Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin


*Institutul de Biologie si Nutritie Animala, Balotesti, Romania; †Institutul Pasteur-Bucharest, Romania; ‡Institutul Central de Diagnostic si Sanatate Animala, Bucarest, Romania; §INRA, Laboratoire de Virologie et Immunologie Moléculaires, Jouy en Josas, France; and ¶INRA, Laboratoire de Pharmacologie-Toxicologie, Toulouse, France

ABSTRACT: A feeding trial was conducted to evaluate the effect of aflatoxin (AF)-contaminated diets on growth and hematological and immunological parameters. Low doses of aflatoxins (140 and 280 ppb) were included in a corn-soybean diet provided for ad libitum consumption to 36 weanling piglets for a period of 4 wk. A "dose-related" decrease in weight gain was observed in treated animals. This effect was significant \((P < 0.05)\) in the 280 ppb-treated group compared to the control group. Ingestion of AF-contaminated feed at either level had no effect on total red blood cell numbers or on their relative number of lymphocytes, monocytes, neutrophils, basophils, and eosinophils in blood. Likewise, AF did not alter globulin, albumins, or total protein concentrations in serum, nor did AF alter the expression of regulatory cytokines produced by either Th1 (IL-2) or Th2 (IL-4) lymphocyte subsets in phytohemagglutinin-stimulated blood samples. By contrast, AF had a biphasic effect on total white blood cell number; the low dose of AF (140 ppb) decreased the total number of white blood cells, whereas the high dose (280 ppb) had the opposite effect. Consumption of AF also increased the concentration of γ-globulin in the serum. A reduced immune response induced by Mycoplasma agalactiae in the 280-ppb-treated group was also observed. Cytokine mRNA expression in phytohemagglutinin-stimulated blood cells indicated that AF decreased proinflammatory (IL-1β, TNF-α) and increased anti-inflammatory (IL-10) cytokine mRNA expression. These results demonstrate that low doses of AF depress growth and alter many aspects of humoral and cellular immunity in pigs.

Key Words: Aflatoxins, Antibodies, Blood, Body Weight, Cytokines, Pigs

Introduction

Aflatoxins (AF) are toxic metabolites produced by Aspergillus flavus and A. parasiticus, widespread contaminants of foods and feeds. Their high toxicity to both animals and humans makes AF the most dangerous known mycotoxins (Wilson and Payne, 1994).

Acute toxicity following consumption of high doses of aflatoxin is well documented (Wilson and Payne, 1994). In pigs, it is characterized by feed refusal, reduced weight gain, changes in hematological and biochemical parameters, increased prevalence of infectious disease, and liver and kidney lesions (Southern and Clawson, 1979; Miller et al., 1981; Harvey et al., 1988). The effects of chronic low-level exposure to aflatoxins are more difficult to document.

The immunotoxic potential of AF is known in many species, including laboratory and domestic animals (Oswald and Comera, 1998; Bondy and Pestka, 2000). In pigs, AF decreases the blastogenesis response to mitogen, reduces the complement titers, decreases macrophage activation, and depresses delayed hypersensitivity (Miller et al., 1978; Sillvotti et al., 1997; Mocchegiani et al., 1998). However, in other studies, using comparable doses, no depression of the proliferative responses of lymphocytes to mitogens was noted (Panangala et
Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0 to 21 d</th>
<th>22 to 30 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>50.38</td>
<td>61.86</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15.00</td>
<td>17.00</td>
</tr>
<tr>
<td>Powdered milk</td>
<td>20.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Oil</td>
<td>5.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>4.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.40</td>
<td>1.80</td>
</tr>
<tr>
<td>Feed-grade limestone</td>
<td>0.50</td>
<td>1.10</td>
</tr>
<tr>
<td>Vitamin-mineral supplement[^2]</td>
<td>1.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Lysine supplement</td>
<td>0.60</td>
<td>0.40</td>
</tr>
<tr>
<td>Choline supplement</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Methionine supplement</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>21.32</td>
<td>18.61</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.51</td>
<td>1.10</td>
</tr>
<tr>
<td>Methionine + cystine, %</td>
<td>1.06</td>
<td>0.68</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.16</td>
<td>0.95</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.98</td>
<td>0.85</td>
</tr>
<tr>
<td>Metabolizable energy, MJ/kg</td>
<td>14.71</td>
<td>12.85</td>
</tr>
</tbody>
</table>

[^2]: Vitamin-mineral premix per kg diet for the period from 0 to 30 d: 10,000 IU vitamin A, 2,000 IU vitamin D, 30 IU vitamin E, 2 mg vitamin K, 1.96 mg vitamin B1, 3.84 mg vitamin B2, 14.85 mg pantothenic acid, 19.2 mg nicotinic acid, 2.94 mg vitamin B6, 0.98 mg folic acid, 0.03 mg vitamin B12, 0.06 mg biotin, 24.5 mg vitamin C, 40.3 mg Mn, 100 mg Fe, 100 mg Cu, 100 mg Zn, 0.38 mg I, and 0.23 mg Se.

Aflatoxins affect cytokine production in pigs

Aflatoxins affect cytokine production in pigs. The first objective of this experiment was to study the influence of low levels of AF in the diet on body weight, blood parameters, and antibody synthesis in pigs. The second objective was to investigate whether the immunomodulatory effect of AF seen in pigs is produced through the interference of the production of cytokines using an RT-PCR technique developed in our laboratory (Dozois et al., 1997).

Materials and Methods

Animals and Experimental Design

Thirty-six 4-wk-old, crossbred weanling piglets were identified by ear tags and housed in floored indoor pens. The piglets, from 13 litters (21 females and 15 males), were randomly assigned among the treatments. Their initial average body weight was 11.42 ± 0.11 kg. Piglets were fed on a corn-soybean meal basal diet (Table 1) and randomly assigned to one of the three treatments: one control group without AF, a 140 µg AF/kg feed group, and a 280 µg AF/kg feed group. The respective treatments were administrated for 30 d and the various treatments and samplings were done according to the protocol described in Figure 1. During the experiment, piglets were given ad libitum access to water and to the assigned diet. They were observed twice daily and weighed weekly.

Aflatoxin Production

Aflatoxin was produced by Aspergillus parasiticus via fermentation on sterile, polished rice. The mould strain was cultured on a solid Sabouraud medium and incubated at 27°C for 7 d. The culture was then scraped and passed onto rice powder and incubated at 27°C for aflatoxin production. After 7 d, the rice powder was processed and AF extracted with methanol:water 70:30 (vol:vol). For AF determination, an aliquoted volume of the filtered extract was diluted in methanol 10%. Aflatoxin content was measured by ELISA kit (Noack, Darmstadt, Germany) according to the manufacturer’s instructions. This ELISA kit is able to detect all the subclasses of AF. The fungal strain we used produced mainly AFb1 (70% of total AF). The aflatoxin extract thus obtained was incorporated into the basal diet in a manner to provide the desired levels of AF (140 ppb or 280 ppb).

Immunization and Blood Sample Collection

On the 8th and 22nd d of the experiment, all piglets including the control group without AF were immunized by subcutaneous inoculation with 1 mL suspension of Agavac (Institutul Pasteur, Bucharest, Romania). This vaccine consists of a combination of formalin-inactivated Mycoplasma agalactiae strains resuspended in aluminium hydroxide. An additional group of six piglets was used as a control in order to verify the efficacy of vaccination. On d 0, 22, and 30 of the experiment, blood samples were aseptically collected by jugular venipuncture from six animals per treatment. Tubes without anticoagulant were used to collect serum for antibody and biochemical parameter measurements and syringes with lithium-heparin were used for the blood cell counts and cytokine detection.

Hematological and Serum Biochemical Measurements

Erythrocyte and white blood cell numbers were determined using a cell counter (Coulter hemoglobinometer, Coulter Electronics, Hialeah, FL). Differential leukocyte populations were performed on blood smears stained with May Grunwald-Giemsa. This vaccine consists of a combination of formalin-inactivated Mycoplasma agalactiae strains resuspended in aluminium hydroxide. An additional group of six piglets was used as a control in order to verify the efficacy of vaccination. On d 0, 22, and 30 of the experiment, blood samples were aseptically collected by jugular venipuncture from six animals per treatment. Tubes without anticoagulant were used to collect serum for antibody and biochemical parameter measurements and syringes with lithium-heparin were used for the blood cell counts and cytokine detection.
Antibody Measurement

Antibody titers against *M. agalactiae* were assayed by ELISA from blood samples collected on d 0, 22, and 30 after immunization. Blood was allowed to clot at room temperature and stored at 4°C overnight. The serum was collected after low-speed centrifugation then stored at −20°C until it was analyzed. ELISA plates were coated with 100 μL of a 10 μg/mL supernate obtained from ultrasonicated *M. agalactiae* culture. After five washes with 0.01 M PBS, pH 7.4, containing 0.05% Tween 20 (PBS-Tween), serum samples diluted 1/100 in PBS-Tween with 0.5% BSA were added in duplicate to the plates (100 μL/well) and incubated 1 h at 37°C. After incubation for 1 h at 37°C with peroxidase-labeled anti-pig IgG (Institutul Pasteur, Bucharest) the substrate (0.05% peroxide and 0.6 mg/mL 2,2′-azino-bis-[3-ethyl-benzthiazoline-6-sulfonic acid] in citric acid buffer) was added to the wells. Plates were incubated for 30 min at room temperature and the reaction was stopped with 1.5% sodium fluoride, 50 μL/well. The absorbance at 405 nm was recorded using an ELISA plate reader (Multiscan MS Labsystem, Finland) and values were expressed as optical densities.

Cytokine Detection

Cytokines were measured on whole blood cells (d 30) after in vitro stimulation as already described (De Groote et al., 1996). Briefly, blood was diluted 10-fold in RPMI 1640 containing 2 mM L-glutamine, 1 mM pyruvate, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The blood cells were stimulated with 10 μg/mL phytohemagglutinin. These cell culture reagents were purchased from Sigma Chemical (St. Louis, MO). After incubation for 24 h with 5% CO₂ at 37°C, blood samples were centrifuged and cell pellets were resuspended in 1 mL Trizol (Gibco, Life Technologies, Cergy Pontoise, France) then frozen at −80°C until they were used. Total RNA was extracted as recommended by the manufacturer. The RNA was resuspended in 20 to 50 μL ultrapure water containing 0.02% (wt/vol) diethyl pyrocarbonate (DEPC) (Sigma) and 1 mM EDTA. Total RNA was quantified by optical density at 260 nm (OD₃₅₀/OD₂₈₀ ratio, which was between 1.8 and 2. Cytokine mRNA was measured by RT-PCR as previously described (Fournout et al., 2000). Briefly, 1 μg of mRNA was reverse-transcribed with M-MLV reverse transcriptase, RNase H (Promega, Charbonnières, France) for 1 h at 42°C and then amplified with 1 unit Taq DNA Polymerase (Gibco, Life Technologies, Gaithersburg, USA).
Table 2. Oligonucleotides used in this study to specifically detect porcine cytokine and cyclophilin mRNA

<table>
<thead>
<tr>
<th>Gene specificity and primer</th>
<th>Oligonucleotide sequences (5′-3′)</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>S: TGCCAGCTATGAGCCACTTCC</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>AS: TGACGGTGTCGGAATGATGCT</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>S: GATGGCCAGAGGAGGAGTTGAC</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>AS: ATCGGCCCATCCAGAGGAAGAG</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>S: GATTTCACGGTGTCTTTGAA</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>AS: GTTGAGTAGATGCTTTGACA</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>S: TACCAGCAACTTCGTCCAC</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>AS: ATCGTCTTTAGCCTTTGACA</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>S: GCATCCACTTCCCAACCA</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>AS: CTTCCCTCATCTCTAGCTCAT</td>
<td></td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>S: TAACCCCATCGTCTCTTT</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>AS: TGGCCATCAACACACTCAG</td>
<td></td>
</tr>
</tbody>
</table>

*aIL = interleukin; TNF-α = tumor necrosis factor-α.
*bS, sense primer; AS, antisense primer.

MD). Each cycle of amplification was 94°C for 45 s, 54°C for 45 s, and 72°C for 45 s. Primer sequences and numbers of PCR cycles used for each cytokine are given in Table 2. Amplified DNA was analyzed after electrophoresis on 1.2% TBE (Tris-Borate-EDTA) agarose gels, which were stained with ethidium bromide. The level of each PCR product was quantified densitometrically using the Quantity One program (Bio-Rad, Hercules, CA). To compare the relative cytokine mRNA expression levels among samples, the values are presented as the ratio of the band intensity of the cytokine-specific RT-PCR product over that of the corresponding constitutively expressed “housekeeping” gene, cyclophilin.

**Statistical Analysis**

Student’s t-tests were used to analyze the differences in term of weight, average daily gain, cytokine production, and so on, between the control, 140 ppb AF and 280 ppb AF groups. P-values of 0.05 were considered significant.

**Results**

**Effect of Aflatoxin on Animal Performance**

We first investigated the effect of dietary treatment on clinical signs and animal performance. Piglets were fed with control or AF-contaminated diets (140 or 280 ppb) for a period of 4 wk. Control animals as well as piglets fed AF appeared clinically normal during the whole experiment and no deaths resulted from the AF exposure.

![Figure 2. Influence of aflatoxin (AF) on body weight.](image)

Animal weight gains are reported in Figure 2. A dose-related effect of AF upon body weight gain was observed. The body weights of the intoxicated piglets were always numerically lower than those in the control group. However, this difference was significant only for the 280-ppb AF-treated group, and the effect was more pronounced after 3 and 4 wk of intoxication.

As can be observed in Figure 2, the average daily gain during the period from 0 to 22 d was 349 ± 19 g for the control animal vs 218 ± 17 g for the 280-ppb AF-treated animals (P < 0.001). For the period from 0 to 30 d these average daily gains increased to 489 ± 18 g for the control group and 326 ± 17 g for the 280-ppb AF-contaminated group (P < 0.001).

**Influence of Aflatoxin on Hematological Parameters**

The blood samples collected on d 30 of the experiment were used to analyze the effect of AF-contaminated diet on blood cell number and composition. As shown in Figure 3, no effect on red blood cell number appeared as a result of AF ingestion. By contrast, consumption of the low dose of AF (140 ppb AF) decreased the total number of white blood cells, whereas consumption of the high dose (280 ppb AF) had the opposite effect when compared to the values obtained for animals fed the control diet. We then investigated whether the effect produced by AF on leukocyte number was specific to one particular subpopulation. The percentages of the different white blood cell subpopulations in the blood of animals from different groups were within the normal range.
Figure 3. Influence of aflatoxin (AF) on blood cell counts. Pigs received a control diet (open bars) or a diet contaminated with 140 (shaded bars) or 280 (solid bars) ppb of AF. Erythrocyte and leukocyte counts were performed on samples collected on d 30 of the experiment. Results are expressed as number of cells/mm$^3$ from four to five animals (mean $\pm$ SEM). A Student’s $t$-test was performed to compare the differences between groups: a indicates difference ($P < 0.05$) between control and 140 ppb AF treatments; b indicates difference ($P < 0.05$) between 140 and 280 ppb AF treatments.

Influence of Aflatoxin on Serum Parameters

Serum electrophoresis analysis was also performed on the 30th d of the experiment for blood samples from six animals per treatment. Exposure to AF had no effect on the total globulin, albumin, and protein concentrations. By contrast, intoxication with AF induced a dose-dependent increase in $\gamma$-globulin ($P < 0.05$). Gamma-globulin concentration increased from 13 $\pm$ 1 g/L in the control group to 16 $\pm$ 3 and 22 $\pm$ 4 g/L in piglets treated with 140 ppb and 280 ppb AF, respectively (Table 3).

Table 3. Effect of aflatoxin (AF) consumption on serum proteins in piglets after 30 d of AF treatment$^a$

<table>
<thead>
<tr>
<th>Dietary AF</th>
<th>Parameter</th>
<th>0 ppb</th>
<th>140 ppb</th>
<th>280 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein, g/L</td>
<td>60.7 $\pm$ 12.1</td>
<td>54.6 $\pm$ 10.3</td>
<td>61.8 $\pm$ 9.7</td>
</tr>
<tr>
<td></td>
<td>Total globulin, g/L</td>
<td>24.1 $\pm$ 3.3</td>
<td>22.9 $\pm$ 1.6</td>
<td>23.5 $\pm$ 5.8</td>
</tr>
<tr>
<td></td>
<td>Albumin, g/L</td>
<td>36.6 $\pm$ 8.4</td>
<td>31.7 $\pm$ 5.7</td>
<td>38.3 $\pm$ 7.0</td>
</tr>
<tr>
<td></td>
<td>$\gamma$-Globulin, g/L</td>
<td>13.3 $\pm$ 1.6$^x$</td>
<td>16.5 $\pm$ 3.3$^y$</td>
<td>22.0 $\pm$ 4.5$^z$</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as mean $\pm$ SEM, n = 6.

$^{x,y,z}$Within a row, means without a common superscript letter differ ($P < 0.05$).

Influence of Aflatoxin on Specific Antibody Response

To investigate the influence of 140 and 280 ppb AF on the immune response the control and the AF-treated piglets were immunized with *M. agalactiae*. Serum antibody levels were measured by ELISA after the primary and the secondary injections (d 22 and 30) for the six animals per treatment. As expected, the injections increased ($P < 0.05$) the antibody levels. This increase was observed in the immunized control piglets as well as in the intoxicated animals (Figure 4). Nevertheless, ingestion of AF decreased the primary and the secondary immune response; antibody levels against *M. agalactiae* were always numerically lower in AF-fed animals compared to immunized control piglets, although the difference was not significant.

Influence of Aflatoxin Contamination on the Expression of Cytokines

The ability of AF exposure to modulate cytokine expression at the transcriptional level was then investigated in whole blood samples (d 30) after mitogenic stimulation. The synthesis of regulatory cytokines (in-
terleukin (IL)-2 and IL-4) and proinflammatory (IL-1β, tumor necrosis factor-α (TNF-α)) and anti-inflammatory cytokines (IL-10) was measured by RT-PCR. The mRNA synthesis of regulatory cytokines produced by Th1 (IL-2) and Th2 (IL-4) lymphocyte subsets was not modified by the contamination with AF, although variations from animal to animal were observed (Figure 5). By contrast, AF treatments altered the synthesis of the mRNA of inflammatory cytokines. We observed a slight, but nonsignificant, decrease in TNF-α and a significant decrease in IL-1β mRNA synthesis (P < 0.05). By contrast, an increased IL-10 mRNA synthesis (P < 0.05) was noted (Figure 6). For these three cytokines a high variability among the piglets was also observed.

Discussion

In this trial, diets containing 0, 140, or 280 ppb AF were fed to pigs for 4 wk. Although these levels are high compared to the limit authorized in most European countries (50 ppb), they are well within the range for animal feeds within the United States (20 to 300 ppb, depending on the feed). In particular, for swine the maximum tolerated level for aflatoxin is 200 ppb (FAO, 1997). In developing countries, where long-term storage is often inadequate in terms of heat and humidity, much higher levels of contamination of animal feed with AF can be observed. We found that ingestion of low doses of AF alters animal performance. Indeed, weight gains were reduced by 17.6% after ingestion of 280-ppb AF-contaminated diets for 4 wk. This result is consistent with that of van Heugten et al. (1994), who reported a 31% decrease in growth of piglets fed a diet contaminated with 280 ppb of AF. Reduced growth rates have also been observed in piglets (Schell et al., 1993), barrows (Davila et al., 1983; Harvey et al., 1988, 1994), and growing or finishing swine (Southern and Clawson, 1979) exposed to higher doses of mycotoxin (740 to 2500 ppb).

There is limited information available concerning the biochemical effects of low AF exposure (Southern and Clawson, 1979). In the current study it was observed that although γ-globulin concentrations increased, most of the hematological parameters did not change or were too inconsistent to draw conclusions. An increase of γ-globulin has been observed in pigs, but the available data are limited to animals fed with high doses of AF (Annau et al., 1964; Cysewski et al., 1978; Miller et al., 1981). This alteration of γ-globulin levels has also been observed in lambs (Fernandez et al., 1996), goats (Clark et al., 1984), guinea pigs (Thurston et al., 1974), cattle (Brucato et al., 1986), and poultry (Richard et al., 1973; Fernandez et al., 1997). Most authors have also found increases in red blood cells in AF-intoxicated animals (Fernandez et al., 1996), which may explain the increase observed in most hematological parameters in these experiments. We did not find any changes in red blood cell numbers in piglets exposed to low doses of AF, but we noticed a biphasic effect of AF on white blood cell numbers. Consumption of a high dose of AF (2.5 ppm) increased white blood cell numbers in pigs (Harvey et al., 1990) and in other species (Fernandez et al., 1996; Bortell et al., 1982).

It is known that the immune system is very sensitive to AF (reviewed by Pier 1992; Oswald and Comera 1998; Bondy and Pestka 2000). In swine, attempts to evaluate the effect of AF on specific cellular or humoral immune responses have so far provided conflicting results. Our results from the present study indicated that pigs fed low doses of AF tend to have lower antibody levels to M. agalactiae than control pigs; however, the difference was not significant. Joens et al. (1981) observed significantly lower hemagglutination titers to Treponema hyodisenteriae in pigs that received AF compared to control pigs. By contrast, ingestion of AF did not alter the humoral response of weaning pigs to sheep red blood cells (van Heugten et al., 1994) or to Erysipelothrix rhusiopathiae (Panangala et al., 1986).

To our knowledge this is the first report that investigates cytokine production during AF intoxication in pigs. Previous experiments in pigs have shown that AF decreases lymphocyte proliferation (van Heugten et al., 1994; Harvey et al., 1995). In the present study we did not find any effect of AF on regulatory cytokines produced by either the Th1 or the Th2 subset of lymphocytes (Figure 4). This is in agreement with results ob-

Figure 5. Influence of aflatoxin (AF) on the regulatory cytokine production. Piglets were fed a control diet or diets contaminated with 140 or 280 ppb AF for 30 d. Blood samples were taken on d 30 of treatment and cultured for 24 h. Total RNA was isolated and assayed for expression of regulatory cytokines (IL-2, IL-4) and the cyclophilin housekeeping genes by RT-PCR. Quantification of the relative cytokine mRNA levels for each sample are expressed in arbitrary units (AU) as the ratio between the cytokine-specific RT-PCR product and the corresponding cyclophilin band intensity. Results of individual animals (closed triangles) and the means (± SEM) of each group (vertical bars) are shown.
Figure 6. Influence of aflatoxin (AF) on the inflammatory cytokine production. Quantification of the relative cytokine mRNA levels for the inflammatory cytokines was done as shown for Figure 5. Student’s t-test was used to compare the means of AF-treated pigs with controls. The asterisk indicates a difference (P < 0.05).

tained in mice that received oral gavage with 30 to 700 μg AF/kg body weight (Dugyala and Sharma, 1996) or in rats that received a weekly dose of 0.1 or 1 mg of AF by intragastric inoculation (Watzl et al., 1999).

We found a profound depression on inflammatory cytokine IL-1β and a slight, but not significant, decrease for TNF-α in weanling pigs fed an AF-contaminated diet. This was associated with an increase in the anti-inflammatory cytokine IL-10. Indeed, it has been demonstrated that IL-10 was able to decrease the production of several inflammatory cytokines, including IL-1β and TNF-α (Waal Malefyt et al., 1991). Thus, the down-regulation of inflammatory cytokines could be a consequence of the induction of IL-10. The inhibition of inflammatory cytokines has been observed in rodents during respiratory aflatoxicosis (Jakab et al., 1994) or after oral intoxication (Dugyala and Sharma, 1996; Moon et al., 1999), although certain differences were noted. Peritoneal macrophages of AF-treated mice and rats produced less TNF-α, IL-6, and IL-1 at the protein level. The AF effect on mRNA encoding for cytokines was more complex; a low (30 μg/kg body weight) to medium dose (145 μg/kg body weight) of this mycotoxin significantly increased the mRNA levels, whereas high doses (700 μg/kg body weight) had the opposite effect (Dugyala and Sharma, 1996). In contrast, we demonstrated an inhibition of mRNA encoding for both IL-1 and TNF in blood cells from intoxicated pigs. This discrepancy can be due to differences in host response (mouse vs pig). In vitro studies have also demonstrated a suppressive effect of AF on inflammatory cytokine levels in mice (Moon et al., 1999), humans (Rossano et al., 1999), and cattle (Kurtz and Czuprynski, 1992).

In conclusion, this study demonstrates that subclinical exposure of young swine to AF in the diet is associated with a number of effects manifested by a reduction in weight gain, changes in several blood parameters, and alteration of both humoral and cellular immune responses. Several papers have demonstrated that ingestion of high doses of AF increases susceptibility to infectious diseases (Pier, 1992; Oswald and Comera, 1998; Bondy and Pestka, 2000). Ours results suggest that, even when present at low doses, AF alters the immune response and this may predispose pigs to infectious diseases.

Implications

Mycotoxins are secondary metabolites of fungi that grow on a variety of feed and foodstuffs consumed by animals and humans. Among them, aflatoxins are the most abundant and toxic metabolites produced by Aspergillus molds. In this study we evaluated the effects of two low-aflatoxin-contaminated diets on growth and hematological and immunological parameters of weanling piglets. The contamination with aflatoxin caused a dose-related decrease in weight gain, modifications in some blood parameters, and alterations of the immune response, in particular of cytokine expression. These results show that even subclinical exposure to aflatoxin may result in economic losses due to decreased performance and in impairment of cellular and humoral immune functions. This latter effect could decrease host resistance to infections and also could induce a breakdown in vaccine immunity even in properly vaccinated animals.

Literature Cited


