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SHORT COMMUNICATIONS

Effect of diet on the lipid-depressing activity of aflatoxin B_1 in the Nigerian monkey

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The aflatoxins have been reported to inhibit phospholipid synthesis in rat liver and labelled acetate incorporation into rat liver cholesterol [1, 2]. Dietary aflatoxin also decreased serum lipids in chickens [3]. Accumulation of lipids in the liver has been reported in ducklings [4] after multiple administration of sublethal amounts of aflatoxin. The composition of diet has been reported to influence the metabolism and toxicity of aflatoxin in some species [5, 6]. A high-fat diet has been found to have a mortality sparring effect on aflatoxicosis in turkeys [7]. High-protein diets have been similarly reported to protect the liver from necrosis in rats fed high-protein diets and aflatoxin [5]. This paper deals with the influence of dietary fat on effects arising from the administration of aflatoxin to the Nigerian monkey (*Cercopithecus aethiops, Tantalus*).

Monkeys were obtained from the primate colony of the Department of Biochemistry of Ibadan University. They had weights between 2.8 and 3.5 kg. Their ages were not known because none of them was born in captivity. They were in apparent good health and were individually housed in cages which were supplied with food trays. All monkeys were maintained on the stock, low-fat diet used at the primate colony (Table 1). Feed and tap water were available *ad libitum*.

The stock diet was inoculated with a strain of *Aspergillus flavus* (UI.81), using a method of Bassir [8] and fed *ad libitum* to two groups of three male monkeys for 15 days. The amount of food consumed was measured daily throughout the feeding period. Another set of two groups of three male monkeys was kept as control and fed daily with an equivalent amount of the stock diet only. The diet was analyzed before use to ensure freedom from aflatoxin. No other food supplements were provided. The experimental designs were completely randomized.

At the end of the feeding period, blood was taken from the femoral vein of unanaesthetized monkeys. Blood from each animal was collected in a sterile tube and allowed to clot undisturbed for 1 hour at 37 ± 1°. The sera were harvested by centrifuging at 3000 r.p.m. Total serum cholesterol was measured by the method of Sperry and Webb [9]. Serum total phospholipids were assayed according to the method of Whitehorn [10]. Serum total lipids were determined by the method of Kunkel *et al.* [11].

In another experiment, six groups of six monkeys were given *ad libitum* for 15 days either the stock, low-fat (2.2%) diet (groups 1, 2 and 3) or a high-fat (18.1%) diet (groups 4, 5 and 6) (Table 1) and vitamin C was added to each diet [12]. The experimental designs were completely randomized. Two feeding tests each lasting 15 days were done. At the end of feeding, groups 2 and 5 were given a single i.p. dose (60 μg/kg body wt) of aflatoxin B_1 while groups 3 and 6 received 100 μg aflatoxin B_1/kg body wt. Aflatoxin was dissolved in 0.5 ml of dimethylsulphoxide. Groups 1 and 4 were kept as controls and received 0.5 ml of the vehicle. Aflatoxin B_1 was produced by the method of Shotwell *et al.* [13] as modified by Llewellyn *et al.* [14]. The crude extract was purified on thin layer plates and quantitatively estimated by the method of Rodricks and Stoloff [15]. Blood was obtained from the control and aflatoxin-treated monkeys by venepuncture and serum lipid determinations carried out as stated above [9–11].

The data from these experiments were treated statistically with an analysis of variance using the F-ratio as the measure of significance.

The mean daily intake of the aflatoxin-contaminated diet (75 g) was extracted with chloroform using the method of Llewellyn *et al.* [14]. Aflatoxins B and G were identified under ultraviolet light at 365 μm; their concentrations were found to be 13.22 and 13.14 p.p.m., respectively. The serum lipid levels (mg/100 ml) of monkeys subsisting on the contaminated diet were found to be: total cholesterol,

Table 1. Composition of low-fat and high-fat diets

Ingredients	Low-fat (%)	High-fat (%)
Ground yellow maize	76.6	60.3
Corn starch	7.0	7.0
Brown fish meal (67% N × 6.25)	3.0	3.0
Casein	4.0	5.3
Bewer's dried yeast	1.4	1.4
Rice bran	5.0	5.0
Sodium chloride	0.3	0.3
Dicalcium phosphate	2.2	2.2
Vitamin mixture*	0.5	0.5
Lard	—	15.0

* Pfizer Products (Ikeja) comprising: vitamin A (10 million units), vitamin D_3 (2 million units), vitamin E (7000 million units), vitamin B_{12} (10 μg), vitamin K (2000 mg), riboflavin (4200 mg), nicotinic acid (25,000 mg), pantothenic acid (10,000 mg), folic acid (1000 mg), lysine (454 g), cobalt (492.8 mg), copper (24,868 mg), iodine (2016 mg), manganese (34,720 mg), zinc (100,800 mg) and iron (100,800 mg).

Table 2. Effect of dietary fat on aflatoxin-induced depression of serum cholesterol, phospholipid and total lipid levels

Treatment	Total cholesterol (mg/100 ml)	Phospholipid (mg/100 ml)	Total lipids (mg/100 ml)
2.2% fat:			
Control	128.33 ± 11.20	265.08 ± 17.73	631.67 ± 21.58
AFB (60)*	87.42 ± 8.04‡ (31.9)	163.73 ± 10.81§ (38.2)	477.67 ± 25.12§ (24.4)
AFB (100)†	58.28 ± 8.05§ (54.6)	143.02 ± 10.06§ (46.1)	428.17 ± 21.41§ (32.2)
18.1% fat:			
Control	189.50 ± 11.50 140.33 ± 10.1	291.33 ± 15.75	763.01 ± 25.26
AFB (60)*	1§ (26.0)	250.63 ± 10.19‡ (14.0)	652.17 ± 24.60§ (14.5)
AFB (100)†	122.01 ± 7.72§ (35.6)	227.98 ± 24.13§ (21.8)	613.67 ± 26.11§ (19.6)

Figures in parentheses indicate percentage decreases. Results are expressed as means ± standard deviation.

* Aflatoxin B_1 at 60 μg/kg body weight.

† Aflatoxin B_1 at 100 μg/kg body weight.

§ Significantly different from the control monkeys given the low-fat or high-fat diet ($P < 0.01$); ‡ ($P < 0.05$).

86.21 ± 5.62 (control, 105.26 ± 3.39); total phospholipids, 170.01 ± 9.53 (control, 213.75 ± 5.50) and total lipids, 586.60 ± 12.68 (control, 657.10 ± 8.30). These values represent the means of 6 determinations ± standard deviation. Statistical analysis of the data showed that the serum lipid levels were significantly ($P < 0.01$) lowered in all monkeys given the stock diet which was contaminated with a strain of *A. flavus*.

The effect of dietary fat on aflatoxin-induced depression of serum lipids is shown in Table 2. The results obtained show evidence that aflatoxin B_1 depressed the total serum cholesterol, phospholipid and total lipid levels significantly in monkeys given the low-fat and high-fat dietary regimens. A factorial analysis of variance revealed that there was a significant ($P < 0.05$) interaction between aflatoxin and dietary fat concentration such that the lipid-depressing activity of aflatoxin was decreased when it was administered to monkeys on the high-fat diet. The mechanism of this effect is not yet clear. The results suggest that the level of fat in the diet has a significant effect on aflatoxin-induced depression of serum lipid levels. Decreases in serum lipid levels may be attributable to impairment of lipid transport and increased degradation of serum lipids as a consequence of aflatoxin administration. Dietary aflatoxin was reported to impair lipid transport in chickens [3, 16]. Secondly, the serum lipid levels were not lowered to the same extent by any given dose of aflatoxin B_1. Depression of total cholesterol appeared to be most profound (Table 2). The differential depression of serum lipid levels may be due to the different proportions of lipid components in the different lipoprotein classes. An inhibition of very low density lipoprotein transport by aflatoxin would have different inhibitory effects on low density lipoprotein or high density lipoprotein transport. More work is being done to clarify the above observations.

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