Effects of Moniliformin on Performance and Immune Function of Broiler Chicks

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ABSTRACT Three trials were conducted to evaluate the effect of moniliformin (M) on performance and immune function in chicks. Day-old chicks were randomly assigned to four dietary treatments (0, 50, 75, or 100 mg M/kg diet). In Trial 1, chicks were placed on treatments for 3 wk and were injected intravenously with \(4.6 \times 10^6\) Escherichia coli on Day 21. Blood samples were collected at 60, 120, and 180 min after inoculation, and liver, spleen, and lung were collected at 180 min postinjection. Compared with control chicks, chicks fed 75 and 100 mg M/kg diet had higher \((P < 0.05)\) numbers of E. coli colonies in the circulation, liver, and spleen. In Trial 2, chicks were placed on diets for 4 wk and were injected with 0.5 mL Newcastle disease virus (NDV) vaccine intramuscularly on Weeks 2 and 3 of the experiment. The primary and secondary anti-NDV antibody titers were measured 7 d after each injection. Chicks fed 100 mg M/kg diet had lower \((P < 0.05)\) secondary antibody titers than did control chicks. In Trial 3, lymphocyte proliferation in chicks exposed to M in vivo and in vitro was determined. Results of the in vivo study showed that cell proliferation in response to mitogens from control- and M-fed chicks did not differ \((P > 0.05)\). For the in vitro study, lymphocyte proliferation decreased linearly \((P < 0.01)\) with increased concentrations of M. In all three trials, chicks fed 100 mg M/kg diet had lower \((P < 0.05)\) feed intake and weight gain than did control chicks. Data from the current study suggested that M decreased performance and immune response in chicks at the level of 75 mg/kg diet.

(Key words: chicks, moniliformin, Escherichia coli, Newcastle disease, lymphocyte proliferation)

INTRODUCTION

Moniliformin (M) is a water-soluble fungal metabolite produced by several Fusarium species. Moniliformin appears to be a growth regulator in plants and is phytotoxic in corn and tobacco (Vesonder and Golinski, 1989). This fungal metabolite is highly toxic and results in rapid death in chicks and rats. Cole et al. (1973) reported oral lethal dose \(\left(\text{LD}_{50}\right)\), which is the dosage of toxin that on average will kill 50% of the test animals, of 3.68 mg M/kg body weight in day-old chicks and 41.6 mg M/kg body weight in male and female rats. Burmeister et al. (1979) reported \(\text{LD}_{50}\) values of 5.4, 29.1, and 20.9 mg M/kg body weight in day-old chicks and male and female mice, respectively.

Although M was discovered more than two decades ago (Cole et al., 1973), active investigations on the toxic effects of M in poultry have only been initiated in recent years. Javed et al. (1993) and Ledoux et al. (1995) observed 70 and 83% mortality in chicks fed 154 and 300 mg M/kg diet, respectively. Engelhardt et al. (1989) reported 80 to 100% mortality in chicks, pouls, and ducklings fed 144, 288, or 575 mg M/kg diet. Cardiac injury with alterations in cardiac electrical conductance was shown to be the primary cause of mortality in these birds (Nagaraj et al., 1996; Reams et al., 1997). Feeding low levels of M to birds has been shown to cause poor growth performance, increased serum pyruvate levels, enlarged hearts, and cardiac lesions (Ledoux et al., 1993, 1995; Morris et al., 1997; Reams et al., 1997).

The toxic effects of M on growth response, biochemistry, and pathology are well established; however, there is very little information regarding its effect on the immune system of chicks. It is known that chicks exposed to aflatoxin, T-2 toxin, or ochratoxin have suppressed growth performance and immune responses (Giambrone et al., 1978; Chang and Hamilton, 1979a,b; Pier et al., 1980; Dwivedi and Burns, 1984a,b; Pier and Mcloughlin, 1985; Corrier et al., 1987). The immune system may also be a

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Abbreviation Key: M = moniliformin; NDV = Newcastle disease virus; ConA = concanavalin A; PWM = pokeweed mitogen; LPS = lipopolysaccharide; SI = stimulation index.
target for M toxicity. Although the effect of M on the immune system in poultry has not been previously reported, culture material containing fumonisin B₁, fumonisin B₂, and M has been shown to suppress immune response in chicks (Dombbrink-Kurtzman et al., 1993; Javed et al., 1995; Qureshi et al., 1995). The objective of this study was to determine the effect of M on the immune system in chicks.

MATERIALS AND METHODS

Birds and Diets

One-day-old Arbor Acres × Arbors Acre male broiler chicks were used in all experiments. Chicks were individually weighed, wing-banded, and randomly assigned to pens in a stainless-steel battery. Chicks were maintained on a 24-h constant light schedule and allowed to consume feed and water ad libitum. Dietary treatments containing M were prepared by substituting ground Fusarium fujikuroi culture material, which contained 10,000 mg M/kg culture material, for ground corn in a typical corn-soybean meal basal diet. Four dietary treatments containing 0, 0.5, 0.75, and 1% moniliformin culture material, respectively. The levels of M used in the present study were based on those that did not cause any mortality as reported in the literature. Because moniliformin is highly toxic, feeding high levels of M (≥150 mg M/kg diet) to chicks can result in death, which was not desirable for the current study. Diets were formulated to be isocaloric, isonitrogenous, and either met or exceeded the nutrient requirements of broiler chicks as recommended by the National Research Council (1994). Diets were screened for the presence of mycotoxins and were found to be free of aflatoxin, citrinin, vomitoxin, sterigmatocystin, zearalenone, ochratoxin A, and fumonisins (Rottinghaus et al., 1982, 1992). Chicks were individually weighed, and feed intake and feed efficiency were determined for each pen on a weekly basis. Chicks were checked daily for signs of disease and mortality. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

Experiment 1: Bacterial Clearance Rate

Escherichia coli challenge was employed to evaluate the ability of chicks fed M to clear the bacteria from circulation. This E. coli isolate originated from a case of colibacillosis in commercial turkeys. Day-old male broiler chicks were randomly assigned to one of four dietary treatments with six replicates of two chicks per treatment for 3 wk. At the end of the feeding period, chicks were intravenously injected with 1 mL bacterial suspension containing 4.6 × 10⁶ E. coli.

Blood (1 mL) samples were collected from the jugular vein into heparinized tubes at 60, 120, and 180 min postinjection. Prior to E. coli inoculation, blood samples from each bird were collected and checked for the presence of E. coli to ensure no preexisting infections. At 180 min postinjection, chicks were euthanatized, and tissue samples of liver, spleen, and lung were taken for quantitative bacterial determination.

Whole blood samples were serially diluted with sterile PBS, and 100 µL of each dilution at each time point were plated onto MacConkey agar plates. Approximately 1 g tissue sample was homogenized with 5 mL sterile PBS. Serial dilutions of tissue suspension (100 µL) were plated onto MacConkey agar plates. Each sample was run in duplicate plates. Plates were incubated at 37 C for 18 h, and E. coli colony-forming units were enumerated. Colonies grown on the plates were confirmed to be E. coli colonies by previously reported methods (Patten et al., 1995). Final bacterial concentrations were calculated as the numbers of colony-forming units per milliliter of blood and as colony-forming units per gram of harvested tissue.

Experiment 2: Antibody Response to Inactivated Newcastle Disease Vaccine

Primary and secondary antibody response to inactivated Newcastle disease virus (NDV) vaccine was used to examine the humoral immune response of chicks fed M. Day-old male chicks were placed on dietary treatments (six replicates of two chicks each per treatment) for 4 wk. At the end of Weeks 2 and 3 of the experiment, chicks were injected intramuscularly with 0.5 mL inactivated NDV vaccine.

Blood samples were withdrawn from the jugular vein 7 d after each injection for determination of primary and secondary antibody response. Serum samples were harvested and stored at −20 C until analysis. Serum samples were analyzed for anti-NDV antibody titers by ELISA. The ELISA was performed with commercial kits according to manufacturer’s recommendations.

Experiment 3: Mitogen-Induced Lymphocyte Proliferation

Feeding Trial. A [³H]-thymidine uptake assay was used to assess the proliferation of chick lymphocytes in response to three mitogens, including concanavalin A (ConA), pokeweeds mitogen (PWM), and lipopolysaccharide (LPS). These mitogens were chosen such that proliferative response of T and B lymphocytes could be distinguished. Concanavalin A is known to stimulate T-cell proliferation, whereas bacterial lipopolysaccharide selectively stimulate B-cell proliferation. However, it has been reported that chick lymphocytes respond poorly to LPS (Fritsche et al., 1991). Thus, PWM, which induces a strong

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3Stover Hatchery, Stover, MO 65078.
4Remel, Lenexa, KS 66215.
6IDEXX Laboratory, Inc., Westbrook, ME 04029.
7Sigma Chemical Co., St. Louis, MO 63178-9916.
B-cell response, was also chosen. Day-old chicks were randomly assigned to either control or 100 mg M/kg diet. Each of the two diets were fed to four pens of four chicks per pen for 3 wk.

At the end of the feeding period, lymphocytes were isolated from peripheral blood. Peripheral lymphocytes were adjusted to a final concentration of 2 × 10⁷ cells/ml in RPMI-1640 cultured medium (RPMI-1640 plus 2 mM L-glutamine, 100 U penicillin/mL, and 100 µg streptomycin/mL). One hundred microliters of cell suspension (2 × 10⁶ cells) were added to each well in 96-well microtiter plates. Cells were incubated with optimal concentrations of either ConA (20 µg/mL), PWM (10 µg/mL), or LPS (5 µg/mL) and 5% homologous chick serum. Cell cultures were incubated at 40 C for 48 h in a humidified incubator with 5% CO₂. For the final 16 h of incubation, 1 µCi [³H]-thymidine in 10 µL RPMI-1640 cultured medium was added to each well. Upon completion of incubation, cells were harvested, and radioactivity in each well was determined in a liquid scintillation counter. Each sample was added to each well in 96-well microtiter plates. Cells were incubated with optimal concentrations of either ConA (20 µg/mL), PWM (10 µg/mL), or LPS (5 µg/mL) and 5% homologous chick serum. Cell cultures were incubated at 40 C for 48 h in a humidified incubator with 5% CO₂. For the final 16 h of incubation, 1 µCi [³H]-thymidine in 10 µL RPMI-1640 cultured medium was added to each well. Upon completion of incubation, cells were harvested, and radioactivity in each well was determined in a liquid scintillation counter. Each sample was run in triplicate wells, and the values were averaged and expressed as mean cpm. A stimulation index (SI) was calculated as the mean cpm of tritium incorporated in cells treated with mitogens divided by the mean cpm of tritium incorporated in cells not treated with mitogens.

In Vitro Trial. Twelve 1-d-old chicks were used to determine the mitogenic response of chick lymphocytes exposed to M in vitro. Chicks were fed an NRC corn-soybean meal diet for 21 d. On Day 21, peripheral blood lymphocytes were isolated, and 2 × 10⁶ cells were incubated with various concentrations of purified M (0 to 10 µg/mL) and ConA (20 µg/mL). Purified M was purchased from Sigma Chemical Co. Procedures used in this study were the same as described in the in vivo study.

Statistical Analysis

Data were subjected to ANOVA, and means for treatments showing significant differences in the ANOVA were compared by Fisher’s protected least significant difference procedure (Snedecor and Cochran, 1967). Significance was accepted at P < 0.05. Data of bacterial clearance and antibody response were logarithmically transformed prior to analyses to achieve homogeneity of variance. Data of lymphocyte proliferation (in vitro study) were also analyzed by polynomial regression analysis. All analyses were conducted on a Macintosh computer using version 1.11 of SuperANOVA.¹¹

RESULTS

The effects of dietary M on feed intake, body weight gain, and feed conversion are summarized in Table 1. Compared with controls, chicks fed 100 mg M/kg diet had lower (P < 0.05) feed intakes and body weight gains over the total 3-wk period (Experiments 1 and 3) and 4-wk period (Experiment 2). In the second experiment, chicks fed 75 mg M/kg diet also had significantly lower feed intake and body weight gain than controls. Feed efficiency was not affected by M in any experiment.

The effects of dietary M on bacterial clearance from the blood system are presented in Table 2. Microbiological cultures from blood samples taken before E. coli injection were sterile in all groups. Dietary M had a significant influence on systemic bacterial clearance in chicks. Compared with controls, chicks fed 100 mg M/kg diet had higher (P < 0.05) numbers of E. coli colonies in the blood over the 180-min period. Compared with controls, chicks fed diets containing 75 mg M/kg had a higher (P < 0.05) number of E. coli colonies at 120 and 180 min postinjection. Numbers of E. coli colonies in the blood cultures from chicks fed control and 50 mg M/kg diet diet decreased with increased postinjection time. More than 50% of control chicks and chicks fed 50 mg M/kg diet were able to clear bacteria from the circulation by the end of the observation period. Compared with controls, systemic bacterial clearance was delayed (P < 0.05) in chicks fed either 75 or 100 mg M/kg diet. An interaction of treatment by time was observed in the present study.

In comparison with the results in the controls, the delayed systemic bacterial clearance in chicks fed 75 and 100 mg M/kg diet was associated with significantly higher numbers of viable bacteria in organs (Table 3). Compared with controls, higher (P < 0.05) numbers of E. coli colonies were observed in liver and spleen samples in chicks fed 100 mg M/kg diet, whereas chicks fed 75 mg M/kg diet had higher (P < 0.05) bacteria numbers only in the spleen.

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¹¹Amersham Corp., Arlington Heights, IL 60005.
³Beckman Instruments, Inc., Schaumburg, IL 60173-3833.
cultures. The numbers of viable bacteria in lung cultures were similar in all groups.

The primary and secondary antibody production against inactivated NDV in chicks fed M is shown in Table 4. Dietary M did not influence (P > 0.05) primary antibody production against inactivated NDV. Compared with controls, mean secondary antibody titer response to inactivated NDV was lower (P < 0.05) in chicks fed 100 mg M/kg diet.

Effects of dietary M on chick lymphocyte proliferation in response to mitogens are shown in Figure 1. In the absence of mitogens, lymphocyte proliferation was 1,125 ± 87 (SE) cpm in control chicks and 1,045 ± 96 (SE) cpm in M-fed chicks. Chick lymphocyte proliferation in response to ConA, PWM, and LPS was not affected (P > 0.05) by dietary M. Results of the in vitro study showed a dose-dependent response in cells exposed to M (Figure 2). Increasing levels of M resulted in a linear (P < 0.01) decline in lymphocyte proliferation in response to ConA. Proliferation of cells exposed to 1.25 µg M/mL was decreased by 50%. Compared with controls, cells exposed to 10 µg M/mL showed 95% inhibition of proliferation.

**DISCUSSION**

In the present study, feed intake and body weight gain were decreased at 100 mg M/kg. Reductions in feed intake and body weight gain were observed in all three experiments. Decreased body weight gain observed in the present study appears to be a result of reduced feed intake because the difference in body weight gain was related to the difference in feed intake. Reductions in feed intake and body weight gain were also reported in previous studies with chicks, poults, and ducklings fed moniliformin (M)-contaminated diets.

### TABLE 2. Systemic bacterial clearance in chicks fed diets containing moniliformin (M)

<table>
<thead>
<tr>
<th>Treatment (mg/kg diet)</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>Pooled mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.87 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.08 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.10 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>2.84 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.97 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>2.94 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.91 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.61 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.82 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled mean</td>
<td>2.95 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.61 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.86 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Source of variation: Treatment: 0.001, Time: 0.001, Treatment × time: 0.001.

### TABLE 3. Counts of viable bacteria in tissue homogenates taken 180 minutes after *Escherichia coli* injection in chicks fed diets contaminated with moniliformin (M)

<table>
<thead>
<tr>
<th>M</th>
<th>Spleen</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/kg diet)</td>
<td>(Log&lt;sub&gt;10&lt;/sub&gt; cfu/g tissue)&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>5.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>5.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>5.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.05</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values within columns with no common superscripts are different (P < 0.05).

### TABLE 4. Primary and secondary antibody production against inactivated Newcastle disease vaccine in chicks fed moniliformin (M)-contaminated diets

<table>
<thead>
<tr>
<th>M</th>
<th>Primary Antibody titers (log&lt;sub&gt;10&lt;/sub&gt;)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Secondary Antibody titers (log&lt;sub&gt;10&lt;/sub&gt;)&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/kg diet)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.57</td>
<td>4.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>3.41</td>
<td>4.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>3.53</td>
<td>4.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>3.46</td>
<td>4.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.07</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values within columns with no common superscripts differ (P < 0.05).

<sup>b</sup>Data are presented as means of six pen replicates.

<sup>2</sup>Antibody titers were logarithmic-transformed to achieve homogeneity of variance and were expressed as log<sub>10</sub> antibody titer.
FIGURE 1. Effect of dietary moniliformin (M) on chick lymphocyte proliferation in response to mitogens (20 µg concanavalin A/mL, 10 µg pokeweed mitogen/mL, and 5 µg lipopolysaccharide/mL). Data are expressed as mean stimulation index; error bars represent the standard error. Each value is the mean of four pen replicates of four chicks each. *Stimulation index is calculated as the mean cpm of tritium incorporated in cells treated with mitogens divided by the mean cpm of tritium incorporated in cells not treated with mitogens. Radioactivity in the control wells (without mitogens) was 1,145 ± 87 and 1,045 ± 96 cpm for control and M groups, respectively.

FIGURE 2. Mitogenic response of chick peripheral lymphocyte exposed to moniliformin in vitro and cocultured with 20 µg concanavalin A (ConA)/mL. Data are expressed as mean stimulation index; error bars represent the standard error. Each value is the mean of 12 chicks per dose. Means that do not share a common letter are different (P < 0.05). Linear effects of moniliformin were also observed (P < 0.01). *Stimulation index is calculated as the mean cpm of tritium incorporated in cells treated with mitogens divided by the mean cpm of tritium incorporated in cells not treated with mitogens. Radioactivity in the control wells (cells not treated with ConA) was 1,238 ± 56 cpm.

of the feeding period. In Experiment 2, chicks were placed on experimental diets for 4 wk, whereas chicks were only fed experimental diets for 3 wk in Experiment 1. According to calculation, in the second experiment, chicks fed 75 mg M/kg diet consumed 112 mg M over the total 4-wk period, which is greater than the amount of M ingested by the chicks of the same group in the first experiment (63 mg). The lower concentration of M may have a more pronounced effect when chicks are exposed to the toxin over an extended period.

In the E. coli clearance study, chicks fed 75 and 100 mg M/kg diet had significantly delayed systemic bacterial clearance and higher numbers of bacterial colonies in the liver and spleen, suggesting a reduction in resistance to infections. Reduced bacterial clearance has been shown in chicks fed aflatoxin, and the reduction in bacterial clearance was associated with suppressed phagocytic potential of heterophils, monocytes, and tissue macrophages (Chang and Hamilton 1979a,b; Neldon-Ortiz and Qureshi, 1992). Reductions in systemic bacterial clearance observed in the current study may also be a result of dysfunction of the mononuclear phagocyte system. In addition to the delayed bacterial clearance in the blood, higher numbers of bacterial colonies in the tissue cultures of chicks fed 75 and 100 mg M/kg diet also suggest that the ability to kill bacteria is impaired. It is speculated that tissue macrophages in M-fed chicks were unable to digest bacteria within macrophages, leading to higher numbers of bacteria present in the tissues. As a result, systemic bacterial clearance was delayed. Decreased numbers of macrophages and phagocytic ability of macrophages were also observed in chicks fed diets containing M (Qureshi et al., 1995).

Apart from dysfunction of the mononuclear phagocyte system and diminished bacterial killing of tissue macrophages, depression in performance might have contributed to the delayed systemic bacterial clearance observed in the present study. Compared with controls, chicks fed 100 mg M/kg diet had significantly lower feed intake, resulting in decreased body weights. In this study, chicks were receiving the same challenge dose of E. coli, regardless of body weights. Thus, chicks fed 100 mg M/kg diet received a higher dosage of E. coli per kilogram of body weight compared with controls, which may have had an impact on whole body bacterial clearance rate. It should be noted, however, that body weight gain was reduced by 20% in Trial 1, whereas bacterial clearance was decreased 100-fold at 180 min post-E. coli injection. The magnitude of the changes in body weight gain and bacterial clearance would suggest a true deficit in the mononuclear phagocyte system. Furthermore, chicks fed 75 mg M/kg diet also had a significant decrease in bacterial clearance (Table 2), whereas no statistical difference was observed in feed intake and body weight (Table 1), suggesting that immune response may be more sensitive than the growth response for M toxicity.

The current study also demonstrated that M suppressed humoral immunity. Chicks fed 100 mg M/kg diet had significantly lower secondary anti-NDV anti-
body titers compared with control chicks. Decreased antibody production has been previously shown in chicks fed diets containing M. Qureshi et al. (1995) fed chicks a diet containing 61 mg fumonisin B₁/kg, 10.5 mg fumonisin B₂/kg, and 42.7 mg M/kg for 6 wk and found a significant reduction in anti-NDV antibody titers was also observed in chicks fed Fusarium proliferatum culture material containing 61 mg fumonisin B₁/kg, 14 mg fumonisin B₂/kg, and 66 mg M/kg for 3 wk (Javed et al., 1995). The culture material used in the Javed et al. (1995) studies contained more than one toxin; thus, the lower immune response observed could be caused by any of the individual toxins or by additive or synergistic effects of the toxins.

Results of the feeding trial showed that lymphocyte proliferation was not affected by M consumption. This result was surprising because other immune responses were suppressed in chicks fed 100 mg M/kg diet. Because the medium used to culture the lymphocytes from this study did not contain added M, cells were able to proliferate when they were removed from exposure to M. However, it is unclear whether the homologous serum in the medium contained M. It should also be mentioned that fewer lymphocytes (20 to 25%) were obtained from the peripheral blood of the M-fed birds compared with controls during the lymphocyte isolation, suggesting that fewer lymphocytes were circulating in the blood of the M-fed chicks. It has been shown that chicks fed diets containing M had a significant reduction in white blood cell counts and numbers of viable lymphocytes (Dombrink-Kurtzman et al., 1993; Javed et al., 1995). In addition, results of the in vitro trial showed that lymphocytes exposed to M in vitro decreased lymphocyte proliferation in a dose-dependent manner. Decreased cell proliferation observed in this study could be a result of cell death. Further, it is known that M is cytotoxic to cardiac cells. Thus, it is possible that M is also cytotoxic to lymphocytes.

The current study demonstrated that dietary M not only suppressed performance but also suppressed the immune system. Depressed growth and immune responses observed in the present study may relate to inhibition of energy metabolism. The mechanism of action of M was demonstrated to be impaired energy production, resulting from inhibition of the activity of mitochondrial pyruvate and α-ketoglutarate dehydrogenase complex (Thiel, 1978). Energy is required for biosynthesis of numerous molecules that participate in many biochemical reactions in the body, including those involved in growth and the immune system. Therefore, reduced ATP formation is likely to affect growth and the synthesis of the cellular components and antibodies of the immune system. In fact, growth retardation and suppression in humoral and cell-mediated immunity have been documented in humans and chicks with energy deficiency (Glick et al., 1981, 1983; Latshaw, 1991; Chandra and Kumar, 1994).

Taken together, data of the present study suggest that M is immunosuppressive in chicks. Levels of M in excess of 75 mg/kg diet resulted in reduced humoral immunity and decreased bacterial clearance. Because M-producing fungi are commonly found on corn and other cereal grains used in feed, occurrence of M in poultry rations should be suspected. To date, there have been only two reports on the naturally occurring levels of M (Thiel et al., 1982; Logrieco et al., 1993). In these two reports, the levels of M in all samples tested ranged from 16 to 425 mg M/kg. Until more data on the naturally occurring levels of M are reported, the significance of this mycotoxin to the poultry industry is difficult to assess.

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