Mediated Immune Response in Japanese Quail

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ABSTRACT A total of 390 one-day-old quail chicks (Coturnix coturnix japonica) were divided into 4 groups (3 replicates per treatment), viz. CX, FX, MX, and FM, containing 75, 105, 105, and 105 birds, respectively. Birds in the control group (CX) were fed quail mash alone, whereas birds in group FX were fed 200 ppm of fumonisin B1 (FB1) from Fusarium verticillioides culture material; group MX was fed 100 ppm of moniliformin (M) from Fusarium fujikuroi culture material; and group FM was fed a combination of 200 ppm of FB1 and 100 ppm of M. Diets were fed from d 1 to 35 to study clinical signs, growth response, serum biochemical changes, and cell-mediated immune response. Birds fed FB1 (FX) showed ruffled feathers and poor growth. Birds in group MX appeared more stunted than those in group FX and exhibited signs of poor feathering and decreased feed and water intake. Clinical signs observed in group FM were more or less similar to those observed in groups FX and MX.

Key words: Fusarium verticillioides culture material, Fusarium fujikuroi culture material, fumonisin B1, moniliformin, Japanese quail

INTRODUCTION

Fumonisin B1 (FB1), a water-soluble Fusarium metabolite, is the most abundant member of the recently discovered group of mycotoxins called fumonisins (Bezuidenhout et al., 1988). The fumonisins were isolated for the first time from the fungus Fusarium moniliforme (now renamed Fusarium verticillioides) in South Africa. A number of fumonisins have since been isolated and characterized, but FB1 remains the most toxic compound (Gelderblom et al., 1991). Fumonisin B1 has been reported to occur naturally in corn and corn screenings at levels as high as 195 and 330 mg of FB1/kg, respectively (Ross et al., 1991). Similar studies carried out by various researchers in India have also found high levels of FB1 in maize, poultry feeds, or both (Shetty and Bhat, 1997; Jindal et al., 1999).

Fumonisin B1, either in naturally contaminated maize or maize-based feeds or in purified form, has been reported to cause equine leukoencephalomalacia (Marasain et al., 1988), porcine pulmonary edema, and hydrothorax syndrome (Harrison et al., 1990). Fumonisin B1 also causes liver toxicity and liver cancer in rats, and atherosclerosis in monkeys (Norred, 1993). Fumonisins have been found to be epidemiologically associated with a high incidence of esophageal cancer in South Africa and China (Rheedar et al., 1992; Yoshizawa et al., 1994). In chicks, FB1 has been associated with poor performance, elevated free sphinganine:sphingosine ratios, increased organ weights, decreased immune responses, and organ lesions (Ledoux et al., 1992; Qureshi and Hagler, 1992; Javed et al., 1993; Weibking et
In poultry rations (including quail). In one of our earlier studies, active investigations on the toxic effects of M in poultry have been initiated only in recent years. Cardiac injury, with alterations in the cardiac electrical conductance, was shown to be a primary cause of mortality in birds (Nagaraj et al., 1996; Reams et al., 1997). Prolonged feeding of M to birds has been shown to cause poor growth performance, increased serum pyruvate levels, and cardiopathy (Ledoux et al., 1993; Morris et al., 1997; Reams et al., 1997). Although M was discovered more than 2 decades ago (Cole et al., 1973), acute mortality and gross lesions, including asites, hydropneumocardium, and myocardial pallor, have been observed in broilers, turkeys, and ducklings (Engelhardt et al., 1989).

Although most research has concentrated on the individual effects of FB1 and M in poultry, these mycotoxins are often present simultaneously in moldy corn samples (Sydenham et al., 1990). It has been shown that the toxicity of some individual mycotoxins can be increased or decreased when they are present as cocontaminants in feed (Huff et al., 1988; Kubena et al., 1988, 1995). Fumonisins B1 and M fed in combination to chicks resulted in an additive negative effect on performance (Javed et al., 1993), whereas in turkey pouls, no synergistic or additive effects were reported for any parameter measured (Bermudez et al., 1997). Poult fed a combination of FB1 and M had lower primary and secondary antibody responses and lower relative thymus weights (Li et al., 2000b). Both renal and cardiac lesions were observed in chickens fed a combination of FB1 and M (Ledoux et al., 2003), but the interactive effects of FB1 and M were not synergistic and were less than additive in nature. Quail farming is increasing in certain parts of India and corn is frequently used up to 50 to 60% in poultry rations (including quail). In one of our earlier studies, the disease process in quail after FB1 feeding was shown to be different from that of chickens and turkeys, as characterized by signs of nervousness (Asrani et al., 2006). The susceptibility of quail to M is not well known. Hence, in the present study the individual and combined effects of FB1 and M were evaluated in Japanese quail.

**MATERIALS AND METHODS**

**Cleaning of Experimental Room and Cages**

Before the arrival of unvaccinated 1-d-old Japanese quail (Coturnix coturnix japonica) in the department, the experimental room, cages, trays, and cage stands were first washed with tap water and then thoroughly cleaned with 2.5% phenol. This cleaning process was repeated 3 times at intervals of 10 to 15 d. After drying in the sunlight, the cages, trays, and stands were flamed with dry heat by using a blowtorch. The waterers and feeders were thoroughly washed with potassium permanganate solution and sun dried. After placing the cages and other accessories in their designated areas in the room, the room was fumigated with formaldehyde gas just 2 d before arrival of the quail chicks. The cages were cleaned daily with 2.5% phenol until the end of the experiment.

**Experimental Birds**

The present study was conducted on 390 one-day-old Japanese quail chicks procured from the Central Poultry Development Organization (Chandigarh, India). The quail chicks used in the present study were from the same breeding flock. Excreta swabs taken on d 1 from 20 randomly selected chicks, before dividing them into various groups, were streaked on brilliant green agar plates and examined for bacterial growth after 48 h of incubation at 37°C. The excreta samples were found to be negative for *Salmonella* on bacteriological examination. The birds were kept under strict hygienic conditions in departmental animal housing throughout the period of the experiment. The animal care and experimental protocol were approved by the university and by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

**Feeding Schedule**

The quail chicks were maintained on chick mash (quail mash procured from Him Vet Feed Industry, Palampur, India) from d 1 until the end of the experiment. Feed was autoclaved for 15 min at 15 pounds per square inch before feeding or mixing with *Fusarium* culture material(s). Boiled and subsequently cooled water was given to the birds throughout the experiment. Feed and water were given ad libitum, and no medication was given during the entire period of the experiment. Before feeding, representative samples of chick mash were submitted to the Animal Feed Analytical and Quality Control Laboratory, Veterinary Hospital Campus (Namakkal, Tamil Nadu, India), and to the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Sciences, CCS Haryana Agricultural University (Hisar, Haryana, India), for analysis of common mycotoxins. The feed samples were found to contain aflatoxins at permissible limits (6 μg/kg) and were free of citrinin, FB1, M, ochratoxin A, T-2 toxin, and zearalenone. The mycotoxins for the present study, FB1 and M, were supplied by *F. verticillioides* M-1325 culture material (FCM) and *Fusarium fujikuroi* M-1214 culture material (MCM), respectively (courtesy of G. E. Rottinghaus, University of Missouri, Columbia, MO). *Fusarium verticillioides* M-1325 culture material containing 6,200 mg of FB1/kg and MCM containing 10,000 mg of M/kg was incorporated at the rate of 3.25 and 1% into the chick mash to supply 200 mg of FB1/kg of feed and 100 mg of M/kg of feed, respectively. The FCM and MCM were not incorporated into the feed of the control group.
Experimental Design

A total of 390 one-day-old Japanese quail chicks were randomly divided into 4 groups, fumonisin B1 alone (FX), moniliformin alone (MX), fumonisin B1 and moniliformin (FM), and chick mash alone (CX), with 105 birds in each group for groups FX, MX, and FM, and with 75 birds in group CX. The present study was conducted with 3 pen replicates of 25 quail per pen in group CX. The present study was conducted with 3 pen replicates of 35 quail per pen in each of the 3 groups FX, MX, and FM, and with 75 birds in group CX. The present study was conducted with 3 pen replicates of 35 quail per pen in each of the 3 groups FX, MX, and FM, and with 75 birds in group CX. Although the duration of the experiment was up to 35 d postfeeding (DPF), clinical signs, growth response, and serum biochemical changes were studied only up to 28 DPF because it was thought that the application of 1-chloro-2,4-dinitrobenzene (DNCB) for the contact hypersensitivity test at 28 DPF and onward might interfere with some clinical parameters. The various dietary treatments, starting from d 1 until 35 d in different groups, are presented in Table 1.

Clinical Findings, Growth Response, and Serum Chemistry

The birds in all the groups were closely observed, at least 3 times daily, for the development of clinical signs and mortality. To see the individual and combined effect of FB1 and M on BW, 9 randomly selected quail from each treatment group (3 quail per replicate) were weighed at 0, 7, 14, 21, and 28 DPF. After weighing, 2 to 3 mL of blood was collected (at 7, 14, 21, and 28 DPF) via cardiac puncture from each of these birds for determination of serum aspartate transaminase (AST), alanine transaminase (ALT), total serum proteins (TSP), albumin, cholesterol, and creatinine. Determination of lactate dehydrogenase (LDH) and creatine kinase (CK) was done only at 28 DPF. All the biochemical determinations were done by using diagnostic kits (Bayer Diagnostics India Ltd., Baroda, India) in a fully automated Blood Chemistry Analyzer (RA-50 Auto Chemistry System, Bayer Diagnostics) according to the manufacturer’s recommendations. The birds were killed after collection of blood samples at each of the aforementioned intervals.

Contact Hypersensitivity Test

Thirty-six birds from each group (CX, FX, MX, and FM) were subjected to contact hypersensitivity testing, which was assessed by skin hypersensitivity reaction to DNCB. At 28 DPF, birds were first sensitized with DNCB (SRL Sisco Research Lab., Mumbai, India) and subsequently challenged 1 wk later (i.e., at 35 DPF). During this time, birds were continued on their respective dietary treatments. Birds were sensitized by painting 100 µL of 2.5% DNCB dissolved in acetone (4:1) vehicle on one side of the abdominal skin. The challenge was performed by applying 20 µL of 1% DNCB on the other side of the abdominal skin 7 d after sensitization. Before challenge, skin thickness was measured in individual birds from each of the treatment groups. After challenge, the degree of contact hypersensitivity was determined by measuring the change in thickness of the skin caused by swelling. Skin thickness was measured at 1, 2, 3, 6, 12, 24, 48, and 72 h after challenge by using a vernier caliper (Hauptner, Solingen, Germany) on 9 birds from each group (3 quail per replicate). After measuring the skin thickness, 3 birds from each of the treatment groups (CX, FX, MX, and FM; 1 quail per replicate) were euthanized by cervical dislocation at 0 (before challenge), 1, 2, 3, 6, 12, 24, 48, and 72 h after challenge, and pieces of skin tissue were collected in 10% neutral-buffered formalin for histological studies. The percentage increase in skin thickness was calculated by using the following formula to indicate the degree of swelling:

\[
\text{Skin swelling} = \frac{[\text{skin thickness (mm) after challenge} - \text{skin thickness (mm) before challenge}]}{\text{skin thickness (mm) before challenge}} \times 100.
\]

Bacteriological Isolations

Sections of liver tissue from a few dead and killed birds from each of the treatment and control groups at different intervals were subjected to bacteriological isolations in selenite broth and brilliant green agar plates.

Statistical Analysis

Data were subjected to statistical analysis for drawing inferences by using a standard procedure (Snedecor and Cochran, 1967). Treatment means were compared by using Duncan’s multiple range test (2-way ANOVA) to determine the effect of treatment, age, and their interactions (Duncan, 1955). All levels of significance were based on the 95% level of probability.

Table 1. Dietary treatments in different groups of quail chicks from d 1 to 35

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total level of culture material(s) used (%)</th>
<th>Level of mycotoxin(s) supplied (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX</td>
<td>Chick mash alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FX</td>
<td>Fumonisin B1 alone</td>
<td>3.25</td>
<td>200</td>
</tr>
<tr>
<td>MX</td>
<td>Moniliformin alone</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>FM</td>
<td>Fumonisin B1 and moniliformin</td>
<td>4.25</td>
<td>200 fumonisin B1 + 100 moniliformin</td>
</tr>
</tbody>
</table>
RESULTS

Clinical Signs

None of the birds from the CX group exhibited any clinical signs throughout the course of the experiment. In group FX, clinical signs were detected as early as 3 d after feeding of FB1 began, when nearly 5% of the birds were seen passing dark tan-colored semisolid excreta. Occasionally, droppings revealed mild to moderate levels of mucus. Subsequently, the percentage of birds exhibiting diarrhea increased rapidly, and by 2 wk post-FCM feeding, a large number of birds had shown increased mucus contents in the droppings during cleaning operations of the cage floor. Although the number of birds exhibiting mucus diarrhea reduced thereafter, the excreta continued to have a pasty consistency until the end of the experiment. In addition, clinical signs of ruffled feathers and stunted growth were observed in almost all birds from 1 wk onward. Occasionally, birds were found sitting on their hocks. In general, the clinical signs in group MX, discernible as early as 2 d post-MCM feeding, were similar to those observed in group FX, except that the diarrhea in group MX revealed a more watery consistency and was observed in a majority of birds up to the second week, and mucus was occasionally seen in the droppings. The diarrheic excreta also appeared whitish in color. The number of diarrheic birds reduced significantly thereafter but fluffy, cotton-like, white, mild pasty droppings, which appeared to be predominantly urates, were recorded from the third week onward. Clinical signs of poor body growth, ruffled feathers, and low feed and water intake were observed consistently throughout the experiment in this group. Although feed consumption was not measured in the present study, daily observations revealed less feed intake by birds fed MCM, FCM, or both. Droopiness of wings was observed in nearly 10% of the birds. The birds in group FM revealed signs more or less similar to those observed in groups FX and MX. Although there was no significant difference in the severity of diarrhea when compared with other groups (FX and MX), feed refusal was comparatively higher in this group, as detected during cleaning of cage floors. Diarrhea was recorded as early as 2 d post-FCM and post-MCM feeding, when a few birds (nearly 5%) were seen passing slightly loose excreta, which appeared dark in color. From d 4 onward, droppings appeared watery, with increased mucus content. Subsequently, after 2 wk, excreta appeared to have a semisolid consistency, with slight mucus seen occasionally and, in some cases, with a whitish tinge. On d 4, a typical posture of resting against the sternum and raising the posterior was seen in some birds, but this was not a consistent feature. The clinical signs of hyperexcitation and ataxia with backward motion to gain balance were observed in only 2 birds in the second week.

Mortality

No mortality was observed in quail of group CX throughout the experiment. Mortality was observed in all other treatment groups (FX, MX, and FM; Figure 1). In group FX, mortality started as early as 3 DPF. Of the 3 birds that died during the first week of the experiment, 2 birds died on d 3 and 1 died on d 4 post-FCM feeding. A total mortality of 2.8% was recorded in the first week, followed by 0.9% in the second week. The mortality in this group was highest (6.6%) during the third week, followed by 1.9% in the last week of the experiment. The overall mortality throughout the course of the experiment in group FX was 12.3%. In group MX, mortality started as early as 2 DPF. A mortality of 4.7% was recorded during the first week, followed by 2.8% in the second week. No mortality was recorded in this group after 11 d of M feeding. The overall mortality throughout the course of the experiment in group MX was 7.6%. In group FM, the highest mortality (12.4%) was recorded during the first week of the experiment and began as early as 2 DPF. Out of a total of 13 birds that died during the first week, 1 bird each died on d 2 and 3, 7 birds died on d 4, and 4 birds died on d 5 post-FCM and post-MCM feeding. A mortality figure of 1.9% was observed in group FM during the second week, followed by 3.8% in the third week and 2.9% in the last week of the experiment. The overall mortality was 20.9% (22 birds), which was higher than the groups fed either FB1 or M alone.

Growth Response

The mean BW of birds at different intervals among groups CX, FX, MX, and FM are shown in Table 2. A steady increase in BW of quail was recorded in all 4 groups throughout the period of the experiment. However, the increase in BW was found to be significantly lower in groups FX, MX, and FM in comparison with group CX. By 28 DPF, BW was only 74.3, 65.1, and 60.9% that of group CX in groups FX, MX, and FM, respectively. Although the mean BW in group FM was found to be lower as compared with groups FX and MX from 14 DPF onward, the difference was significant (P ≤ 0.05) only at 28 DPF when compared with group FX only. A significant difference in the mean BW was observed between groups FX and MX at 7 DPF only; thereafter, the values were more or less comparable. A mean treatment effect at the end of the experiment revealed a significant decrease in BW of quail fed FB1 or M, either alone or in combination, when compared with group CX. The interaction between the different treatments and the age of the quail was also highly significant (P ≤ 0.01).

Serum Biochemistry

The mean TSP, albumin, and cholesterol concentrations in the sera of Japanese quail from different groups are given in Table 3. In general, the TSP values increased with advancing age of the birds in almost all groups. Although mean serum protein values were higher in groups FX, MX, and FM as compared with group CX at almost all intervals, the difference was only statistically significant (P ≤ 0.05).
in the combination group FM at 7 and 14 DPF, and in groups MX and FX at 7 and 21 DPF, respectively.

In general, there was an increase in albumin concentration in all mycotoxin treatment groups (FX, MX, and FM), although not all treatment groups differed significantly from group CX at all time intervals evaluated. The mean albumin concentration was significantly higher ($P \leq 0.05$) in group FX from 14 DPF onward when the values were compared with group CX. In the combination group FM, the mean albumin concentration tended to decrease with advancing age, whereas the serum albumin of group FX tended to increase up to 21 DPF. The mean albumin concentration value of group FM was significantly higher ($P \leq 0.05$) than those of groups CX and FX at 7 DPF, and from that of group MX at 14 DPF, whereas it was significantly lower than that of group FX at 21 DPF. The overall treatment effect at the conclusion of the experiment across the age of birds indicated significantly higher values for all treatment groups (FX, MX, and FM) compared with group CX. However, differences in albumin values among treatment groups were not significant.

The serum cholesterol values throughout the study were generally higher in the treatment groups (FX, MX, and FM) when compared with those of group CX. The data clearly showed that mean cholesterol values were significantly higher ($P \leq 0.05$) in groups FX and FM throughout the period of study as compared with those in groups CX and MX. Although serum cholesterol levels were higher in group MX as compared with group CX from 14 DPF throughout the experiment, differences were not statistically significant. The mean age effect was a significant increase in serum cholesterol values up to 21 DPF, followed by a decrease at 28 DPF. The interaction between age and treatment was significant ($P \leq 0.01$) for both albumin and cholesterol.

Mean values of ALT, AST, and creatinine for each of the experimental groups are presented in Table 4. In general, mean ALT activity in groups FX and FM was higher than that in groups CX and MX throughout the course of the experiment, but the difference in values was significantly higher ($P \leq 0.05$) only in group FM at 7 DPF and in groups FX and FM at 14 and 21 DPF. The addition of

### Table 2. Effects of fumonisin B1 and moniliformin, supplied by *Fusarium verticillioides* and *Fusarium fujikuroi* culture material respectively, on body weight (g) in Japanese quail

<table>
<thead>
<tr>
<th>Group</th>
<th>Days postfeeding</th>
<th>Mean treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>CX</td>
<td>$8.52 \pm 0.17^a$</td>
<td>$29.66 \pm 0.62^a$</td>
</tr>
<tr>
<td>FX</td>
<td>$8.55 \pm 0.17^a$</td>
<td>$25.00 \pm 1.92^b$</td>
</tr>
<tr>
<td>MX</td>
<td>$8.55 \pm 0.24^c$</td>
<td>$19.67 \pm 1.84^c$</td>
</tr>
<tr>
<td>FM</td>
<td>$8.66 \pm 0.27^c$</td>
<td>$23.22 \pm 1.71^bc$</td>
</tr>
<tr>
<td>Mean age effect</td>
<td>$8.57 \pm 0.11^E$</td>
<td>$24.39 \pm 0.98^{D}$</td>
</tr>
<tr>
<td>Age x treatment effect</td>
<td>$7.57^{16}$</td>
<td></td>
</tr>
</tbody>
</table>

*Values within columns (between groups CX, FX, MX, and FM) with different superscripts are significantly different by ANOVA ($P \leq 0.05$).

$^a$–$^c$Values within a column with different superscripts showing mean treatment effect are significantly different by ANOVA ($P < 0.01$).

$^A$–$^E$Values within a row with different superscripts showing mean age effect are significantly different by ANOVA ($P \leq 0.05$).

$^{16}$Statistically significant F-value indicating the interaction between different treatments and age of quail chicks (HS = highly significant) by ANOVA ($P \leq 0.01$).

1Data are means ± SE of 3 replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B1; MX = birds fed moniliformin; FM = birds fed fumonisin B1 and moniliformin.
Mean values of LDH and CK for each of the experimental groups observed at 7, 14, 21, and 28 days postfeeding are given in Table 5. Mean LDH values were significantly higher \((P \leq 0.05)\) in all 3 mycotoxin groups (FX, MX, and FM) as compared with group CX. The mean value in group FM was comparable to those in groups FX and MX. Mean CK values were significantly higher \((P \leq 0.05)\) in groups FX and MX as compared with those in groups CX and FM.

**Cell-Mediated Immune Response**

**Skin Thickness.** The mean percentage increase in skin thickness recorded in quail from each of the experimental groups at 1, 2, 3, 6, 12, 24, 48, and 72 h after challenge with DNCB is presented in Table 6. In group CX, there was a marked increase in skin thickness up to 3 h, followed by a plateau phase up to 12 h. The maximum thickness was recorded at 24 h, followed by a reduction in thickness at 48 and 72 h postchallenge. In group FX, skin thickness continued to increase gradually up to the end of the observation period, that is, 72 h postchallenge. In group MX, a gradual increase in skin thickness was observed up to 6 h, and it persisted with the same intensity up to 12 h. Another increase in skin thickness was observed in this group at 24 h, which persisted with the same intensity until the end of the experiment. In group FM, skin thickness increased gradually up to 24 h postchallenge. It then decreased slightly at 48 h but showed a peak response at 72 h postchallenge. When mean values were compared among treatment groups, the percentage increase in skin thickness in group FX was significantly lower \((P \leq 0.05)\) compared with group CX at 1, 2, 3, and 24 h, and was lower compared with group MX throughout the study except at 3 and 12 h. The values of group FM fell between those of groups FX and MX but appeared significantly higher \((P \leq 0.05)\) than those of groups CX and FX only at 72 h postchallenge. The mean treatment effect at the conclusion of the experiment. Compared with group CX at 1, 2, 3, 6, 12, 24, 48, and 72 h after challenge with DNCB is presented in Table 6. In group CX, there was a marked increase in skin thickness up to 3 h, followed by a plateau phase up to 12 h. The maximum thickness was recorded at 24 h, followed by a reduction in thickness at 48 and 72 h postchallenge. In group FX, skin thickness continued to increase gradually up to the end of the observation period, that is, 72 h postchallenge. In group MX, a gradual increase in skin thickness was observed up to 6 h, and it persisted with the same intensity up to 12 h. Another increase in skin thickness was observed in this group at 24 h, which persisted with the same intensity until the end of the experiment. In group FM, skin thickness increased gradually up to 24 h postchallenge. It then decreased slightly at 48 h but showed a peak response at 72 h postchallenge. When mean values were compared among treatment groups, the percentage increase in skin thickness in group FX was significantly lower \((P \leq 0.05)\) compared with group CX at 1, 2, 3, and 24 h, and was lower compared with group MX throughout the study except at 3 and 12 h. The values of group FM fell between those of groups FX and MX but appeared significantly higher \((P \leq 0.05)\) than those of groups CX and FX only at 72 h postchallenge. The mean treatment effect at the conclusion of the experiment.
ment across the time period revealed a significantly lower thickness response in group FX as compared with the other groups (CX, MX, and FM). Likewise, the thickness response in group MX as compared with the other groups (CX, FX, and FM) was significantly higher. The mean age effect among different treatments revealed a significant (CX, FX, and FM) was significantly higher. The mean age effect among different treatments revealed a significant increase in percentage thickness up to 24 h postchallenge.

**Histological Changes in Skin.** Before the challenge (0 h), no inflammatory cells or exudate was noticed in any of the 4 treatment groups.

**After Challenge.** Up to 6 h, in groups CX and MX the increase in skin thickness (evident as early as 1 h) was due to the accumulation of large amounts of serous exudate and heterophils just below the epithelium. Blood vessels appeared prominent and congested, and hemorrhages were noticed in some places. Lymphocytes started appearing as early as 2 h after challenge and areas close to blood vessels and below the epithelium in both of these groups showed heavy infiltration with lymphocytes. At 3 h postchallenge, there was massive serofibrinous exudate and infiltration mainly with heterophils, but the number of lymphocytes increased significantly in subsequent stages in both of these groups. In contrast, groups FX and FM revealed only slight exudate and a mild inflammatory response with heterophils as the main inflammatory cells up to 6 h, although mild aggregations of lymphocytes could be seen close to blood vessels as early as 2 h postchallenge.

From 12 to 24 h, in comparison with the previous stage, the thickness of the skin in group CX was mainly attributed to an increase in the number of lymphocytes and macrophages. Lymphoid nodules were present in massive numbers throughout. However, a mild serofibrinous exudate was also observed along with occasional infiltration of heterophils. On the other hand, serofibrinous exudate infiltrated with a massive number of heterophils contributed greatly toward the increase in skin thickness in group FX. The mononuclear inflammatory cell response was poor as compared with group CX at this stage. In group MX, skin response was found to be more cellular and less exudative in all birds at this stage. The mononuclear cell response in group MX was comparatively higher than in group FX at 12 h, whereas it appeared to be more or less comparable to group FX at 24 h. A mild serofibrinous exudate was evident along with scattered heterophils immediately below the epithelium only. In contrast to groups CX, FX, and MX, the increase in skin thickness in group FM was found to be mainly due to excessive accumulation of serofibrinous exudate. A large number of heterophils were observed.

### Table 4. Effects of fumonisin B₁ and moniliformin, supplied by *Fusarium verticillioides* and *Fusarium fujikuroi* culture material, respectively, on serum alanine transaminase, aspartate transaminase, and creatinine levels in Japanese quail

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days postfeeding</th>
<th>Mean treatment effect</th>
<th>Age × treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Alanine transaminase (IU/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>17.44 ± 0.86abc</td>
<td>19.00 ± 1.78b</td>
<td>15.33 ± 1.14b</td>
</tr>
<tr>
<td>FX</td>
<td>20.55 ± 1.32ab</td>
<td>26.33 ± 2.10a</td>
<td>23.33 ± 1.19a</td>
</tr>
<tr>
<td>MX</td>
<td>16.55 ± 1.08b</td>
<td>19.22 ± 1.41b</td>
<td>15.00 ± 1.30b</td>
</tr>
<tr>
<td>FM</td>
<td>22.22 ± 1.73a</td>
<td>37.17 ± 3.11a</td>
<td>25.44 ± 2.87a</td>
</tr>
<tr>
<td>Mean age effect</td>
<td>19.19 ± 0.73b</td>
<td>24.08 ± 1.38a</td>
<td>19.78 ± 1.16b</td>
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<tr>
<td>Aspartate transaminase (IU/L)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CX</td>
<td>242.22 ± 6.69ab</td>
<td>213.44 ± 19.92b</td>
<td>268.44 ± 21.87b</td>
</tr>
<tr>
<td>FX</td>
<td>289.88 ± 23.26ab</td>
<td>259.88 ± 17.11b</td>
<td>316.55 ± 14.83b</td>
</tr>
<tr>
<td>MX</td>
<td>322.11 ± 22.51a</td>
<td>354.44 ± 25.48a</td>
<td>301.22 ± 24.53a</td>
</tr>
<tr>
<td>FM</td>
<td>290.11 ± 16.34ab</td>
<td>266.17 ± 14.86b</td>
<td>323.91 ± 15.42a</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>0.34 ± 0.01a</td>
<td>0.31 ± 0.02a</td>
<td>0.56 ± 0.02a</td>
</tr>
<tr>
<td>FX</td>
<td>0.33 ± 0.02a</td>
<td>0.31 ± 0.01b</td>
<td>0.58 ± 0.02a</td>
</tr>
<tr>
<td>MX</td>
<td>0.32 ± 0.02a</td>
<td>0.38 ± 0.01a</td>
<td>0.53 ± 0.02a</td>
</tr>
<tr>
<td>FM</td>
<td>0.34 ± 0.01a</td>
<td>0.31 ± 0.02b</td>
<td>0.55 ± 0.03a</td>
</tr>
<tr>
<td>Mean age effect</td>
<td>0.33 ± 0.01c</td>
<td>0.32 ± 0.01c</td>
<td>0.55 ± 0.01a</td>
</tr>
</tbody>
</table>

a,b,cValues within a column with different superscripts are significantly different by ANOVA (P ≤ 0.05).

### Table 5. Effects of fumonisin B₁ and moniliformin, supplied by *Fusarium verticillioides* and *Fusarium fujikuroi* culture material, respectively, on serum lactate dehydrogenase and creatine kinase levels in Japanese quail at 28 DPF

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactate dehydrogenase</th>
<th>Creatine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX</td>
<td>588.66 ± 80.19a</td>
<td>158.68 ± 39.83a</td>
</tr>
<tr>
<td>FX</td>
<td>1,520.57 ± 111.29a</td>
<td>372.67 ± 50.86a</td>
</tr>
<tr>
<td>MX</td>
<td>1,042.79 ± 171.25b</td>
<td>487.00 ± 77.91a</td>
</tr>
<tr>
<td>FM</td>
<td>1,334.44 ± 195.79ab</td>
<td>212.66 ± 18.18b</td>
</tr>
</tbody>
</table>

a,bValues within columns (between groups CX, FX, MX, and FM) with different superscripts are significantly different by ANOVA (P ≤ 0.05).

Data are means ± SE of 3 replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B₁; MX = birds fed moniliformin; FM = birds fed fumonisin B₁ and moniliformin.
### Table 6. Mean percent increment in skin thickness at various intervals post-1-chloro-2,4-dinitrobenzene (DNCB) challenge in various groups of Japanese quail

<table>
<thead>
<tr>
<th>Hours post-DNCB challenge</th>
<th>Mean treatment effect</th>
<th>Age × treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CX</td>
<td>72.66 ± 16.30[^a^]</td>
</tr>
<tr>
<td></td>
<td>MX</td>
<td>74.11 ± 17.12[^b]</td>
</tr>
</tbody>
</table>

[^a^] Values within columns between groups (CX, FX, MX, and FM) with different superscripts are significantly different by ANOVA (P ≤ 0.05).

[^b^] Values within a column showing mean treatment effect with different superscripts are significantly different by ANOVA (P ≤ 0.05).

[^c^] Values within a row showing a mean age effect with different superscripts are significantly different by ANOVA (P ≤ 0.05).

[^NS^] Value indicating interaction between different treatments and age of quail chicks (NS = nonsignificant) by ANOVA (P ≤ 0.01).

1 Data are means ± SE of 3 replicate pens of 3 quail each. CX = birds fed quail mash alone; FX = birds fed fumonisin B 1; MX = birds fed moniliformin; FM = birds fed fumonisin B1 and moniliformin.
study suggests that M has some damaging effects on the kidneys. Kidney lesions of varying severity have been reported in M-fed broiler chicks (Ledoux et al., 1995). In an experimental study on 21-d-old broilers fed 546 ppm of FB₁, 98 ppm of FB₂, and 367 ppm of M for 7 d, Javed et al. (1993) observed whitish diarrhea that appeared to be predominantly urates and bile in several birds within hours of feeding. Clinical signs of ruffled feathers and stunted growth were observed in almost all the birds fed with culture material-amended diets, either alone or in combination, from 1 wk onward and were consistent with previous studies (Javed et al., 1993; Ledoux et al., 1996; Bermudez et al., 1997; Li et al., 2000b; Asrani et al., 2006). In contrast, Weibking et al. (1993a,b) and Bermudez et al. (1997) showed that feed intake and BW gains of chicks and poulets were not affected by dietary FB₁ at levels less than 325 mg of FB₁/kg of feed. However, in the study by Bermudez et al. (1997), both feed intake and BW gain were significantly reduced in turkey poult fed rations containing 100 mg of M/kg. Reductions in feed intake and BW gain were also reported in previous studies with chicks, poult, and ducklings fed 100 mg of M/kg of diet (Javed et al., 1993; Ledoux et al., 1993, 1995; Morris et al., 1997). Reams et al. (1997) reported significant reductions in the BW of birds receiving 80 to 330 mg/kg of M by 8 d. In the present study, feed refusal was comparatively more severe in groups fed either MCM alone or in combination with FC as compared with the group fed FC alone, which implies that MCM feeding is specifically responsible for feed refusal, because FB₁-fed birds had normal feed intake. Although BW in the MCM-fed and combination groups were lower than in the group fed FC alone, no additive or interactive effects were evident between FB₁ and M. In an experimental study using purified FB₁ and M, Javed et al. (1993) also observed that M-fed groups had lower weight gains than FB₁-fed groups. Decreased BW in MCM and the combination group in the present study could be the result of greater feed refusal because of the presence of M in the diet (Li et al., 2000a). Reductions in BW in the FC-fed group may be linked to a decreased efficiency of feed utilization (Kubena et al., 1997), which was most likely associated with a disruption in sphingolipid biosynthesis by FB₁ (Wang et al., 1991). These sphingolipids are involved in the regulation of cell surface receptors, ion pumps, and other systems vital for cell function and survival (Merrill et al., 1996). The presence of diarrhea and intestinal lesions (goblet cell hyperplasia) may have been additional factors contributing to the reduction in BW in all the culture material-amended groups.

Mortality was found to be highest in quail fed FC alone during the third week. None of the earlier studies has reported on mortality in either broiler chicks or turkey poult caused by feeding FC supplying varying levels of FB₁ (Brown et al., 1992; Weibking et al., 1993b; Ledoux et al., 1996). Mortality of 12.3% was recorded in the present study in the group fed FC alone, which suggests a higher susceptibility of quail to the toxic effects of FB₁ when compared with broiler chicks and turkeys. Previous studies have also shown mortality in quail depending on the level of FC incorporated into the diet. A mortality of 2.25% was observed in quail when FC was incorporated at a level of 2.5% (supplying 150 ppm of FB₁) in the ration from d 5 of age (Deshmukh et al., 2005a), whereas mortality was 5% when FC was added at a level of 5% (supplying 300 ppm of FB₁) in the diet from d 1 (Asrani et al., 2006). Mortality in the group fed MCM alone was observed only up to 11 DPF, and it was highest during the first week, which is consistent with an earlier report by Engelhardt et al. (1989). An overall mortality of 7.6% was observed in the group fed MCM alone. Ledoux et al. (1995) observed 13 and 83% mortality in broiler chicks fed MCM supplying 150 and 300 mg of M/kg, respectively. Javed et al. (1993) reported 40 and 70% mortality, respectively, in chicks fed 27 and 154 mg of pure M/kg. The lower mortality figure can be ascribed to the fact that the source of M in the present study was F. fujikuroi culture material used at a level of 1%, which supplied 100 mg of M/kg of diet. The higher mortality observed by Javed et al. (1993) might be linked to the use of pure M in their studies. It is possible that pure M has a greater bioavailability than the M present in culture material. In culture material, a certain amount of M will be physically trapped in the undigested culture material, and this M has no opportunity to be absorbed by the chick. The overall mortality (20.95%) was found to be highest in the combination group (FCM + MCM) as compared with the groups fed either FC or MCM alone. Like MCM alone, the mortality in the combination group was also highest (12.4%) during the first week, in contrast to the mortality caused by FB₁ in the present study, which was highest in the third week. Results of a previous study indicating that M may be more toxic than FB₁ to young broilers (Javed et al., 1993) suggest that high mortality in the combination group in the first week might possibly be due to the presence of M in the diet. A greater reduction in feed intake, as observed in the present study in the combination group and the group fed MCM alone, may also partially explain the lower mortality in these groups in the later stages because chicks with reduced feed intake would have ingested less M (Ledoux et al., 1995). A reduction in mortality after 7 DPF in the MCM-fed group may also be linked to the development of cell-mediated immune response because M feeding in the present study did not seem to suppress the immune response and the percentage skin thickness values were also more or less comparable to those of the control group (CX) throughout the duration of experiment. A detailed investigation on this aspect merits serious consideration. In the present study, the interaction between FB₁ and M indicated additive effects in terms of mortality. The histopathological examination of mortality during the first week from these groups (results not presented) clearly suggested that the presence of M in the diet led to severe heart lesions in the combination group, but the presence of FB₁ did not appear to lead to severe liver lesions in the combination group. In previous studies, FB₁ has been shown to be associated with cardiac lesions of mild intensity in turkey poult (Ledoux et al., 1996). The muscle-specific serum chemistry in the present study also suggests that higher levels of CK in the group fed ...
FCM alone may indicate that FB1 does alter heart function, whereas unaltered levels of serum ALT throughout the period of the experiment in the group fed MCM alone may suggest that M is probably not hepatotoxic. The results of the present study thus suggest that in young quail, the cardiotoxic effect of M may be enhanced in the presence of FCM and might eventually lead to high mortality. Similarly, Javed et al. (1993) indicated additive effects in terms of mortality and the onset of clinical signs in chickens fed FB1 and M. In their studies, a mortality of 37% was observed in chicks fed a combination of 61 mg of FB1/kg and 66 mg of M/kg. The higher mortality in broiler chicks in their studies could have been because these authors used a higher amount of culture material (5.1 to 45.5%) in their diets compared with the amounts (1.0 to 4.25% culture material) used in the present study, or may indicate a difference in mycotoxin toxicity between quail and chickens.

Biochemical parameters revealed a significant variation between quail fed chick mash alone and those given culture material-amended diets. Total serum protein values were higher in the groups fed MCM, either alone or in combination with FCM, compared with the group fed FCM alone up to 14 DPF, and this phase coincided with the period during which diarrhea was more severe in all treatment groups. During this period, maximum mortality was observed in the combination group and the group fed MCM alone, and the birds in both groups suffered an acute phase of the disease, with heavy mortality within this period. Hence, an increase in TSP with comparatively higher values in the combination group may be attributed to dehydration or to the synthesis of acute phase proteins that occurs 2 to 5 d after tissue injury, inflammation, or necrosis (Duncan et al., 1994). An increase in TSP could also have been caused by an increase in the albumin fraction, the values for which were higher in the combination group as compared with the other groups up to 14 DPF, and this might have occurred concurrently with an increase in globulin concentration during dehydration (Duncan et al., 1994). Both TSP and albumin values were higher in the group fed FCM, either alone or in combination with MCM, compared with the group fed MCM alone after 14 DPF, and this phase coincided with the period during which mortality was higher in the group fed FCM alone; the inflammatory and necrotic lesions in the liver were also severe in this group from 14 DPF onward. Total serum protein and albumin values in the combination group from 14 DPF onward were, however, between those of the groups fed either FCM alone or MCM alone, which could be attributed to reduced feed intake and could have been caused by the mycotoxins. An increase in TSP as a result of feeding FB1 or M-amended diets has also been reported previously (Bermudez et al., 1996, 1997; Asrani et al., 2006).

Serum ALT and cholesterol levels were found to be significantly higher in the groups fed FCM alone and in the combination group as compared with the other 2 groups. Decreases in serum cholesterol have been reported earlier in FB1-fed chickens (Weibking et al., 1993a) and turkeys (Weibking et al., 1993b), whereas the values remained unchanged in ducklings (Bermudez et al., 1995). In quail, however, serum cholesterol levels have been shown to be significantly higher following FB1 feeding, and levels remained elevated throughout the experiment period (Asrani et al., 2006). Increases in the levels of serum cholesterol and ALT in the group fed FCM alone or the combination group at all intervals in the present study could be due to hepatic effects of FB1, which is characterized by severe hepatic lesions at 150 mg of FB1/kg of diet (Deshmukh et al., 2005b). The results of the present study clearly suggest that increases in the levels of serum cholesterol and ALT in the combination group were possibly the result of the presence of FCM in the diet because these biochemical parameters remained unchanged in the group fed MCM alone. In a previous study, serum cholesterol levels also remained unchanged with feeding of 100 mg of M/kg to turkeys (Bermudez et al., 1997).

Serum AST, LDH, and CK activity were found to be higher in both the groups fed FCM or MCM alone and in the combination group compared with the group fed chick mash only (CX). Increases in serum AST activity have been observed previously in FB1-fed broiler chicks (Ledoux et al., 1992), turkeys (Weibking et al., 1993b; Bermudez et al., 1997), and quail (Asrani et al., 2006), and in M-fed chicks (Javed et al., 1995) and turkeys (Bermudez et al., 1997). Significant increases in LDH have also been reported earlier in FB1- and M-fed chickens (Javed et al., 1995) and turkeys (Bermudez et al., 1997). Significant increases in CK activity in the groups fed FCM and MCM alone suggest that both FB1 and M are toxic to striated or cardiac muscle. The higher values of LDH and AST in the group fed FCM alone compared with the group fed MCM alone probably reflect significant liver lesions in the presence of FCM because the ALT values remained unchanged in the group fed MCM alone. The only noticeable observation from the AST results was an indication of an interaction between FB1 and M at 14 DPF, which appeared to be less than additive in nature because the AST values were significantly higher in the combination group than in either the group fed FCM or the group fed MCM alone. After 14 DPF, the values of these biochemical parameters (AST, LDH, and CK) in the combination group were either lower or in between those of groups fed either FCM or MCM alone. The lower values in the combination group compared with the group fed FCM alone could possibly be linked to the greater reduction in feed intake caused by the incorporation of MCM because the quail with reduced feed intake from the third week onward would have ingested less FB1 and M.

Serum creatinine values were significantly higher in the combination group and in the group fed MCM alone only at 28 DPF, which can be attributed to kidney damage, possibly caused by the presence of M in the diet, because no change in creatinine values was observed in the group fed FCM alone as compared with quail in the CX group.
The whitish-colored excreta, which appeared to have an increased urate content in the latter stages of the experiment, suggested involvement of the kidneys in the group fed MCM alone and in the combination group. Kidney lesions of varying severity have been reported earlier in M-fed broiler chicks (Ledoux et al., 1995) and in chickens fed a combination of FB1 and M (Javed et al., 2005).

In the present study, a delayed type of hypersensitivity reaction was used to study the acquired cellular immune response in vivo. Skin hypersensitivity is an immunological reaction in which antigens cause a release of lymphokines that mediate an inflammatory response, and the extent of inflammation gives the status of cell-mediated immune response. In the current study, groups fed chick mash alone and MCM alone revealed maximum skin thickness at 24 h postchallenge, and the inflammatory response was more or less comparable. Histological studies of these 2 groups revealed massive inflammatory edema in the early stages, usually accompanied by heterophils, but within 24 h, there was the presence of a large number of lymphocytes and macrophages. There was no indication of suppressed cellular immune response by feeding M to quail in the present study; rather, the mononuclear inflammatory response at 12 h postchallenge for the M group was greater compared with all other treatment groups. Results of previous studies, however, have shown a reduction in lymphoid organ weights (bursa, spleen, and thymus) when diets containing varying levels of M were fed to chicks and turkeys (Ledoux et al., 1995; Qureshi et al., 1995; Bermudez et al., 1997; Li et al., 2000a). Decreased bursal weights have been correlated with a reduction in humoral immune response, and broiler chicks and turkey pouls fed 100 mg of M/kg of diet revealed significantly lower secondary anti-Newcastle disease virus antibody titers compared with control birds (Li et al., 2000a,b). Significant reductions in white blood cell counts and numbers of viable lymphocytes in earlier studies with chicks fed diets containing M suggested a suppression of cell-mediated immune response in the presence of M (Dombrink-Kurtzman et al., 1993; Javed et al., 1995). However, no immunosuppressive effect of M was evident in the present study. Similarly, the results of a feeding trial conducted by Li et al. (2000a) in broiler chicks showed that lymphocyte proliferation was not affected by 100 mg of M/kg of diet, and the histological changes in spleen, thymus, and bursa in the present studies in the group fed MCM alone did not provide any evidence of lymphoid cell depletions in these organs (data not presented). In comparison with groups fed chick mash alone and MCM alone, both the FCN-fed group and the combination group revealed a gradual increase in skin thickness up to 72 h, and histological studies suggested that the mononuclear inflammatory response was poor until 72 h postchallenge, which could possibly explain the delayed cellular response in these groups. The values for percentage skin thickness and histological response in the combination group were found to fall between those of MCM alone (higher) and FCN alone (lower), which suggested that the poor cell-mediated immune response was possibly due to the presence of FCN (FB1) in the diet. Earlier studies have shown significantly lower lymphocyte proliferation responses to mitogens in calves (Osweiler et al., 1993) and chicks (Li et al., 1999) fed FB1. In a previous study, concanavalin A-stimulated proliferation in poult fed 200 mg of FB1/kg was reduced by 22%, which suggested that T-cell proliferation in poult fed FB1 was affected (Li et al., 2000b). The low blood lymphocyte counts reported in our recent study with quail chicks also support this finding (Deshmukh et al., 2005a). The mechanism of action of FB1 has been demonstrated to involve the disruption of complex sphingolipids and accumulation of free sphinganine and sphingosine (Wang et al., 1991). Inhibition of sphingolipid synthesis by FB1 has been shown to correlate with suppressed cell proliferation (Wang et al., 1991; Yoo et al., 1992). Sphingolipid breakdown products were also recognized as antiproliferative lipids and were demonstrated to decrease cell-mediated and humoral immune response (Felding-Habermann et al., 1990; Hannun and Linardic, 1993; Martinova, 1996). Thus, the poor lymphocyte and macrophage infiltration response in the present study in both groups receiving FCN in the diet might be attributed to depletion of complex sphingolipids and accumulation of free sphinganine and sphingosine caused by FB1.

Studies on combinations of mycotoxins have received significant attention recently because toxicity of combinations of mycotoxins cannot always be predicted based on their individual toxicities. Further, there is the possibility that one mycotoxin that is relatively nontoxic to poultry might be causing severe damage when present in combination with other mycotoxin(s). Similarly, the presence of one mycotoxin may reverse the damaging effects of other mycotoxins. Previous studies have shown additive toxicity effects with FB1 and T2 toxin (Kubena et al., 1995), M and aflatoxin (Kubena et al., 1997), and M and deoxynivalenol (Harvey et al., 1997). With the exception of mortality and serum AST values (at 14 DPF only), no additive or synergistic effects were observed in any response variable parameter measured in the current study, in which all statistical differences were attributed to either one mycotoxin or the other. Minor differences between the results of the present study and previous studies by Javed et al. (1993) and Bermudez et al. (1997) could be due to differences in the susceptibility of quail as compared with broiler chicks and turkeys. Although the results of the present study suggest that FB1 was the sole cause of immunosuppression and that M appeared to play no role in immunosuppression, additional studies are needed to confirm the phagocyte functional activities in the presence of M. In the present study, however, the presence of M in the diet appeared to reverse the effects of FB1, particularly at later stages, which may be attributed to significant feed refusal, but further studies are indicated.

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REFERENCES


