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Suppression of Serum Iron-Binding Capacity and Bone Marrow Cellularity in Pigs Fed Aflatoxin

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Flavus-parasiticus species of the genus Aspergillus are recognized as the primary producers of aflatoxins B_1, B_2, G_1, and G_2, hereafter referred to as aflatoxin (AF). The effects of feeding AF-contaminated diets to growing and finishing pigs have been described with changes in clinical performance, serum biochemistry, histology, and hematology attributed to aflatoxicosis (Edds 1973; Hoerr et al. 1983). However, most of these studies evaluated AF-induced changes for a single AF dosage at a given point in time. The present study was designed to characterize how various AF dosages influence bone marrow histology, hematology, prothrombin and activated thromboplastin times, serum amino acids, and serum iron binding capacity during aflatoxicosis in growing pigs.

MATERIALS AND METHODS

Twenty-five, 6-week-old crossbred (Yorkshire X Landrace X Hampshire) weaned barrows (mean wt=11.9 kg) were housed in concrete-floored covered outdoor pens, and provided starter diet and water ad libitum. Corn/soybean starter diets (20% protein) met or exceeded the levels of critical nutrients recommended by the National Research Council (1979). The experimental design consisted of 5 dietary treatments of 0, 1, 2, 3, or 4 mg AF/kg of feed fed to 5 pigs per treatment for 28 days. Aflatoxin was produced through the fermentation of rice by Aspergillus parasiticus NRRL 2999 as described by Shotwell et al. (1966) and modified by West et al. (1973). The fermented rice was autoclaved to kill the fungus, ground to a powder, and AF content determined by spectrophotometric analysis (Nabney 1965; Wiseman 1967). The rice was then incorporated into starter diets to provide the AF concentration desired, and the rice powder did not exceed 1% of the diet. Basal diets were analyzed for mycotoxins and had no detectable levels of AF, deoxynivalenol, zearalenone, ochratoxin, or cyclopiazonic acid. Treatment diets were analyzed and parent AF (which was spiked into basal ration) was structurally confirmed via capillary GC/quadrupole mass spectrometry (Clement et al. 1985).

Pigs were fed their respective diets for 28 days, observed twice a day, and weighed weekly. Group feed consumption was recorded once a week. Vena caval blood samples were obtained weekly in the morning for serum and hematologic analyses.

Hematologic parameters measured included erythrocyte count (RBC), leukocyte count (WBC), WBC differential, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Serum measurements included prothrombin time (PT), activated partial thromboplastin time (APTT), serum amino acids (AA), serum iron (SI), unsaturated iron binding capacity (UIBC), and total iron binding capacity (TIBC). Procedures for RBC, WBC, PCV, Hb, and MCV were performed on automated equipment according to manufacturer's recommendations and have been described previously (Harvey et al. 1984; Kubena et al. 1985). Mean corpuscular hemoglobin and MCHC were calculated. Serum iron and TIBC procedures (No. 565, Sigma Diagnostics, St. Louis, MO) were performed manually. Measurements for PT and APTT were accomplished by following methods described by Steel et al. (1976). Serum AA analysis was performed on an amino acid autoanalyzer according to the manufacturer's recommendations (LKB Instruments, Inc., Gaitherburg, MD). On day 29 of the study, all pigs were euthanatized (T-61, American Hoechst Corp, Somerville, NJ). Samples of bone marrow (1-2 g) from mid-shaft right femur were fixed in neutral buffered 10% formalin, embedded into paraffin, sectioned at 5μm, and stained with hematoxylin and eosin for histologic examination.

Group means for RBC, WBC, WBC differential, PCV, Hb, MCV, MCH, MCHC, PT, APTT, SI, UIBC, TIBC, and AA were compared statistically according to the general linear model procedure for analysis of variance and ranked by the Duncan's multiple range test ($P<.05$) (SAS Institute Inc., 1982).

RESULTS AND DISCUSSION

Pigs fed the diets containing 0, 1, and 2 mg AF/kg appeared clinically normal throughout the study whereas the pigs given feed containing 3 and 4 mg AF/kg were slightly dehydrated and listless, had roughened haircoats, consumed less feed, and did not gain weight as efficiently as the first 3 groups (Harvey et al. 1987). Two pigs in the group fed 4 mg AF/kg of feed became icteric, moribund, and were euthanatized (day 25 and 28). Analysis of hematologic data (Table 1) for day 28 revealed that when compared to controls, RBC, PCV, WBC, and Hb were increased and MCV and MCH were decreased by AF treatments. Aflatoxin treatments also produced a decrease in granulocytic cellularity of the bone marrow (Fig. 1B) when compared to control (Fig. 1A).

Table 1. Hematologic values of pigs fed aflatoxin-contaminated diets for 28 days.

Parameter*	Dietary Aflatoxin (mg/kg feed) 0 N=5	1 N=5	2 N=5	3 N=5	4 N=3
RBC X 10^6 cells/µl	6.34±0.18^{d}	6.73±0.31^{d}	7.33±0.23^{bc}	7.77±0.23^{b}	8.53±0.12^{a}
MCV (μm^3)	56.6±0.60^{a}	58.2±0.60^{a}	58.2±0.60^{a}	57.0±1.00^{a}	49.0±1.15^{b}
PCV (%)	37.0±0.92^{c}	39.0±0.89^{bc}	43.0±1.21^{ab}	45.0±1.59^{a}	41.0±0.33^{bc}
WBC X 10^3 cells/µl	15.26±1.74^{b}	24.06±5.58^{b}	20.08±0.73^{b}	28.22±2.39^{ab}	41.37±12.84^{a}
Hb (g/dl)	12.2±0.36^{c}	13.0±0.32^{bc}	14.3±0.39^{ab}	15.0±0.55^{a}	13.6±0.51^{ab}
MCH (pg)	19.3±0.33^{a}	19.4±0.60^{a}	19.5±0.26^{a}	19.3±0.30^{a}	15.9±0.40^{b}
MCHC (%)	32.8±0.30^{a}	33.3±0.63^{a}	33.5±0.46^{a}	33.4±0.42^{a}	33.4±1.00^{a}
PT (seconds)	11.02±0.31^{c}	11.08±0.17^{c}	12.62±0.99^{b}	13.30±0.53^{b}	18.50±1.46^{a}
APTT (seconds)	18.72±1.45^{b}	21.24±1.74^{ab}	16.34±0.91^{b}	16.72±0.46^{b}	24.53±3.29^{a}

*RBC=erythrocytes, MCV=Mean corpuscular volume, PCV=packed cell volume, WBC=leukocytes, Hb=hemoglobin, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, PT=prothrombin time, APTT=activated partial thromboplastin time

a,b,c Expressed as mean values (± SEM). Values for the same parameter in the same row with different superscripts significantly ($P<0.05$) differ.

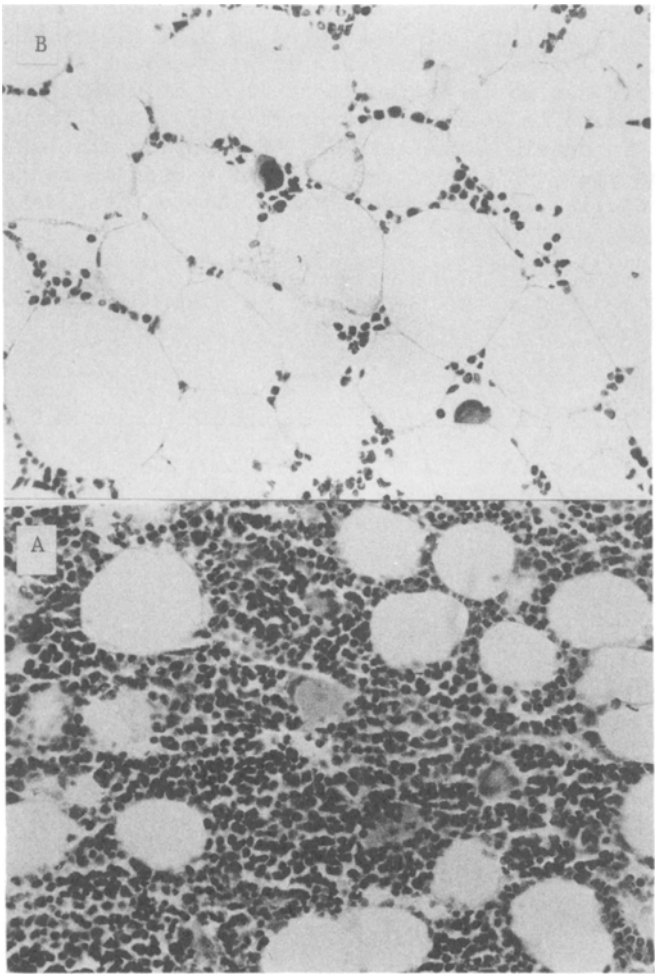

Fig. 1 Bone marrow granulocytic cellularity of pigs fed (A) control diet or (B) diet contaminated with 4 mg aflatoxin/kg feed for 28 days.

Aflatoxin-induced increases in circulating RBC, Hb, and WBC are at variance with bone marrow histopathology. However, reductions in MCV and MCH demonstrate that circulating RBC were smaller and probably older cells and support a diagnosis of bone marrow suppression. The etiology of increased RBC, Hb, and WBC (2, 3, and 4 mg AF/kg) in pigs is unknown. Hemoconcentration could produce these changes; however, clinically, only slight dehydration of pigs was observed. Hematologic changes noted in

aflatoxicosis have been variable and include declines in PCV and a rise, then a decline, of leukocytes in pigs (Cysewski et al. 1968); lymphopenia and eosinopenia in pigs (Hoerr et al. 1983); increased PCV and Hb in ponies (Bortell et al. 1983); increased RBC, PCV, and Hb in goats (Clark et al. 1984); and increases in PCV and Hb in cattle (Wyatt et al. 1985; Ray et al. 1986). In the present study, higher doseages of AF caused increases in WBC, neutrophils, and monocytes (Table 2) and these data agree with that reported (Hoerr et al. 1983).

Table 2. WBC differential (absolute) of pigs fed aflatoxin-contaminated diets for 28 days

Aflatoxin levels mg/kg feed	WBC*	Lymph	Seg	Mono
0	15.24(1.74)^{b}	7.405(0.413)^{b}	6.144(1.340)^{b}	1.088(.173)^{b}
1	24.06(5.58)^{b}	10.030(0.849)^{b}	9.858(3.590)^{b}	1.003(.322)^{b}
2	20.00(0.72)^{b}	10.355(1.476)^{b}	6.262(0.745)^{b}	1.425(.072)^{ab}
3	28.24(2.40)^{ab}	15.240(1.351)^{a}	8.842(0.849)^{b}	2.069(.649)^{ab}
4	41.37(12.84)^{a}	11.639(3.849)^{ab}	23.641(11.323)^{a}	2.601(.849)^{a}

* Values expressed as group means (± SEM) X 10^3 cells of 5 pigs/group except 4mg/kg (N=3)

^{ab} Values with different superscripts differ significantly ($P<0.05$)

Serum AA profiles at day 28 in AF-fed pigs were not significantly different from controls (data not shown). Prothrombin and APTT were elevated in all AF fed groups (Table 1). The increases noted in PT and APTT are consistent with results noted in previous studies (Osuna et al. 1982; Bortell et al. 1983). Anticoagulant properties of AF are related to its hepatotoxic nature and its ability to interfere with vitamin K utilization. Furthermore, hypoproteinemia, commonly associated with aflatoxicosis, may cause decreases in plasma fibrinogen levels thereby reducing clotting capabilities (Bortell et al. 1983). Reduced protein synthesis may also have contributed to reduced granulocytic cellularity of the bone marrow (Harvey et al. 1987) in pigs fed AF-contaminated diets..

Few differences are noted in SI throughout the present study, however, progressive reductions in UIBC and TIBC occur with increasing AF treatments (Table 3). Aflatoxin dosages of 2, 3, or 4 mg AF/kg feed caused decreases in UIBC and TIBC as early as 14 days. Serum iron is that amount of iron bound to transferrin (siderophilin). Transferrin is a beta-1 globulin with a molecular weight of approximately 83,000, and each molecule is able to bind two atoms of ferric iron, and has a half life of about 10 days (Henry 1969). It is documented that AF interferes with protein synthesis and causes decreases in total

protein, α globulin, β globulin, and γ globulin (Hoerr et al. 1983; Harvey et al. 1987). Because transferrin is a β globulin, aflatoxicosis should cause decreased transferrin levels, a higher iron saturation of remaining transferrin, and hence reduced UIBC and TIBC. This, in fact, was observed in the present study (Table 3).

Table 3. Serum iron, unsaturated iron binding capacity and total iron binding capacity of pigs fed aflatoxin-contaminated diets for 28 days.

Treatment	Time	SI*	UIBC	TIBC	Saturation
mg aflatoxin/kg feed	(days)	----------	g/dl	----------	%
0		135±23^{a}	332±38^{a}	467±22^{a}	29
1		126±21^{a}	352±46^{a}	479±40^{a}	26
2	7	125±36^{a}	291±52^{a}	416±33^{a}	30
3		154±19^{a}	242± 7^{a}	396±25^{a}	39
4		142±17^{a}	256±20^{a}	398±15^{a}	36
0		87±32^{a}	396±29^{a}	484±24^{a}	18
1		62±25^{a}	323±18^{ab}	385±26^{b}	16
2	14	70±24^{a}	303±19^{b}	374±27^{b}	19
3		111±14^{a}	245±16^{bc}	356±17^{b}	31
4		142±31^{a}	211±44^{c}	353±20^{b}	40
0		143±28^{a}	368±34^{a}	511±30^{a}	28
1		159±23^{a}	304±36^{a}	464±18^{ab}	34
2	21	214±52^{a}	176±36^{b}	391±36^{bc}	55
3		173±22^{a}	180±30^{b}	353±12^{cd}	49
4		162±15^{a}	118±47^{b}	280±36^{d}	58
0		105±21^{c}	378±33^{a}	483±24^{a}	22
1		132±42^{bc}	358±27^{a}	490±48^{a}	27
2	28	224±22^{a}	117±45^{b}	341±41^{b}	66
3		204±10^{ab}	91±20^{b}	295±13^{bc}	69
4		189±28^{abc}	28± 2^{b}	217±27^{c}	87

* SI=serum iron; UIBC=unsaturated iron binding capacity; TIBC=total iron binding capacity

a,b,c,d Values are expressed as mean (± SEM). Values with different superscripts significantly ($P<0.05$) differ. N=5 for each treatment except 4 mg/kg feed treatment on day 28 (N=3).

Transient reductions in TIBC and transferrin have been reported for pigs inoculated with Salmonella cholerae-suis (Kramer et al. 1986). However, it is uncertain if host-bacterial interactions that affect iron binding capacity differ from mechanisms of action of aflatoxin on iron binding capacity. Diminished iron binding capacity has been observed in acute and chronic infections, pernicious anemia, hemolytic anemia, cirrhosis of the liver, uremia, malignancy (Wintrobe 1965), and in conditions of decreased circulating protein (Henry 1969). In the author's opinion, TIBC may have potential as a diagnostic or prognostic test when dealing with suspected aflatoxicosis in pigs.

In summary, the hematologic and hematopoietic systems of growing pigs are affected when pigs are fed diets containing 1 to 4 mg AF/kg feed for 28 days. Aflatoxin treatments resulted in bone marrow suppression and reduced MCV, MCH, UIBC, and TIBC; whereas, WBC, PT, and APTT were increased. Total iron binding capacity was more sensitive than other measured variables to the effects of dietary AF.

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