Effects of Aflatoxin on Reproduction in Swine. (32387)

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Aflatoxins, metabolites of *Aspergillus flavus* have been demonstrated to be toxic to several species of animals(1). It has also been suggested that levels of aflatoxin below the level required for acute toxicity may induce hepatic carcinomas(2). Hintz *et al*(3) reported that Duroc pigs (12-14 weeks old at start of trial) fed rations containing 450 parts per billion (ppb) of aflatoxin B_1 did not exhibit decreased rate of gain or feed conversion. There were no specific gross lesions but microscopic lesions were observed in 3 of 9 animals when the pigs were autopsied at market weight (90 kg) Gagné *et al* (to be published). The weight gains of pigs fed rations containing 615 ppb were decreased and pigs fed 810 ppb had even larger decreases in weight gains and one of 10 pigs died.

LeBreton *et al*(4) reported that aflatoxin B_1 caused fetal growth retardation and fetal death in rats and Elis and Dipaolo(5) reported that aflatoxin B_1 was teratogenic in hamsters. The trial reported herein was conducted to study the effect of feeding a ration containing 450 ppb aflatoxin B_1 on reproductive performance of swine.

Materials and methods. The growth rate and reproductive performance of 5 Duroc gilts and 5 Duroc boars fed a ration containing 450 ppb aflatoxin B_1 was compared to the performances of 9 gilts and 7 boars fed standard rations. The ration consisted (in percentage) of 75 ground barley, 15 peanut meal (contained 3000 ppb aflatoxin B_1)†‡, 4 meat and bone meal, 5 alfalfa meal, 0.5 salt, 0.5 premix which supplied 220 mg of $ZnSO_4$ and 1300 IU vitamin A/kg of ration.

Since the degree of toxicity produced from a given intake of aflatoxin is apparently reduced as the level of protein in the diet is increased(7), it should be mentioned that in this work a practical type basal ration was used containing 19-20% crude protein on a dry matter basis.

The animals were 12-14 weeks old at the start of the trial and were fed *ad libitum* until they weighed approximately 130 kg. At that time, the daily intake was restricted to 2.2 kg per animal to prevent over-fatness. The boars were restricted to this level until the end of the trial but the gilts were returned to *ad libitum* feeding during lactation.

The gilts were bred when they were approximately 8 months old. One of the boars fed aflatoxin was randomly selected and used to breed the gilts fed aflatoxin, and semen samples were obtained from the other 4 boars and 2 control boars *via* artificial vagina. The boars fed aflatoxin were slaughtered and autopsied 248 days after the start of the trial. When the litters were about 6 weeks old (325 days after the start of the trial), the gilts fed aflatoxin and 3 piglets from each of their litters and a total of 6 piglets from control litters were slaughtered and autopsied.

The following tissues were collected from each animal at necropsy, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin: turbinate, trachea, bronchial lymph node, lung, tonsil, mandibular salivary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, liver, gall bladder, kidney, urinary bladder, spleen, testes or ovary, prostate, urethra, penis, accessory sex glands, uterus, mammary gland, thyroid gland, pancreas, thymus, adrenal, pituitary, psoas muscle, diaphragm, myocardium, atrium, aorta, skin, eyes, cerebral cortex, basal ganglia, pons, cerebellum and medulla.

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‡ The aflatoxin was produced on a rice substrate at the Northern Regional Research Lab., Peoria, Ill., by O. L. Shotwell *et al* (6). The procedure used for spiking the peanut meal at the Southern Regional Research Lab., New Orleans, La., was described previously (3).

Results. Confirming our previous work (3), growth was in the normal range for the boars and gilts fed rations containing 450 ppb of aflatoxin during the growing-finishing period (23-90 kg). Specifically, the 5 boars fed the aflatoxin ration gained an average of 0.72 kg/day compared to a gain of 0.73 kg/day for 7 control boars, and the 5 gilts fed aflatoxin gained 0.71 kg/day compared to a gain of 0.74 kg/day for 9 control gilts.

Aflatoxin B_1 had no significant effect on sperm motility, per cent of live sperm or per cent of abnormal sperm. Further, aflatoxin B_1 had no significant effect on the reproductive performance of the gilts. Thus, an average of 8.6 live pigs and 0.6 dead pigs were farrowed per litter, the average litter weight at birth being 10.9 kg, and at weaning (42 days) there were 6.8 pigs per litter weighing 63.5 kg. For comparative purposes, from 9 control litters an average of 8.2 live pigs and 0.2 dead pigs were farrowed per litter, the average litter weight at birth being 10.8 kg and at weaning (42 days) there were 7.3 pigs per litter weighing 68.2 kg.

At autopsy, liver and kidney weights of the control and experimental pigs were very similar. The mean values were 1215 and 216 g per 100 lb body weight, respectively, for 6 pigs from sows fed aflatoxin and 1215 and 224 g per 100 lb body weight for 6 control pigs.

Seven pigs from the sows fed aflatoxin were used in a subsequent feeding experiment unrelated to aflatoxin. These pigs gained an average of 0.68 kg/day during the growing-finishing period while a group of control pigs fed a similar ration gained 0.69 kg/day. Thus, the growth performance of the offspring from sows fed aflatoxin was not affected.

The average total intake of aflatoxin B_1 per animal was 275 mg for the boars and 425 mg for the gilts.

Macroscopic lesions (boars, sows, piglets) were: scarring of most livers as a result of ascarid migration, abscess formation associated with castration, congenital cyst of one kidney and focal petechial hemorrhages which were agonal in origin. Two livers contained an abscess and an infarct was present in one kidney. None of these lesions was associated with aflatoxin.

Microscopic lesions resulting from aflatoxin were present in the liver of each sow. The lesions were minimal and consisted of cellular enlargement, abnormal cellular alignment, clumping or loss of cytoplasmic material and a minor accumulation of bile pigment within a few hepatocytes and Kupffer cells. Cellular hyperplasia, biliary hyperplasia and tumor formation were absent.

Hepatocytes in the liver of 3 boars lacked uniformity of staining and were irregular in alignment. Both lesions are suggestive of aflatoxicosis and would be placed in the $\pm$ category as described by Gagné *et al* (to be published). The remaining 2 boar livers had no detectable lesions. Microscopic examination of tissues from all body organs of the 15 piglets failed to reveal any lesions indicative of aflatoxicosis. Hepatic cells and nuclei were normal in size, appearance, staining quality and character. Cellular alignment was normal and sinusoidal spaces contained the usual compliment of endothelial cells and Kupffer cells. There was no evidence that prior cellular damage or degeneration had occurred and had undergone repair.

Discussion. Numerous reports indicate that young animals are more sensitive than mature animals to the toxic effects of aflatoxin. According to Allcroft(1) pigs from 3-12 weeks of age, and pregnant sows are very susceptible.

Our results provide definitive information on 3 phases of the life cycle of swine during exposure to aflatoxin. First, the 5 young boars fed a ration containing 450 ppb aflatoxin continuously (starting at 90 days of age) for a period of 248 days showed no abnormalities as regards growth rate and spermatogenesis. The only abnormality detected in 3 of the 5 boars involved a questionable microscopic lesion of the hepatocytes. Secondly, 5 gilts fed the same ration for 325 days all grew at a normal rate, farrowed normal litters, and lactated in a normal manner. Microscopic lesions were present in the liver of each sow at autopsy, the lesions

being minimal in severity. Thirdly, the piglets which were weaned at 6 weeks of age from the sows fed aflatoxin were found to be normal in size and number per litter and no microscopic lesions were found in the liver or other organs. Since no reproductive abnormalities were encountered when gilts were fed a ration containing 450 ppb aflatoxin before and during pregnancy and lactation, it may tentatively be concluded that this level of aflatoxin was non-toxic even with the added stress of pregnancy.

Butler and Wigglesworth(8) reported the liver of pregnant rats was apparently more susceptible to aflatoxin damage during late pregnancy than when the rats were not pregnant. However, it should be pointed out that the gilts in the present study received aflatoxin from weaning through lactation. Magwood *et al*(9) reported that low levels of aflatoxin induced a tolerance to toxic levels in turkeys. Therefore, the possibility exists that pregnant gilts that have not received aflatoxin prior to pregnancy may have a different toxic threshold level than pregnant gilts that have received non-toxic amounts of aflatoxin from weaning.

It has now been clearly established that the milk from lactating cows and ewes fed aflatoxin contains an aflatoxin metabolite designated aflatoxin M(10). Assuming the same would be true for lactating sows, it follows that the suckling pigs received an undetermined amount of aflatoxin M from the milk, supplemented by the aflatoxin consumed as the pigs ate the sows' ration. However, as stated above, there was no evidence of harmful effects being produced in the piglets under these conditions.

Summary. Five male and 5 female Duroc pigs 12-14 weeks of age were fed a practical type of ration containing 450 parts per billion aflatoxin B_1. At 8 months of age a reproduction experiment was conducted while the animals were continued on the same aflatoxin ration. Weight gains and spermatogenesis were normal in the 5 young males which were autopsied after 248 days on test. A questionable microscopic lesion of the hepatocytes in 3 of 5 boars was the only microscopic abnormality detected. The 5 females were autopsied after 325 days on test which included the periods of growth, pregnancy, and lactation, all of which were normal. Microscopic lesions were present in the livers of each sow, but were minimal in severity. Pigs weaned at 6 weeks of age from the females fed the aflatoxin ration were normal in size and number per litter and no microscopic lesions were detected.

The authors wish to thank P. T. Cupps for help with the semen examinations.

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Received May 17, 1967. P.S.E.B.M., 1967, v126.