INFLUENCE OF DIETARY PROTEIN, FAT OR AMINO ACIDS ON 
THE RESPONSE OF WEANLING SWINE TO AFLATOXIN B11,2,s 

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ABSTRACT 
Two experiments were conducted using corn from clean or aflatoxin B1 (AFB1)-contami-
nated (182 ppb) sources. Weanling pigs (28 d) were fed one of eight dietary treatments ar-
ranged in a 2 x 2 x 2 factorial design. In Exp. 1 (192 pigs), treatments varied in corn source 
(clean or AFB1-contaminated), CP level (18 or 20%) and added fat (0 or 5%). At the end of 
the 28-d growth trials, plasma samples were obtained. An AFB1 x CP level interaction was 
detected (P < .05) for growth rate (ADG), feed intake (FI) and feed/gain ratio (F/G). Feeding 
AFB1 reduced (P < .05) ADG (.30 vs .37 kg/d) and FI (.57 vs .66 kg/d) and increased 
F/G (1.88 vs 1.78) of pigs fed 18% CP diets. Performance of pigs fed 20% CP diets was not 
altered by AFB1. Adding 5% fat to diets improved (P < .05) F/G but did not improve ADG 
of pigs fed AFB1. There was an AFB1 x CP x fat interaction (P < .05) for plasma chole-
sterol. Adding fat or increasing the CP level prevented the depression of plasma cholesterol in 
pigs fed AFB1. In Exp. 2 (96 pigs), all diets contained 18% CP and the treatments varied in 
corn source (clean or AFB1-contaminated), added L-lysine HCl (0 or .25%) and added DL-
methionine (0 or .15%). Feeding AFB1 reduced (P < .05) ADG of pigs fed the 18% CP diet 
(.44 vs .50 kg/d) but not of pigs fed diets supplemented with .25% lysine. The ADG of pigs 
fed added methionine was intermediate between that of pigs fed clean or AFB1 corn without 
amino acid supplementation. There was an AFB1 x methionine interaction (P < .05) for 
plasma triglyceride level. Adding methionine increased (P < .05) plasma triglycerides of 
pigs fed clean corn but not of pigs fed AFB1 corn. These data confirm interactions between 
nutrients and the response of swine to AFB1; therefore, tolerances for AFB1 in swine can-
not be established without considering levels of important dietary nutrients. 
(Key Words: Pigs, Aflatoxins, Nutrition, Proteins, Dietary Fat, Amino Acids.) 


Introduction 
Aflatoxins are produced by the fungi Asper-
gillus flavus and Aspergillus parasiticus. Afla-

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toxin B1 (AFB1) is the most abundant and 
most toxic of the aflatoxins (Pier, 1981). The 
acute toxic effects include hemorrhaging and 
death. The subacute or chronic effects of ex-
posure can affect growth, feed efficiency and 
general well-being. Reductions in the concen-
tration of lipid components in the blood are 
indicative of aflatoxicosis (Tung et al., 1972) 
and are a result of lipid malabsorption (Hamil-
ton, 1977). The more subtle effects of AFB1 
may go unnoticed, but over time they are 
costly. 

Attempts have been made to lessen the 
effects of AFB1 by altering dietary nutrient 
content. Increasing dietary protein above the 
required concentration diminished the reduc-
tion in body weight of chicks fed 5.0 ppm 
dietary AFB1 (Smith et al., 1971). Less than 
opimal protein concentration in the diet of
10-wk-old pigs increased the toxic effects of aflatoxin. Younger pigs (4 wk old) were even more sensitive to protein level and aflatoxicosis (Sisk and Carlton, 1972). The effects of protein above the dietary requirement on the response of pigs to AFB₁ have not been investigated.

Addition of dietary fat decreased broiler mortality during aflatoxicosis (Hamilton et al., 1972). Also, the detrimental effects of aflatoxin on feed conversion were lessened by increasing the amount of linoleic acid in the diet (Lanza et al., 1981). The effect of added fat on the response of pigs to AFB₁ has not been examined.

The objective of these experiments was to determine whether modifying the nutrient content of swine diets would enhance resistance to dietary AFB₁; specifically, our goal was to examine the effects of manipulating fat and protein concentration and to determine whether the lysine or methionine content of diets could be modified to enhance resistance to AFB₁.

**Materials and Methods**

Two experiments were conducted using air-dried yellow dent corn from clean or AFB₁-contaminated sources. In both experiments, 28-d-old weanling pigs (7.2 kg) were allotted to one of eight dietary treatments based on weight, sex and ancestry. The treatments were arranged in a 2 x 2 x 2 factorial design. In Exp. 1, treatment diets varied with respect to corn source (clean or AFB₁-contaminated), CP content (18 or 20%) and added fat (0 or 5%). There were two trials utilizing 96 pigs in each (six pigs/pen and two replicate pens/treatments in each trial). In Exp. 2, 96 pigs (six/pen and two replicate pens/treatment) were fed corn-soybean meal diets containing 18% CP. The treatment diets varied in corn source (clean or AFB₁-contaminated), added L-lysine HCl (0 or .25%) and added DL-methionine (0 or .15%). In both experiments, sex was balanced in each replicate; each pen contained three barrows and three gilts. Composition of the diets is given in Table 1.

The ingredients were analyzed for nutrient and mycotoxin contents prior to diet formulation, and enough ingredients were stored to conduct all trials. Corn and soybean meal were analyzed for DM according to AOAC procedures (1984) and for CP with an automatic analyzer⁷. Aflatoxin B₁, B₂, G₁, G₂, zearalenone and deoxynivalenol were determined by the methods of Hutchins and Hagler (1983), Swanson et al. (1984) and Romer (1986). All ingredients were free of aflatoxin G₁, G₂, zearalenone and deoxynivalenol. The soybean meal, clean and AFB₁-contaminated corn contained 0 ppb AFB₁, 48.7% CP, 89.9% DM; 6 ppb AFB₁, 8.49% CP, 89.5% DM and 182 ppb AFB₁, 9.04% CP, 89.0% DM, respectively.

Pigs were housed in an environmentally regulated nursery in pens with slotted concrete floors (1.52 m x 2.13 m) and wire mesh sides. Temperature in the enclosed nursery was regulated by fan ventilation. Pig weights and pen feed consumption were determined weekly over the 28-d trials. At the end of the trials, pigs were fasted for 24 h and blood samples were obtained by jugular venipuncture to monitor changes in plasma constituents that have been reported to be altered by aflatoxin exposure. Concentration of plasma cholesterol was determined spectrophotometrically⁸ by using a cholesterol reagent kit⁹ based on the Lieberman-Burchard reaction, and triglycerides were determined by using a kit¹⁰ based on the ultraviolet procedure of Bucolo and David (1973). Plasma urea was analyzed using the modified assay of Chaney and Marbach (1962) and plasma protein by the procedure of Lowry et al. (1951).

Data were analyzed on a pen basis as a randomized complete block design containing a 2 x 2 x 2 factorial arrangement of treatments (Steel and Torrie, 1980) using least squares analysis of variance according to the General Linear Models procedure of SAS (1982). Single degree of freedom contrasts were used to examine significant (P < .05) interactions.

**Results and Discussion**

The effects of dietary treatment on swine performance and plasma constituents in Exp. 1 are shown in Table 2. There were no interactions.
### TABLE 1. COMPOSITION OF TREATMENT DIETS, %

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>72.79</td>
<td>66.72</td>
<td>66.87</td>
<td>61.80</td>
</tr>
<tr>
<td>Soybean meal (48%)</td>
<td>24.28</td>
<td>25.34</td>
<td>30.24</td>
<td>30.31</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-lysine HCl(^b)</td>
<td></td>
<td></td>
<td>.25</td>
<td></td>
</tr>
<tr>
<td>DL-methionine(^c)</td>
<td></td>
<td></td>
<td>.25</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>.42</td>
<td>.37</td>
<td>.48</td>
<td>.42</td>
</tr>
<tr>
<td>Defluorinated phosphate</td>
<td>1.64</td>
<td>1.70</td>
<td>1.55</td>
<td>1.61</td>
</tr>
<tr>
<td>Salt</td>
<td>.35</td>
<td>.35</td>
<td>.35</td>
<td>.35</td>
</tr>
<tr>
<td>Vitamin-trace mineral premix(^d)</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Antioxidant(^e)</td>
<td>.0125</td>
<td>.0125</td>
<td>.0125</td>
<td>.0125</td>
</tr>
<tr>
<td>Antibacterials(^f)</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
</tbody>
</table>

\(^a\) As-fed basis. Formulated with clean and aflatoxin-contaminated corn for a total of eight treatments in each experiment.

\(^b\) Contained 78% L-lysine.

\(^c\) Contained 98% DL-methionine.

\(^d\) Contained per kg of premix: vitamin A, 4,400,000 IU; vitamin D\(_3\), 1,056,000 IU; vitamin E, 8,800 IU; vitamin K, 2.2 g; vitamin B\(_{12}\), 13.2 mg; riboflavin, 2.6 g; d-pantothenic acid, 8.8 g; niacin, 17.6 g; choline chloride, 308 g; folic acid, 264 mg; d-biotin, 44 mg; Zn, 10 g; Fe, 110 g; Mn, 55.4 g; Cu, 8.8 g; I, 1.1 g; Co, 440 mg; Se, 264 mg.

\(^e\) Ethoxyquin.

\(^f\) Contained per kilogram of antibiotic premix: chlortetracycline, 44 g; sulfathiazole, 44 g; penicillin, 22 g.
TABLE 2. EFFECTS OF DIETARY FAT AND CRUDE PROTEIN LEVEL ON PERFORMANCE AND PLASMA CONSTITUENTS (EXPERIMENT 1) OF SWINE FED CLEAN AND AFLATOXIN-CONTAMINATED CORN

<table>
<thead>
<tr>
<th>Item</th>
<th>No aflatoxin</th>
<th>Aflatoxin&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% fat, 18% CP</td>
<td>0% fat, 18% CP</td>
</tr>
<tr>
<td>ADG, kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.38</td>
<td>.37</td>
</tr>
<tr>
<td>Average daily feed intake, kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.68</td>
<td>.67</td>
</tr>
<tr>
<td>Feed/gain&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.81</td>
<td>1.84</td>
</tr>
<tr>
<td>Plasma constituents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dl&lt;sup&gt;d&lt;/sup&gt;</td>
<td>114.5</td>
<td>89.9</td>
</tr>
<tr>
<td>Triglycerides, mg/dl&lt;sup&gt;e&lt;/sup&gt;</td>
<td>47.7</td>
<td>60.5</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>12.5</td>
<td>11.4</td>
</tr>
<tr>
<td>Protein, g/dl</td>
<td>6.4</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Aflatoxin-contaminated diets were mixed with corn containing 182 ppb aflatoxin B₁.

<sup>b</sup>Aflatoxin × CP interaction (P < .05).

<sup>c</sup>Fat effect (P < .05).

<sup>d</sup>Aflatoxin × CP × fat interaction (P < .05).

<sup>e</sup>CP × fat interaction (P < .05).
(P > .05) between the experimental factors and trial; therefore, the data from both trials were combined. The AFB1 × CP interaction (P < .05) was significant for ADG, average daily feed intake and feed to gain ratio (F/G). Feeding corn containing 182 ppb AFB1 reduced (P < .05) ADG (.30 vs .37 kg/d) and average daily fed intake (.57 vs .66 kg/d) and increased F/G (1.88 vs 1.78) of weanling pigs fed 18% CP diets. In contrast, AFB1 did not affect performance of pigs fed 20% CP. Adding 5% poultry fat to diets improved (P < .05) feed efficiency regardless of protein content or the level of AFB1 in the diet, but added fat did not improve (P > .05) growth rate of pigs fed AFB1.

There was an AFB1 × CP × fat interaction (P < .05) for plasma cholesterol level. Feeding AFB1-contaminated corn lowered (P < .05) cholesterol of pigs fed the diet containing 18% CP diets without added poultry fat. The addition of either fat or protein prevented the depression in cholesterol level in pigs fed AFB1-contaminated corn. There was a CP × fat interaction (P < .05) for plasma triglyceride. Adding 5% fat to 18% CP diets increased (P < .05) plasma triglyceride regardless of corn source, but adding fat to diets containing 20% CP did not change triglyceride level. Plasma urea and protein concentration were not (P > .05) affected by dietary treatment.

Aflatoxin and dietary protein influence protein synthesis by the liver (DeRecondo et al., 1977). Sisk and Carlton (1972) speculated that a lack of adequate dietary protein may interfere with the ability of the liver to synthesize enzymes important for the metabolism and detoxification of the aflatoxin molecule, resulting in a longer exposure and increased toxic effects due to aflatoxin.

Because aflatoxins interfered with the digestion of dietary fats and fat-soluble vitamins, increasing dietary fat may counteract an increased dietary requirement for essential fatty acids during aflatoxicosis (Lanza et al., 1981). It has been hypothesized that AFB1 alters the integrity of cellular membranes and that the effect on lipid metabolism is the primary lesion during aflatoxicosis (Tung et al., 1972). Blood levels of triglycerides, phospholipid and esterified cholesterol were lowered by AFB1, apparently due to a general inhibition of lipid transport (Hamilton, 1977). Blood total lipid and phospholipids, which normally are decreased in aflatoxicosis, were restored to normal values by the use of high fat in poultry diets (Hamilton et al., 1972). In the present study, additional fat or protein prevented the characteristic depression of plasma cholesterol concentration due to aflatoxicosis, but added fat did not improve the growth rate of pigs fed AFB1. Feed efficiency was improved as a result of adding supplemental fat. This has been reported previously by numerous authors and is due to the increased energy density of the fat.

The results from Exp. 1 indicated that increasing dietary CP from 18 to 20% prevented the detrimental effects of AFB1 on the performance of weanling swine. To increase dietary protein, a reduction in the corn to soybean meal ratio of the diets was required. Because corn was the source of AFB1, there was a slight reduction in the AFB1 content of the diet (132 vs 121 ppb); it is not likely that this difference influenced the results. Exp. 2 was designed to examine the effect of supplementing L-lysine HCl and(or) DL-methionine to the diet. The corn and soybean meal content of the diets were not altered; therefore, AFB1 essentially was equal across AFB1-contaminated treatments. Wyatt (1985) reported that increasing the methionine level in broiler diets had a sparing effect on the growth depression of AFB1. Methionine is the most limiting amino acid in corn-soybean meal diets for broilers, whereas lysine is the most limiting amino acid for swine.

The effects of supplemental lysine or methionine are shown in Table 3 (Exp. 2). There were no significant main effects on performance or plasma constituents. The AFB1 × lysine level interaction was significant (P < .05) for ADG. Similar to the results of Exp. 1, feeding corn containing 186 ppb AFB1 reduced (P < .05) ADG (.44 vs .50 kg/d) of pigs fed an 18% CP diet. However, AFB1 did not depress the performance of pigs fed diets supplemented with .25% L-lysine HCl. Also, supplementing the 18% CP diet with .15% DL-methionone prevented (P > .05) depression in gains of pigs fed AFB1-contaminated corn. Growth rate was intermediate between that of pigs fed clean or aflatoxin-contaminated corn with no amino acid supplementation.

There was an AFB1 × methionine level interaction (P < .05) for plasma triglyceride level. Adding .15% DL-methionine increased (P < .05) plasma triglycerides of pigs fed clean but not AFB1-contaminated corn.

The method by which dietary lysine functions to protect against aflatoxicosis is not clear. Aflatoxin B1 is metabolized in the liver
TABLE 3. EFFECTS OF SUPPLEMENTAL LYSINE AND METHIONINE ON PERFORMANCE AND PLASMA CONSTITUENTS OF SWINE FED CLEAN AND AFLATOXIN-CONTAMINATED CORN (EXPERIMENT 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>No aflatoxin</th>
<th>Aflatoxin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Aflatoxin&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% Lys&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0% Lys&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.25% Lys&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0% MET&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.15% MET</td>
<td>0% MET</td>
</tr>
<tr>
<td>ADG, kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.50</td>
<td>.51</td>
<td>.49</td>
</tr>
<tr>
<td>Average daily feed intake, kg</td>
<td>.99</td>
<td>1.12</td>
<td>.97</td>
</tr>
<tr>
<td>Feed/gain</td>
<td>2.01</td>
<td>2.03</td>
<td>1.96</td>
</tr>
<tr>
<td>Plasma constituents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>113.1</td>
<td>129.1</td>
<td>117.9</td>
</tr>
<tr>
<td>Triglycerides, mg/dl&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60.3</td>
<td>89.9</td>
<td>76.0</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>15.7</td>
<td>14.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Protein, g/dl</td>
<td>7.2</td>
<td>8.6</td>
<td>7.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Aflatoxin-contaminated diets were mixed with corn containing 182 ppb aflatoxin B<sub>1</sub>.

<sup>b</sup>LYS = L-lysine HCl; MET = DL-methionine.

<sup>c</sup>Aflatoxin × lysine interaction (P < .05).

<sup>d</sup>Aflatoxin × methionine interaction (P < .05).
by mixed-function oxidase enzymes and, in theory, when AFB\textsubscript{1} is metabolized more rapidly, the toxicity may be reduced (McLean and McLean, 1966; Madhaven and Gopalan, 1968). Protein deficiency has been shown to depress mixed-function oxidase activity (Clinton et al., 1977). Perhaps increased dietary lysine, the most limiting amino acid for swine in corn-soybean meal diets, enhances mixed-function oxidase activity and increases the conversion of AFB\textsubscript{1} into less toxic metabolites (Smith, 1989).

Extra dietary methionine may function to protect against the detrimental effects of AFB\textsubscript{1} via glutathione (Mgbodile et al., 1980). Glutathione is a tripeptide synthesized from cysteine, glutamine and glycine. Studies conducted with rats have shown that increasing dietary methionine led to increased liver glutathione (Seligson and Rotruck, 1983), and glutathione has been demonstrated to suppress the effects of AFB\textsubscript{1} in several species (Mgbodile et al., 1980; Novi, 1981). Glutathione conjugates AFB\textsubscript{1} in the liver and renders it nontoxic, after which it is excreted.

These data further demonstrated the interactions between the concentration of dietary protein and the response of swine to AFB\textsubscript{1}. Previous work had shown that suboptimal protein concentration in swine diets resulted in increased toxicity (Sisk and Carlton, 1972). The present studies indicated that, in addition, increasing dietary protein or amino acids above the normal requirement for optimal performance prevented depressed performance due to AFB\textsubscript{1}. There was no effect on performance due to added nutrients in diets not contaminated with AFB\textsubscript{1}; therefore, the basal diets were not deficient.

The level of AFB\textsubscript{1} that is required to produce a consistent depression in performance has not been determined. Allcroft et al. (1963) reported that 280 ppb AFB\textsubscript{1} depressed growth and efficiency of pigs, whereas Hauser et al. (1971) found even more severe effects and clinical symptoms, such as hemorrhaging, in pigs fed 150 ppb AFB\textsubscript{1}. In contrast, other authors have concluded that 300 to 410 ppb AFB\textsubscript{1} were required to consistently depress swine performance (Carnagan and Crawford, 1964; Hintz et al., 1967; Duthie et al., 1968; Monegue, 1977a; 1977b; Southern and Clawson, 1979). Because interactions exist, a minimum AFB\textsubscript{1} tolerance cannot be established without considering levels of critical nutrients in the diet.

**Literature Cited**


Mgbodile, M. U., M. Holscher and A. Neal. 1980. A


