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Influence of Dietary Aflatoxin, Zinc, and Copper on Bone Size, Organ Weight, and Body Weight in Hamsters and Rats

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Aspergillus flavus and A. parasiticus have been shown to produce aflatoxins in stored foodstuffs such as peanuts, cottonseed meal, rice, and grains (Wogan 1965). Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents in a number of animal species (Newberne and Butler 1969, Wogan and Shank 1971). There are four naturally occurring aflatoxins and many metabolites. An increased sensitivity to aflatoxins was demonstrated in male rats (Wogan and Newberne 1967, Butler 1964). Aflatoxin-treated animals, especially those that have not matured, fail to gain body weight as rapidly as controls (Llewellyn and Thomen 1978) and protein synthesis is reduced (Wogan 1968).

Todd et al. (1934) showed zinc was a necessary trace element in the diet. Pullen et al. (1971) reported a range of 20 to 200 ppm required depending upon the species. Slight deficiencies produce growth retardation, an inhibition of the animal's general well-being, and a reduction in feed efficiency. In rats, growth stops almost immediately (Williams and Mills 1970). Many changes observed in bone of zinc deficient animals (Bergman et al. 1972) were similar to those observed with decreased feed intake (Apgar 1979). High levels of zinc are relatively non-toxic (Cox et al. 1969). McQuitty et al. (1970) showed Walker 256 tumor growth was markedly decreased in zinc deficient weanling rat test groups. There is also evidence that zinc plays a major role in the changes associated with cancerous development (Henkin 1979).

Copper is required in the diet for prevention of anemia (Hart et al. 1928). It has been suggested to have a protective effect against several carcinogens (Kamamoto et al. 1973, Fare and Woodhouse 1963), and is an essential component of many oxidative enzyme systems. Barber et al. (1968) reported that pigs fed an AFB_1 test diet, compared with those fed 250 ppm copper sulfate test diet, had an improved weight gain and feed efficiency for the latter.

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In an effort to determine the potential effects of the interaction of metals and aflatoxins, these studies were initiated. Reported herein are body weights, organ weights, bone sizes and radiographic evaluations.

MATERIALS AND METHODS

Weanling male golden hamsters (Mesocricetus auratus) were divided into seven groups, six animals per group, of approximately equal weights. Animals received diets as indicated in Table 1.

TABLE 1. The Treatment Groups, Diets, and Abbreviations Used in the Hamster Study

GROUP	DIET	ABBREVIATION
Control	Purina Meal, 57 ppm Zinc	Cl
High Zinc	3000 ppm Zinc as $ZnCO_3$	HZn
Aflatoxin	14.6 ppm aflatoxins & 57 ppm Zinc	AFT
High Zinc & Aflatoxins	3000 ppm Zn added to aflatoxin diet	HZN + AFT
ICN Control	66 ppm Zn added as $ZnCo_3$	C2
Low Zinc	ICN low zinc diet 21 ppm Zinc	LZn
Low Zinc & Aflatoxins	14.6 ppm aflatoxins added to ICN diet	LZn + Aft

Water and feed were provided ad libitum. Mixed aflatoxins were produced on a coconut substrate by A. parasiticus NRRL 2999 (Samarajeewa 1972). Chloroform extracts were quantitated and then sprayed on feed. The official A.O.A.C. procedure for aflatoxin and metal determinations was used (Horwitz et al. 1975).

Body weight, feed, and water consumption were totaled weekly. At 18 weeks the animals were sacrificed randomly. The liver, heart, lungs, spleen, kidneys, and both testes were washed in Ringer's solution and weighed. The long bones were radiographed on Dupont non-screen film with 150 MAS at 24 KVP with target to film distance 48 cm. The legs were subsequently boiled for 10 minutes and the flesh removed. The femurs were measured in length to ± 0.01 mm).

Four groups of weanling, male Holtzman rats, 10 animals per group, were maintained under the same conditions as the hamsters. Aflatoxins were produced on a rice substrate (Shotwell et al. 1966) and analyzed in a similar manner. Diets fed were as indicated in Table 2.

TABLE 2. The Treatment Groups, Diets, and Abbreviations Used in the Rat Study

GROUP	DIET	ABBREVIATION
Control	Purina Meal, 18 ppm Copper	C
Aflatoxin	7.8 ppm aflatoxins 18 ppm Copper	AFT
Copper Acetate	2600 ppm CuAc	CuAc
Copper & Aflatoxins	2600 ppm CuAc added to aflatoxin diet	CuAc + AFT

Data was recorded as in the hamster study. At the end of 21 weeks rats were sacrificed in random order. Weights of liver, kidneys, heart, spleen, left lung, and left testis were determined after washing in Ringer's solution. Long bones were radiographed and prepared for measurement in the same manner as the hamster bones. The femurs were measured in length and in narrow diameter at the distal end with a micrometer.

RESULTS AND DISCUSSION

The final body weights using the Quetelet Index of Obesity (100 x body weight/naso-anal length) (Billiwicz et al. 1962) were significantly lower in all groups receiving aflatoxins ($p < 0.05$).

The LZn was significantly lower than the control, but not as low as the LZn + AFT (Figure 1). By factoring out the effect of animal size upon organ weight, the relative organ weights were analyzed using Duncan's Multiple Range Test ($p < 0.05$). The HZn, AFT, and LZn + AFT groups had a significant reduction in size of the testes. The HZn + AFT group was not significantly different from the AFT group. There were no significant decreases or increases in relative size of liver, heart, spleen, or kidneys.

The bone radiographs were examined at the Department of Diagnostic Radiology, Medical College of Virginia, for evidence of osteoporosis, osteomalacia, and modeling defects. The evaluator had no prior knowledge of treatment groups. The physes were closed

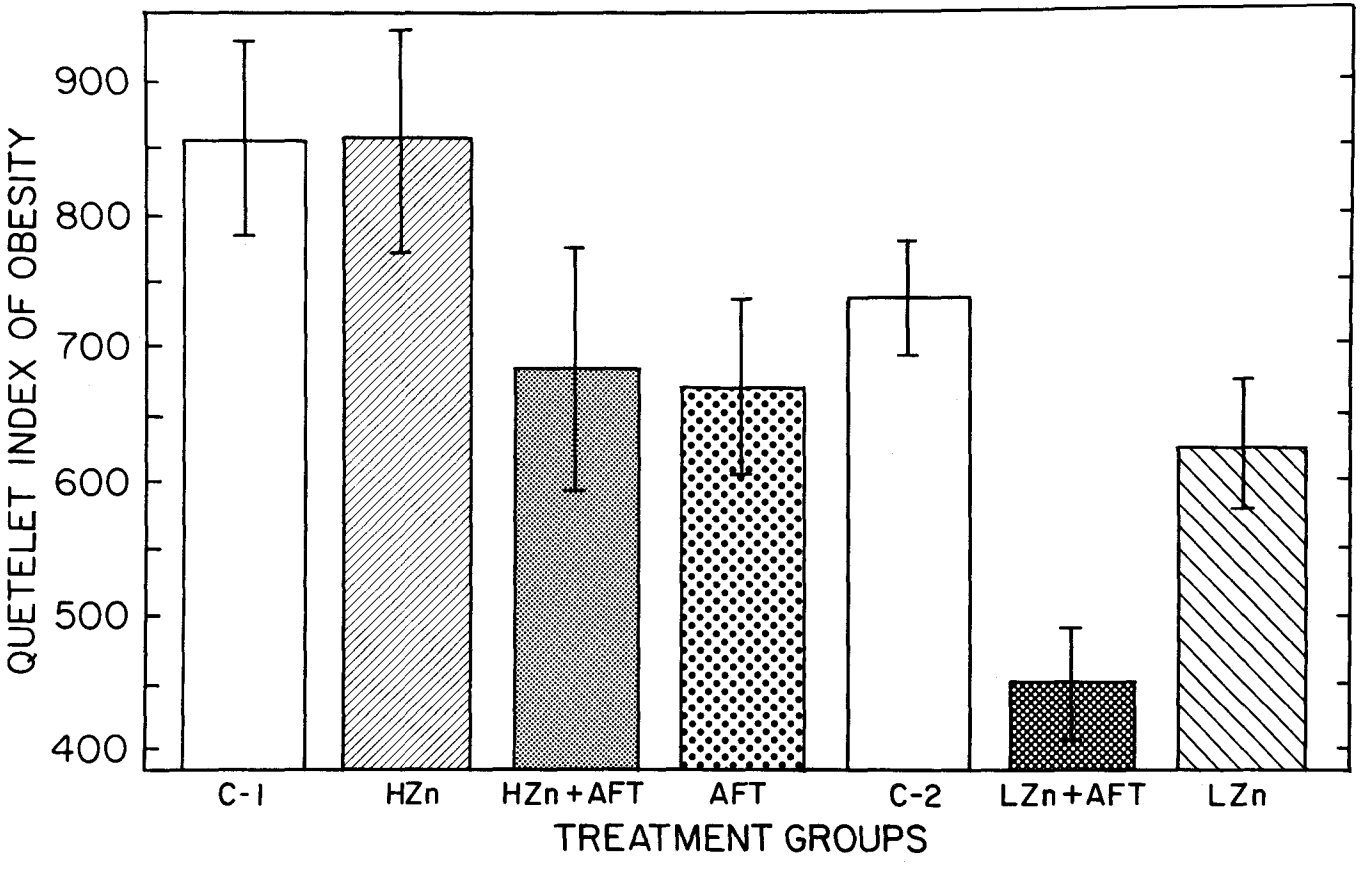

Figure 1. The Quetelet Index of Obesity considered with respect to dietary treatment to the hamsters. This index was measured at sacrifice after the technique of Billiwicz et al. (1962). Means with standard deviations are shown.

indicating no quantitative differences in bones. Using Student's t test ($p < 0.05$), on the measured bones, the animals receiving the double insult diet of LZn + AFT had the only significant decrease in femur diameter.

The body weights of the control and CuAc supplemented groups were consistently greater than the AFT and CuAc + AFT groups during the experimental period. The CuAc group demonstrated a reduction in total growth of 22.9% while the CuAc + AFT and AFT groups were reduced 30.2% and 34.6% relative to the control. The Quetelet Index of Obesity indicated the control group was significantly more obese than both groups receiving AFT (Figure 2). The weights of the heart, spleen, lung and kidney remained unaltered by any of the treatments. The liver weights in both AFT and CuAc + AFT groups was significantly greater than the controls and CuAc groups. The testis of the CuAc group was significantly greater than the control. Again, radiographic data showed there were no qualitative or quantitative differences in bones. The physes were closed indicating skeletal maturity. Utilizing Student's t test ($p < 0.05$) the femur length of animals fed AFT and CuAC diets was deceased relative to the controls (Figure 3).

All AFT treated animals showed significant failure to gain weight as compared to controls. In the hamster study, the smaller femur diameter of animals receiving LZn + AFT was the only effect on bone. This treatment group had significantly reduced body weight compared to other treatment groups. Rats receiving CuAc and AFT were both smaller in body size and had decreased length of the femurs. Comparison of the present data with female albino rats (Fare and Woodhouse 1963) or male hamster studies (Llewellyn and Thomen 1978) suggested that the male Holtzman rat is more sensitive to the chronic effects of CuAc. Petering et al. (1977) reported body weight gain in rats directly related to the level of dietary copper and zinc in physiological doses. Suttle and Mills (1966) report a marked zinc-copper antagonism; thus, the effects of a high copper diet may be similar to those of a low zinc diet. From the radiological evidence, in both the hamster and rat studies, there is no direct metabolic or pathological effect of aflatoxins upon bone growth and development, but rather an apparent concomitant reduction in size because of the reduced feed efficiency associated with aflatoxicosis (Wogan 1965), low zinc, or high copper diets. This response of bone may be species related but copper supplement in rats did reduce the toxic effect although not significantly. Such a toxic-associated response causing failure of bone development was reported by Whittaker (1975) and mentioned by O'Brien (1976). They imply it may have had origins associated with hormonal changes, vitamin D metabolism and/or lowered serum calcium.

The kidneys, heart, lungs, and spleen had no weight changes. The liver and testes were altered in the copper study, the liver being a known target organ for aflatoxins. The CuAc diet caused an

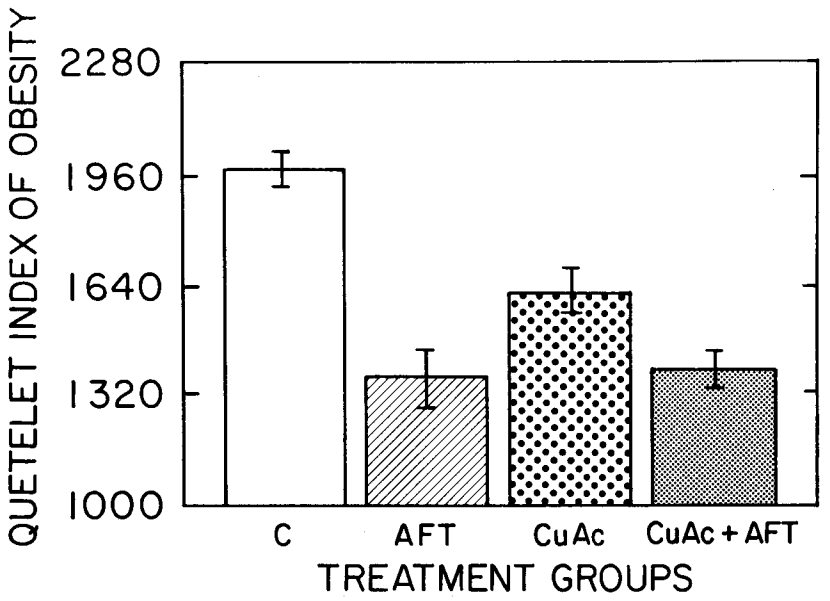

Figure 2. The Quetelet Index of Obesity considered with respect to dietary treatment. Means with standard deviations are shown.

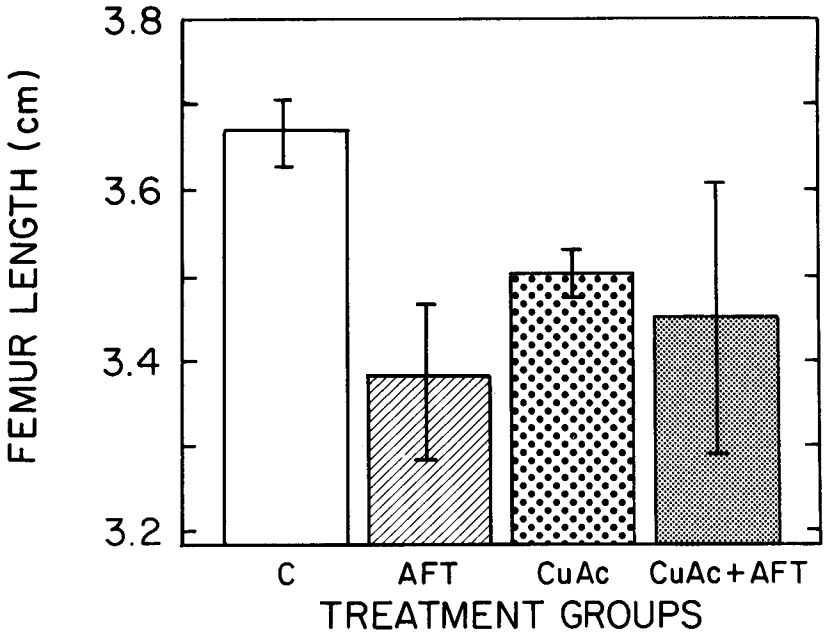

Figure 3. Mean femur lengths of rats shown with standard deviations.

increased testis size, but not so with the AFT and CuAc + AFT diets. In the hamster study the testes were decreased in LZn + AFT, AFT and HZn diet groups. Since the HZn + AFT group was not significantly affected, this may indicate some protection, at least for this organ, by excess zinc in the diet. Also, in this zinc study the liver did not show weight change.

In general, aflatoxicosis not only causes pathological changes in the liver, but also reduced body mass and bone size. Zinc and copper levels showed a slight protective effect against aflatoxins.

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