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# Effect of Aflatoxin and Diacetoxyscirpenol in Ewe Lambs

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Aflatoxins are polysubstituted bisfuranoscoumarins that are secondary fungal metabolites produced by the flavus-parasiticus group of the genus Aspergillus. The toxic effects of aflatoxin (AF) in livestock have been well documented and may include hepatotoxicosis, immunosuppression, reduced performance, oncogenesis, or death (Cast 1989; Shull 1985). Lambs are sensitive to AF and decreased performance, serum biochemical alterations, pathologic hepatic lesions, decreased immune function, and death are effects of AF which can be of economic importance (Harvey et al. 1991b; Suliman et al. 1987; Armbrecht et al. 1970).

Diacetoxyscirpenol (DAS) is a trichothecene mycotoxin produced by several Fusarium species that has been reported in cereal grains, feeds, and agricultural commodities worldwide (Williams 1989). Like T-2 toxin, DAS has been described as radiomimetic with regard to lymphoid tissues and gastrointestinal epithelium, as a contact necrotizing agent for lingual and buccal mucosa, and an inhibitor of DNA and protein synthesis (Williams 1989). It is unknown how sensitive sheep are to DAS, however, T-2 toxin induces toxicity in lambs (Friend et al. 1983) and DAS and T-2 toxin are very similar in their chemical structure and have similar toxicity for most species of animals. It is a common practice to use multiple grain sources in sheep diets and feeding diets contaminated with both AF and DAS is a possibility. The objective of the present study was to investigate the toxicity of AF and DAS and to describe the major effects of feeding diets co-contaminated with AF and DAS to growing lambs.

## MATERIALS AND METHODS

Twenty-four, weaned Rambouillet X Suffolk crossbred ewe lambs (mean wt = 38 kg) were individually identified, treated with an anthelminthic for internal parasites, vaccinated and boosted with a multi-valent Clostridial bacterin, assigned by weight to groups, and randomly assigned to treatments. Lambs were housed in covered outdoor pens, provided ad libitum access to water, and over a 14 day period, were acclimated to an isocaloric-isonitrogenous cottonseed meal/alfalfa-based diet (16% protein, 27% fiber). The experimental design consisted of 4 dietary treatments (3 replicates of 2 lambs each, for a total of 6 lambs/treatment group) of 0 mg AF, 0 mg DAS/kg of feed (control); 2.5 mg AF/kg of feed; 5.0 mg DAS/kg of feed; or 2.5 mg AF plus 5.0 mg DAS/kg of feed. Aflatoxin was produced through the fermentation of rice by

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Aspergillus parasiticus NRRL 2999 as described (Shotwell et al. 1966) and modified (West et al. 1973). The fermented rice was autoclaved, dried, and ground to a powder, and the AF content was measured by spectrophotometric analysis (Hutchins and Hagler 1983). Total aflatoxins in the rice powder consisted of 79% $AFB_1$, 16% $AFG_1$, 4% $AFB_2$, and 1% $AFG_2$. The rice powder was mixed into diets and did not exceed 1% of the diet. The DAS (courtesy, Dr. George Rottinghaus) was 98% pure as determined by nuclear magnetic resonance and mass spectrometry (Sanson et al. 1989). The DAS was incorporated into the diet by dissolving the toxin in 95% ethanol, adding prescribed amounts to a 2-kg aliquot of feed, drying the aliquot, then mixing the aliquot into the remaining portion of the diet. Basal diets were analyzed for mycotoxins and did not have detectable concentrations of AF, DAS, T-2 toxin, zearalenone, deoxynivalenol, ochratoxin, or cyclopiazonic acid. Treatment diets were analyzed, and the presence of the parent AF and DAS (which were added to the basal diets) was structurally confirmed via capillary gas chromatography/quadrupole mass spectrometry (Clement and Phillips 1985). Lambs were fed their respective diets ad libitum and feed consumption recorded daily for 34 days. Lambs were observed twice daily and weighed weekly.

On day 34, blood samples for hematologic, serum biochemical, and immunologic measurements were obtained from the external jugular of each lamb. Hematologic parameters measured included erythrocyte count (RBC), leukocyte count (WBC), hematocrit, hemoglobin, mean cell volume (MCV), mean cell hemoglobin, and mean cell hemoglobin concentration. Serum measurements included prothrombin and activated partial thromboplastin times, enzyme activities of aspartate transaminase (AST), alkaline phosphatase, cholinesterase (ChE), and γ-glutamyltransferase (GGT), and serum concentrations of albumin, calcium, cholesterol (Chol), creatinine, glucose, inorganic phosphorus, total protein (TP), total iron, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), triglycerides, and urea nitrogen. Immunologic evaluation consisted of in vitro mitogen-induced lymphoblastogenesis stimulation indices (uptake of tritiated thymidine) for measurement of cell-mediated immune response. Procedures for RBC, WBC, hematocrit, hemoglobin, and MCV were performed using a Coulter counter (model ZBI counter and model C256 analyzer, Hialeah, Florida) according to manufacturer's recommendations and have been described previously (Harvey et al. 1984). Mean cell hemoglobin and mean cell hemoglobin concentration were calculated (Schalm et al. 1975). All serum biochemical values were determined using a computer-driven autoanalyzer (Gilford Impact 400E, Ciba Corning Diagnostics Corp., Gilford Systems, Oberlin, OH). Testing for prothrombin and activated partial thromboplastin times was done using an automated coagulation instrument according to the manufacturer's instructions (Electra 600 D4, modified with animal clot time option, Medical Laboratories Automation, Mt. Vernon, NY.). Blastogenic responses to phytohemagglutinin (PHA, No. HA17, Burroughs-Wellcome Diagnostics, Research Triangle, NC) were measured utilizing reported procedures (Harvey et al. 1991b; Lee 1978). Stimulation indices for each blood sample were calculated as counts in PHA-stimulated cells, minus counts in non-stimulated cells, divided by counts in non-stimulated cells.

On day 35 of the study, all lambs were euthanized (Sleepaway Euthanasia Solution, Ft. Dodge Laboratories, Ft. Dodge, IA) and necropsied. Liver, left kidney, spleen, and heart were weighed, and organ weight was calculated as a percentage of body weight

(g/100g). Specimens of liver were fixed in neutral buffered 10% formalin, embedded into paraffin, sectioned at 5μm, and stained with hematoxylin and eosin for histologic examination.

Data were grouped by treatment and expressed as mean ± SEM. Pen means for body weight, organ weight, and hematologic, immunologic, and serum biochemical measurements were subjected to ANOVA (Snedecor and Cochran 1967) using the general linear models procedure software (SAS 1985). Significant differences in ANOVA means for treatment groups were compared using the Duncan multiple-range procedure (Duncan 1955). All statements of significance were based on the 0.05 level of probability.

## RESULTS AND DISCUSSION

Lambs fed the control diet appeared clinically normal throughout the feeding trial, whereas lambs fed the DAS-, and AF plus DAS-contaminated diets consumed less feed and lost weight (Table 1). Lambs consuming the AF-contaminated diet had total feed intake that was decreased from control and gained numerically, but not statistically, less weight compared to control (96% reduction). This is in agreement to the 92% reduction in weight gain observed for lambs fed diets containing 2.6 mg AF/kg of feed for 42 days (Harvey et al. 1991b). Inappetance and decreased weight gain induced by DAS and the DAS plus AF diets are similar to that observed for cattle treated with T-2 toxin (Williams 1989) or with swine consuming AF and DAS (Harvey et al. 1991a). The combination of mycotoxins in the present study induced a synergistic interaction for reduced weight gain when compared to the effects of each toxin singly and was similar to the toxic synergy for weight gain observed in broiler chicks fed diets with AF and DAS in combination (Kubena et al. 1993).

In the present study, treatment with AF increased Chol and TIBC, treatment with DAS decreased ChE and urea nitrogen, whereas the combination treatment of AF plus DAS increased Chol, GGT, TP, and TIBC and decreased ChE and urea nitrogen. No treatment differences were observed for prothrombin or activated partial thromboplastin times, for AST or alkaline phosphatase activities, for albumin, calcium, creatinine, glucose, inorganic phosphorus, total iron, UIBC, triglycerides, RBC, WBC, hematocrit, MCV, mean cell hemoglobin, mean cell hemoglobin concentration, mitogen-induced lymphoblastogenesis, or for organ weights of liver, kidney, spleen, or heart (data not shown). Although serum biochemical analytes of the present study were altered by AF, DAS, and AF plus DAS, there appears to be subtle differences between the results of this study and a similar study of AF (2.5 mg AF/kg feed) and DAS (2.0 mg DAS/kg feed) administered to pigs (Harvey et al. 1991a). In that study, AF-treated pigs had increased GGT, ChE, alkaline phosphatase, creatinine, hematocrit, and hemoglobin and decreased urea nitrogen and TIBC concentrations; DAS-treated pigs had decreased TIBC; and AF plus DAS-treated swine had increased GGT, ALP, and hemoglobin and decreased urea nitrogen, UIBC, and TIBC (Harvey et al. 1991a). The reasons for these differences are unclear; however, they are probably related to species specific responses to toxins and the differences in DAS dosages (2 mg DAS/kg feed for pigs and 5 mg DAS/kg feed for lambs). Broiler chicks fed AF and DAS in combination had decreased triglycerides, Chol, glucose, and TP concentrations, decreased activities of lactate dehydrogenase, and increased GGT

Table 1 Performance and serum biochemical values of ewe lambs fed control diet or diets contaminated with aflatoxin (AF), diacetoxyscirpenol (DAS), and AF plus DAS for 34 days.

| | Treatment | | | |
|---|---|---|---|---|
| | Control | AF | DAS | AF + DAS |
| Initial wt (kg) | 38.6 ± 2.1[a] | 37.7 ± 2.6[a] | 38.0 ± 3.7[a] | 38.3 ± 1.9[a] |
| Wt gain (kg) | 2.4 ± 0.9[a] | 0.1 ± 0.5[ab] | -0.6 ± 0.7[bc] | -2.7 ± 0.3[c] |
| Feed Intake (kg/pen/day) | 2.74 ± .03[a] | 2.56 ± .03[b] | 2.48 ± .04[bc] | 2.42 ± .04[c] |
| Cholesterol (mg/dl) | 93 ± 5.2[bc] | 120 ± 3.0[a] | 78 ± 2.9[c] | 125 ± 13.7[a] |
| Urea nitrogen (mg/dl) | 21.4 ± 1.8[a] | 17.9 ± 0.7[ab] | 16.4 ± 1.4[b] | 15.1 ± 1.6[b] |
| Total Protein (g/dl) | 5.97 ± 0.2[b] | 6.38 ± 0.1[ab] | 6.10 ± 0.1[ab] | 6.65 ± 0.2[a] |
| TIBC (μg/dl) | 287 ± 11.2[b] | 358 ± 22.2[a] | 284 ± 2.5[b] | 346 ± 18.1[a] |
| Cholinesterase (IU/l) | 246 ± 9.7[a] | 259 ± 29.5[a] | 167 ± 3.5[b] | 175 ± 7.1[b] |
| γ glutamyltransferase (IU/l) | 43.6 ± 0.9[b] | 54.1 ± 0.4[ab] | 37.5 ± 3.5[b] | 76.2 ± 16.0[a] |

[abc] Values are expressed as mean ± SEM. Values in a row with no common superscript are different ($P<0.05$). N = 3 (3 replicates of 2 lambs each) per treatment group. TIBC = total iron binding capacity.

activities (Kubena et al. 1993). The increases of Chol and TIBC in the AF and AF plus DAS treatments would suggest that these effects were due to AF, and DAS at the dosage used in our study, did not add to this toxicity. Decreased urea nitrogen and ChE in the DAS and AF plus DAS groups could be a reflection of DAS toxicity, and at the dosage used in this study, AF was unable to overcome these effects and express its toxicity. The increased GGT of the AF plus DAS-treated lambs in the present study represent a significant ($P<0.05$) synergistic interaction for AF and DAS. Altered serum concentrations of Chol, TIBC, urea nitrogen, and ChE are probably a result of the combined hepatotoxicity induced by AF (Harvey et al. 1991b) and inhibition of protein and DNA biosynthesis induced by DAS (Williams 1989). T-2 toxin fed to calves is reported to increase prothrombin times (Williams 1989) and serum activities of AST (Williams 1989; Gentry et al. 1984), but in our study serum AST and prothrombin times for DAS treatments were not different from control values. It is unknown why serum TP concentrations of the present study were increased in the AF plus DAS treatment. This is in contrast to decreased serum TP for broiler chicks fed AF or AF plus DAS diets (Kubena 1993), whereas AF diets in lambs and AF plus DAS diets in pigs did not alter TP, but did decrease serum albumin (Harvey 1991a, 1991b). T-2 toxin is reported to induce leukopenia and lymphopenia in lambs (Friend et al. 1983), and to cause a marked decrease in WBC count and a decline in neutrophil count in calves (Gentry et al. 1984), but in our study, no differences were observed for WBC or other hematologic parameters.

Oral and dermal lesions were not induced by DAS in the present study, however, DAS has been reported to cause such lesions in swine (Williams 1989) and broiler chicks (Kubena et al. 1993). Edema and congestion in the gastrointestinal tract are often associated with DAS (Williams 1989), and although we did not see lesions at necropsy in DAS-treated lambs of our study, both of the DAS-treated groups experienced diarrhea for the first week of the study. Although we did not see altered liver weight in

either of the AF treatments of our study, livers were pale, rubbery, and resistant to cutting. Microscopically, mild hepatocellular lipidosis accompanied by early periportal and interlobular fibrosis was consistently present in lambs fed AF and hepatic lesions were not more severe in lambs fed AF plus DAS than in those fed AF alone. These lesions are compatible with aflatoxicosis and agree with those observed in lambs fed AF (Harvey et al. 1991b). Although AF is reported to induce immunosuppression and DAS has been described as radiomimetic (CAST 1989; Harvey et al. 1991b), AF or DAS treatments of the present study did not significantly alter lymphocyte blastogenic responses.

In conclusion, a lamb diet containing 2.5 mg AF/kg of feed resulted in hepatocellular disease and serum biochemical alterations. A diet of 5.0 mg DAS/kg of feed resulted in weight loss and altered serum biochemical values. When AF and DAS were fed in combination, significant synergistic interaction occurred for reduced weight gain and for increased serum activities of GGT. Altered serum urea nitrogen, Chol, and TIBC concentrations and ChE activities induced by the AF and DAS combination under conditions of this study, could best be described as additive or less than additive.

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