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Comparative Dietery Patterns and Body Weight Changes In Adult Male American Cockroaches Fed Pure Aflatoxin B_1

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The aflatoxins are a group of natural, metabolic secretions from the molds, *Aspergillus flavus* and *A. parasiticus* (DIENER and DAVIS 1969). *A. flavus* is one of several fungi contaminating stored agricultural products including peanuts and grains. Aflatoxin B_1 (AFB_1) is a known toxic and carcinogenic agent (BUTLER 1970; NEVINS and GRANT 1971).

Most organisms tested to date have been sensitive to this mycotoxin. Aflatoxin B_1 was found to cause liver damage in ducklings (CARNAGHAN 1965), fish (HALVER 1965), rats (BUTLER 1965), gerbils (HASTINGS and LLEWELLYN 1973) and possibly in man (BOURGEOIS *et al.* 1971; SERCK-HANSEN 1970). Several studies have indicated that the toxin is also pathogenic to various insect populations including *Trichospilus pupivora* (FEER) and *Bracon brevicornis Wesmael* (PEETHAMBARAN *et al.* 1972), the rice weevil, *Sitophilus oryzae* (L.) (SRINATH *et al.* 1973), the velvet mite *Trombidium gigas* (SANNASI and AMIRTHAVALLI 1970), *Dinothrombium giganteum* (SANNASI and OLIVER 1971)and the southern pine beetle, *Denodroctonus frontalis* (MOORE 1971). Investigations using houseflies have demonstrated the possible use of the toxin as a chemosterilizing agent (Al-Adil 1972). Insecticidal properties of the aflatoxins as well as the effects of the toxin on the various stages of the insect life cycle have been reported on the boll weevil (MOORE 1974), the yellow-fever mosquito, *Aedes aegypti* (L.) (MATSUMMURA and KNIGHT 1967), the fruit fly, *Drosophila melanogaster* (CHINNICI *et al.* 1976); LALOR 1976; REISS 1975).

The American cockroach is considered an ubiquitous scavenger and may directly or indirectly affect the human environment by acting as an insect pest of stored grain crops and as a possible vector for various pathogens (ROTH and WILLIS 1960). The present study was undertaken to evaluate further the effects of AFB_1 in the American cockroach which previously has been found less sensitive to the toxin than any other insects examined (MATSUMMURA and KNIGHT 1967 and LLEWELLYN *et al.* 1976).

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MATERIALS and METHODS

Eighteen adult, male American cockroaches, *Periplaneta americana* (L.) were selected at random from our stock colony. Three experimental groups, each containing six animals, received various concentrations of pure AFB_1 (grade B dried *in situ* Calbiochem, LaJolla, Cal.) in their diets of Purina dog chow. The controls were fed a diet of finely ground dog chow without AFB_1. Those in a second group were fed the low concentration of 50 ppm AFB_1 in their diet. The other experimental animals received 200 ppm. All animals were allowed to feed *ad libitum*.

Confirmatory AFB_1 analyses of the various feeds used were carried out at the Virginia Division of Consolidated Laboratory Services, Mycotoxin Laboratory, Richmond, VA. Both quantitative and qualitative analyses utilized a thin layer chromatography procedure modified from the official AOAC Handbook (HORWITZ *et al.* 1975). Concentrations as low as 2 ppb can be determined with this method.

All animals were housed individually in modified plastic cages and food, water and body weight data were recorded regularily. The ambient temperature of the air was 24$\pm$ 2°C (LLEWELLYN *et al.* 1976).

RESULTS and DISCUSSION

The American cockroach has changed very little in respect to factors such as morphology over a long period of time. It is evident that this organism is very adaptable and resistant to many environment variables, possibly including one of the most potent, toxic and carcinogenic compounds known, AFB_1. The results of the study indicate that various cycles or patterns for food ingestion, water ingestion and weight changes occurred in all concentration groups. AFB_1 seemed not to cause a high level of toxic responses in the animals.

When this toxin was introduced in the diets to these animals, it apparently had little affect on them. The feeding cycles occurred and reoccurred in both experimental groups as well as in the control group (Fig. 1). The first 14 weeks of this study may represent an "acclimatizing period" for the animals. During this period, no real differences in food consumption were obvious in the three groups studied. It is of interest to note that all animals had consumed approximately the same mean total amount of food at the midpoint and at the termination of the experiment. The food consumption played a definite role in weight change with a decrease in both mean food and weight occurring about 71% of the time in the controls, 50% of the time in the

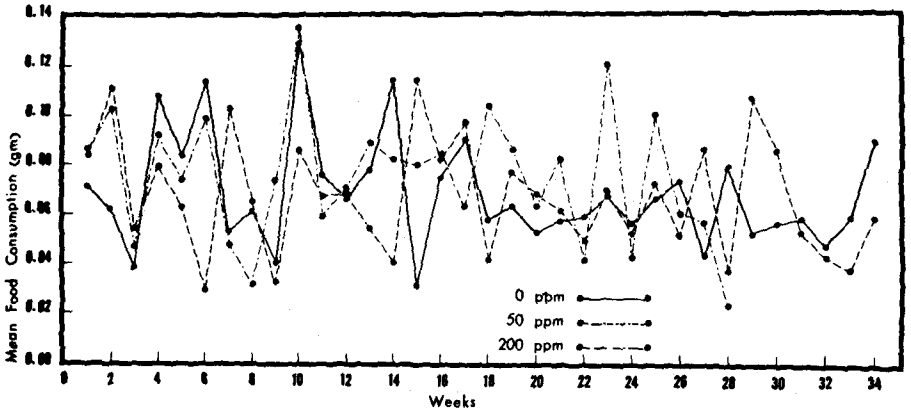

Figure 1. Mean Weekly Food Consumption For All Animals Studied.

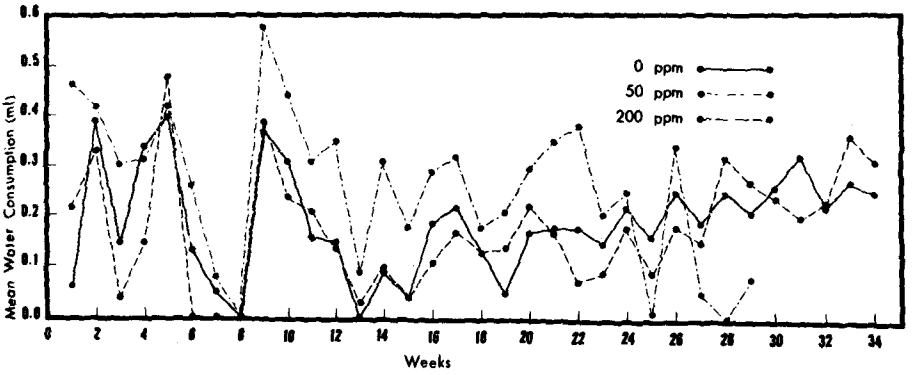

Figure 2. Mean Weekly Levels Of Water Consumption For The Animals.

50 ppm animals and 50% of the time in the 200 ppm animals (Figs. 1 and 2).

Part A, Acclimatization Period: During weeks 1-14 the cyclic nature of ingestion was not drastically affected by the introduction of the toxin into the diets. In the graph (Fig. 2) showing mean water consumption from weeks 4 to 14 the animals drank and stayed in respective positions in reference to total water consumption. The low concentration (50 ppm) animals drank the most followed by the controls and high concentration (200 ppm) animals. Water consumption also played a vital role in the fluctuations of both weight of the animal and food ingestion. The control animals showed a direct relationship between an increase or decrease in both mean water consumption and mean weight change about 50% of the time. The low concentration (50 ppm) animals showed this relationship, also about 50% of the time, while the high concentration (200 ppm) animals showed a direct relationship about 57% of the time. The controls exhibited a direct relationship of an increase or decrease in both food and water consumption about 43% of the time, the low concentration (50 ppm) about 50% of the time, and the high concentration (200ppm) about 57% of the time (Figs. 2&3).

The animal weights were studied concurrently with increases or decreases in mean food and water consumption. A direct relationship existed among the three parameters approximately 36% of the time in the controls, 29% of the time in the low concentration (50 ppm) and 36% of the time in high concentration (200 ppm) animals. This implied that the toxic contamination of the diets may have caused some slight variance in these three factors but was not an obvious deterrent for food and water consumption or weight fluctuations.

All of the animals studied were adults of various ages, selected at random. A consideration of the three deaths (50%) which occurred in the low concentration (50 ppm) during the first 14 weeks is vital in the determination of overall effects, especially on the mean weight. A death in any concentration group would cause an immediate shift in weight for that group unless all animals for that group had equal original body weights. This was noted in the 50 ppm animals and determined to be the main cause for abrupt changes in mean group weights. The obvious changes in mean weights for the low concentration (50 ppm) animals occurred during weeks 2, 9, and 14 which correspond with the death of an individual at each week. These deaths were possibly due to the age of the individuals and/or their sensitivity to the toxin. Other experimental parameters to explain this response are currently being considered. The food consumption in this group was

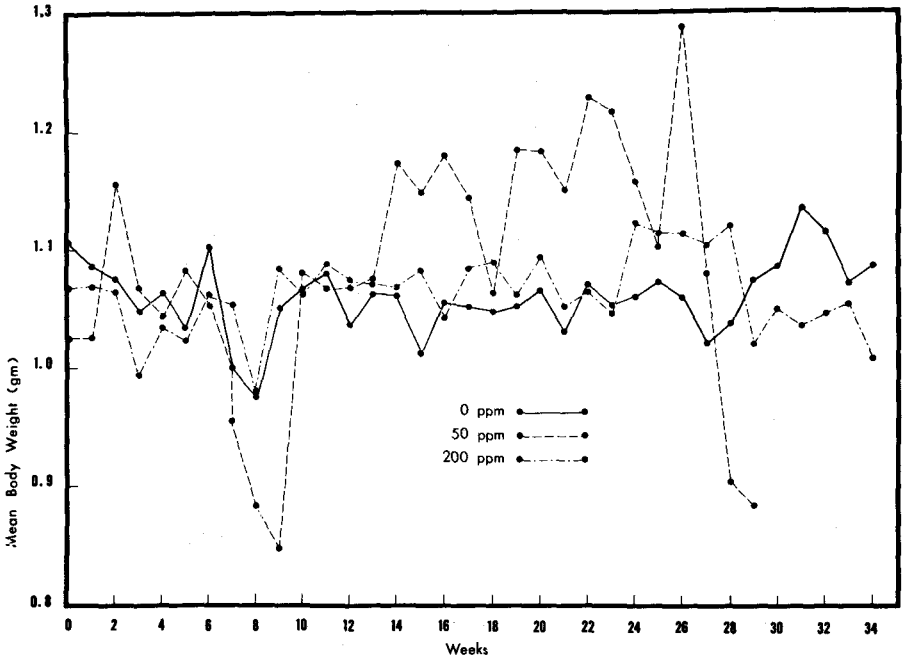

Figure 3. Mean Weekly Body Weights For All Treatment Groups.

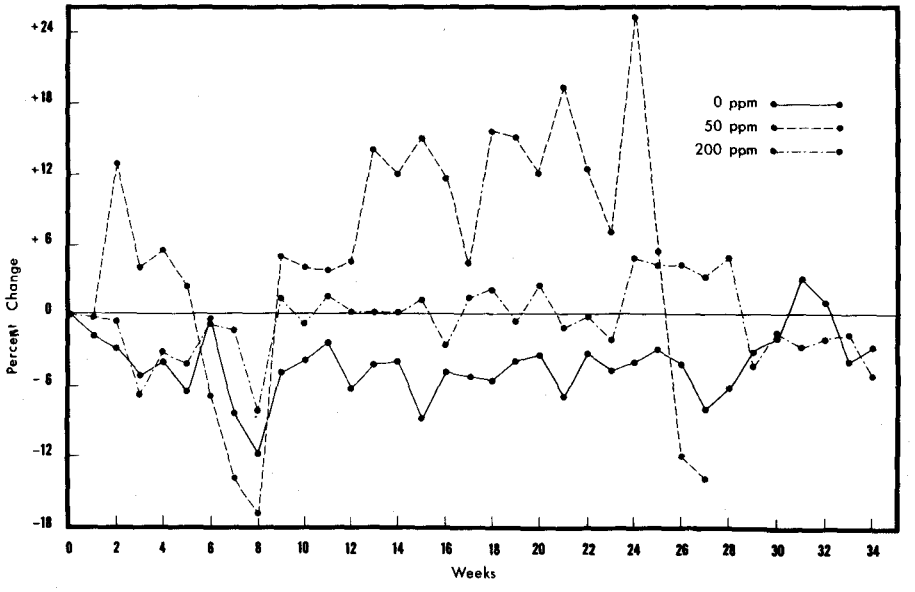

Figure 4. Percent Change In Body Weights As Based On Original Weights.

similar to the other groups but they did drink more water.

Part B, Post Acclimatization Period: During Weeks 15 through 34 the results of the second phase of the total 34-week study were analyzed and compared to the results of the first 14 weeks, Part A, the acclimatizing period.

Both control and experimental animals showed less variance in mean values of water ingestion during the second part (weeks 15-34) of the study. No one group drank consistently more water than the other. The groups did vary from week to week in relation to mean total water consumed. There was a slight tendency in the 50 ppm (low concentration) animals to exhibit increased drinking from weeks 15 to 24. By week 29, all of the 50 ppm animals had succumbed. This possibly explains the obvious decrease in water total consumption from weeks 26-29. At the termination of the study (week 34) the 200 ppm and 0 ppm animals had drunk nearly equal amounts of water.

Mean food consumption also varied from weeks 15-34 but less frequently than in the acclimatizing phase of the study. The most noticeable changes in food consumption occurred in the 50 ppm animals. Similarly, as in water consumption, the great variability in food consumption in this group from weeks 22 to 29 can be explained by the animal deaths during this period. The controls and 200 ppm animals differed by approximately 0.03 g with the control group ingesting the greatest quantity. Taste and/or toxic responses from continued ingestions may have influenced the feeding response.

Mean animal weights from weeks 15 to 34 varied directly with water and food ingestion. Again, the 50 ppm animals exhibited the greatest mean weight fluctuations with the most noticeable changes from weeks 25 to 29. The sharp increase in mean weight for the low concentration animals (week 25 to 26) and corresponding sharp decreases (weeks 26 to 29) probably represents the respective deaths of light and heavy individuals. From weeks 26 to 29 in the low concentration animals a total loss of 0.41 gram of mean weight occurred representing a total of 39.5% change in mean weight. The control and high concentration animals also varied in mean weight after week 14. The control animals at week 15 showed their largest mean weight loss of the study, 0.14 grams, or approximately 9% below their

original mean weight. The 200 ppm animals' final weight (week 34) was also their lowest mean weight recorded (weeks 15 to 34) representing 0.06 grams (2.0%) below their original weight. In relation to their original mean body weights, the 200 ppm animals lost approximately 5.5% while the control animals lost approximately 2.0%. The 50 ppm animals, all dead by week 29, showed a terminal mean body weight of 0.15 g (14%) below their original mean weight.

The AFB_1 ingestion varied directly with the food ingestion. The control animals obviously ingested no AFB_1. The 50 ppm animals consistently ingested less AFB_1 than the 200 ppm animals. One experimental animal showed a range of total aflatoxin ingestion for a week from approximately 6.0 to 23.0 ug of AFB_1. But deaths did not occur following high weekly toxin ingestion levels.

In comparing the results of this study to other work done to determine the effects of aflatoxin ingestion, it becomes apparent that the American cockroach is unusual in its resistance to the toxin. In many cases, chronic administration of aflatoxin have led to an LD_{50} value for the organism studied. LD_{50} values vary from 0.62 mg/kg for 50 gram ducklings (BUTLER 1964b) to 18.2 mg/kg for pigs (BUTLER 1970). Butler (1964; 1970) has also determined the LD_{50} for male rats (7.2 mg/kg), cats (10.55 mg/kg), and guinea pigs (1.4 mg/kg).

A comparison of these reported values to the 50 ppm (50 mg/kg of food) and 200 ppm (200 mg/kg of food) fed to the American cockroach in this study makes it apparent that the adult male cockroach can withstand quantities much higher than any animal tested to date. Additional studies in progress will include the effect of aflatoxin on the stages in the lifecycle of the cockroach, the response of the female American cockroach and the analysis of the fecal material collected herein.

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