Reports of studies on poultry indicate that the decreasing order of sensitivity to aflatoxin in species is ducklings, turkey poults, goslings and chicks (Arafa et.al., 1981). A study was conducted to determine the relative sensitivities of White Leghorn chicks, Broiler chicks, White Chinese goslings, Large White turkey poults and Bobwhite quail chicks to dietary aflatoxins. Aflatoxin was included in the diets at three concentrations (0.7, 1.4 and 2.1 mg aflatoxin B1 equivalent per kg). It was found that there were no significant effects of aflatoxin in broilers and Leghorn chicks. Poults and goslings appear to be most sensitive, quail are intermediate and domestic chickens are the most resistant. Aflatoxin at 0.7 mg/kg decreased the body weight of poults but tended to increase the growth rate of gosling. At 1.4 mg/kg aflatoxin, food consumption, body weight and weight gain of goslings were adversely affected.

The synergistic responses of various mycotoxins have been examined with regard to the possible interaction of aflatoxin B1 (AF B1) and ochratoxin A (OA). Rati et.al. (1981) found synergistic effects on body weight gain and on kidney and liver histology after combined administration of AF B1 and OA. Huff and Doerr (1981) also showed a synergistic response of the two toxins in broiler chickens on growth rate, mortality and kidney lesions at a dose Level of 2.5 µg/g AF B1 and 2.0 µg/g OA. In further work, Huff et.al. (1984) found no synergistic response with regard to the adverse effects of AF B1 (2.5 µg/g) and OA (2.5 µg/g).

A study of turkey poults which were fed on experimental diet containing 2087 µg aflatoxin B1/kg was carried out for 18 days in order to evaluate the concentration of free and conjugated aflatoxins in edible tissues and to determine the rate of elimination when AF B1 was removed from the diet (Gregory III, Goldstein and Edds (1983)) for 18 days. Free and conjugated aflatoxin metabolites were quantified using high performance liquid chromatography (HPLC). Aflatoxin residue levels were greater in Liver than muscle tissues,although all levels were low (range 0.1 to 1.19ng/g tissue).Free and conjugated aflatoxin B1 and M1 were the principal residues. Other metabolites investigated included aflatoxicol (detected) and aflatoxin Q1 (not detected). All aflatoxin residues were rapidly cleared following discontinuation of the dietary aflatoxin B1.

Chen et. al.(1984) designed a study to determine aflatoxin levels in tissues of broiler chickens that had been fed on a diet containing 2057 µg aflatoxin B1 and 1323µg aflatoxin B2/kg for 35 days. Their results revealed that aflatoxins were deposited in all tissues. The highest levels of aflatoxins were present in the gizzards, livers and kidneys. There was evidence that the high levels of aflatoxins B1 and B2 in the gizzards might have been caused by contamination by the gizzard contents during the slaughtering process. After feeding the aflatoxin -contaminated diet for 35 days, mean values for the combined aflatoxins were less than 3µg./kg of tissues.Four days after withdrawal of the aflatoxin-contaminated ration, there was no detectable amounts of aflatoxins in any of the tissues. The results indicated that broiler type of chickens (Hubbard white Mountain) rapidly clear aflatoxins from their tissues once they are transferred to an aflatoxin-free diet.

Aflatoxin B1 was detected in leg, breast, muscle, liver and gizzard tissue at mean levels of 0.09, 0.004, 0.17 and 0.27 µg /kg, respectively. Aflatoxin M1 was also detected in the same organs and tissues at mean levels up to 0.06µg/ kg.

Wolzak et.al (1986) designed a feeding trial using White Leghorn pullets fed a diet contaminated with 3310 µg/ kg aflatoxin B1 and 1680 mg aflatoxin B2/kg feed. At the end of 4 weeks, the livers were pale, enlarged and haemorrhagic. The highest levels of aflatoxin were detected in the gizzard, kidney and liver with average total concentrations of less than 3µg/kg. The lowest residue levels were detected in the breast muscle. Two days after removal of the contaminated feed, aflatoxin residues in all tissues had decreased markedly. No aflatoxin residues were detected in the gizzards of hens killed 8 days after withdrawal,or in the kidneys at 16 days. However, one hen had measurable amounts of aflatoxins B2 in the liver 32 days after withdrawal.(Micco et al, 19988a).

Another study was performed to monitor and determine aflatoxin residues in tissues and organs of male broilers and hens that had been fed a diet contaminated with 50µg/kg aflatoxin B1 after long term administration (169 days). Residue levels of aflatoxin B1, aflatoxicol (Ro), aflatoxin M1 and B2a were determined by an HPLC method, and with the exception of aflatoxin B2a, were detected in the liver, kidney, and thigh of both male broilers and hens. The highest levels found were for Ro in liver (1.1µg/kg for male broilers and 0.6 µg/kg for hens). No detectable amounts of aflatoxins were found in any tissue after withdrawal periods of 14 days in male broilers and 33 days in laying hens (Micco et.al, 1988b).

1.6.2. AFLATOXINS IN HUMANS.

Consumption of foods heavily contaminated with mycotoxins has resulted in acute intoxication episodes in human populations. Conditions enhancing the likelihood of such occurrences include limited kinds of food and food supplies (as in a drought), environmental conditions that favour fungal development in crops and commodities and absence of effective mycotoxin monitoring and control systems (U.S Task Force Report,1989). Such intoxication episodes are likely to occur in Zambia because contaminated and uncontrolled food is permitted in the market, except for that meant for export.

Evidence of possible cases of acute aflatoxicosis in humans has been reported from Uganda,Taiwan,India and Thailand,and the symptoms were vomiting,abdominal pain,pulmonary oedema, fatty infiltration and necrosis of the liver.

In Uganda (Shank 1977;1980; Hannsen, 1970), a teenage boy developed abdominal pains and swelling; four days later he was admitted to hospital with oedema of the legs and a palpable tender liver but no fever. He died two days after admission. At autopsy, liver necrosis and a flabby heart were in evidence. The boy's brothers and sisters became ill at the same time with similar abdominal pains and discomfort.

The diet of these children consisted mainly of cassava and cassava found in their home was mouldy, and on analysis was found to contain 1.7mg/kg aflatoxin.

In two villages in Taiwan, 26 people became ill with symptoms of intoxication after eating mouldy rice, some of which contained approximately 200µg/kg aflatoxin B1. The seven adults involved suffered only a mild general discomfort but the 19 children developed oedema of legs, abdominal pain, vomiting and a palpable liver, but again no fever. Three of the children aged between 4 and 6 years died (Shank 1977;1980)

In India (in 1974), a total of 397 patients of two adjacent states, Gujarat and Rajasthan, were affected by an outbreak of hepatitis. Hundred and six of those affected died a sudden death. The outbreak was associated with the consumption of maize that was shown to contain aflatoxin levels ranging from about 6 to 16µg/kg (Karishnamachari, 1975). These effects were confined to rural areas where the nutritional status of the population was poor. It has also been suggested that aflatoxin may be the causative agent of the so-called Indian childhood Cirrhosis (ICC), a disease of the liver, which frequently affected children eating the same food (in the same family). The disease recognised in Ceylon, Burma, Indonesia, West Africa, The Middle East and the Caribbean affected children between 1 and 1.5 years old.

Other direct, although still circumstantial, evidence linking aflatoxin with acute aflatoxicosis in man comes from Thailand (Burgeois et.al;1971). Reye's syndrome was characterised by vomiting, hypoglycemia, convulsions, hyperammonemia and coma. Many Thai foods have been shown to contain aflatoxin,and the incidence of this disease corresponded with seasonal and geographical variations of dietary aflatoxin (Harwig et.al;1975). Autopsy specimens from 22 of the group of 23 children affected with the disease indicated the presence of aflatoxin B1,the highest concentration being in the liver,intestinal contents and stools.

The possible effect of chronic ingestion of low levels of aflatoxins in "lightly" contaminated food has been investigated in epidemiological studies in a number of countries;The Phillipines (Bulatao-Jayne et.al.,1975),Kenya (Peers and Linsell,1973),Swaziland (Peers et.al.,1976) and Mozambique(Van Rensburg et.al.,1977) and Uganda (Alpert et al, 1969).The results of these studies show a highly significant positive correlation between the level of aflatoxin in the diet and the incidence of primary liver cancer in man.There is therefore considerable circumstantial and direct evidence that aflatoxins are indeed dangerous to humans especially children.The intake of aflatoxin in humans has been determined in Zambia by the collection and analysis of "plate-food samples"(Lovelace et al, 1983).

1.7. AFLATOXINS IN ZAMBIA.

Several studies on aflatoxins in Zambia have been undertaken mainly in Central, Eastern, Lusaka and Southern provinces.These provinces are high production areas for Zambia's basic foods, maize and groundnuts. Most of these provinces, except Lusaka, are predominantly rural in character and are composed of villages.

The crops mainly affected by aflatoxins can be ascertained from data available:

(a) 1975-1977 Village food survey (Eastern Province).
(b) 1977 Farm food survey (Lusaka and areas within 200Km of Lusaka).
(c) 1979-1980 Urine samples (Lusaka and Katete districts).
(d) 1982-1983 Foods from hospitals and schools (Eastern Province).
(e) 1984-1985 Poultry feed samples (Lusaka District).
(f) 1986-1987 Serum and urine samples(Lusaka and Katete districts).
(g) 1986-1987 Groundnuts (Eastern Province)

Research in Zambia on fungal toxins started in 1973, with a joint study by the Ministry of Agriculture Research Station and the University of Zambia (Lovelace et. al., 1988). The first publication noting the presence of aflatoxin in Zambian food stuffs appeared four year later(1977).

1.7.1. FARM FOOD SURVEY.

In this survey, Lovelace and Nyathi (1977) analyzed 61 food samples of beer, maize, malted maize and sorghum collected within 200km of Lusaka. The survey was designed to study the presence of aflatoxin in opaque maize beer in Zambia which was chosen because of the frequent use of mouldy maize in its preparation. Out of the 61 samples of malt and beer analyzed, aflatoxin was found in only one sample of malted maize, representing less than 2% contamination. A much high incidence of the fusarium toxin, zearalenone, was noted (7%).

1.7.2. VILLAGE FOOD SURVEY.

To investigate the level of aflatoxin contamination of foods in Zambia, a "plate-food " study was carried out in five villages of similar social structure in Eastern province. The area was chosen as it is the main groundnut growing area. Analysis of 202 samples gave an aflatoxin incidence of 3% with levels ranging from 1-44 mg/kg (Lovelace and Salter, 1979). The samples that were positive included cooked and dried beans, groundnuts and cooked and dried green vegetables (Table 8).

1.7.3. FOOD FROM HOSPITALS AND SCHOOLS.

Since the previous "plate-food" study was carried out in the late dry seasons of 1975 and 1977, another similar study was carried out in the rainy season (Njapau et al, 1985). There were reports that aflatoxin contamination had been observed to be higher in the rainy season than in the dry season. Plate - food samples were collected from the same areas between February 1982 and April 1983. Two hundred and eleven human food samples were collected from large food preparations from hospitals and schools and analyzed for aflatoxin. The aflatoxin B1 incidence was observed to be higher at 7% as compared to 3% reported by Lovelace and Salter in the dry season. However, the levels of contamination by aflatoxin were not really different as the range of aflatoxin concentration detected was 1-50 µg/kg with a mean contamination around 24µg/kg.Most serious contamination (150µg/kg) was found in germinated maize with a 73% level of contamination (Table 9).

1.7.4. ANIMAL FEEDS.

The concern about the presence of aflatoxin in human foods was soon extended to animal feeds, as maize is the main cereal used to feed animals. During 1984, thirty-four poultry feed samples were collected from the Lusaka district and analyzed for aflatoxin and diplosporin.According to results by Njapau (1985b),this survey showed a high incidence(80%) of fungal contamination,but a low level of aflatoxin at 12-21µg/Kg.The same survey showed that a high incidence of contamination of poultry feed occurred.However,140 milk samples analyzed during 1982 showed no samples with more than 1.5µg/Kg aflatoxin,which was considered insignificant (Njapau,cited by Lovelace et.al.,1988).

Table 8.

AFLATOXIN ANALYSIS BY FOOD TYPE.

Food Type	Number of samples analysed	Number of samples with aflatoxin	Aflatoxin concentration in positive Samples (µg/Kg)	
			Range	Mean
Maize-based	95	0	-	-
Other grains and	12	0	-	-
beverages	6	0	-	-
sweet potato	12	1	-	0.6
Groundnuts	8	1	-	2.4
Beans (Cooked and dried)	3	0	-	-
Meat	66	4	1.7-43.9	14.3
Green Vegetables (Cooked and dried)				
TOTAL SAMPLES	202	6	0.6-43.9	10.0

Lovelace et.al., 1982.

TABLE 9a.

FOOD SAMPLES OBTAINED AT OR NEAR ST. FRANCIS HOSPITAL, KATETE, EASTERN PROVINCE.

COMMODITY	NUMBER OF SAMPLES ANALYSED	NUMBER OF SAMPLES CONTAMINATED BY AFLATOXIN	%
Soaked maize	27	0	0
Shelled dry maize	31	8	26
Germinated maize	11	8	73
Institutional nshima	43	3	7
Institutional beans	34	0	0
Village meals	65	0	0
TOTAL	211	19	9

Njapau et al. 1985.

Table. 9b.

MAIZE PRODUCTS FROM EASTERN COOPERATIVE UNION, CHIPATA, EASTERN PROVINCE.

PRODUCT	NO. OF SAMPLES ANALYSED	NO. OF SAMPLES CONTAMINATED WITH AFLATOXIN	%
Breakfast meal	14	6	43
Roller meal	11	3	27
Maize bran	10	3	30
TOTAL	35	12	34

Samuel, 1987.

TABLE. 9. Cont.

TOTAL AFLATOXIN CONTENT OF VILLAGE FOOD COMMODITIES IN KATETE DISTRICT OF ZAMBIA (1982/1983)

Type of commodity	Source	No. of samples analysed	% positive for aflatoxin	Range of aflatoxins concentration (µg/kg)	Mean of positive (µg/kg)	Overall Mean
Nshima	Village	161	0	-	-	-
Maize (soaked and debraned)	Village	51	2.0	-	14.97[a]	0.29
Maize (whole)	Village	59	15.6	3.9 - 53.8	21.20	3.2
Maize (germinated)	Village	24	58.3	4.2 - 46939.2	55.05[b]	37.82
Nshima maize	School/Hospital	57	8.8	3.3 - 9.8	6.75	0.59
Beans - cooked	Village/Hospital	70	1.4	-	12.65[a]	0.18
Groundnuts - raw and shelled	Village	3	0	-	-	-
Beer (opaque maize beer)	Village	2	100	124 - 170.1	147.1	147.1
Millet - raw and shelled	Village	1	0	-	-	-
	TOTAL	428	7.5	3.9 - 46939.2	42.95	31.76

(a) Result of one positive sample.
(b) Highest reading excluded.

1.7.5. URINE AND SERUM SAMPLES.

Having clearly demonstrated the presence of aflatoxin in human foods and animal feeds in Zambia, investigations were designed to study human consumption of aflatoxin by analyzing urine samples for aflatoxin metabolites. Two hundred and nineteen human urine samples, including patients with liver pathology and controls were collected. Aflatoxin M1 was detected in 3% of the urine samples.A significant seasonal trend was observed, and all but one of the positive samples occurred in the rainy season when the incidence of contamination was higher (6%), Lovelace et.al., 1983) (Table 10).

Another study with 136 urine samples and 34 liver samples from patients with liver pathology and controls at the University Teaching Hospital (U.T.H.), by Dil was completed in 1986. These samples were analyzed for aflatoxin and the results showed an aflatoxin incidence of 3.7% in urine and 14.7% in liver. Aflatoxins B1,M1 and aflatoxicol were detected in the urine samples, while only aflatoxin B1 was detected in the liver samples. Since Lusaka's U.T.H. is usually visited by people from the urban areas, some samples were collected from a hospital in a rural area (St.Francis Hospital, Katete, Eastern Province) for comparison. Twenty-six urine samples were collected and an aflatoxin incidence of 15.4% was obtained, with a mean of 16.5ng aflatoxin per 100ml urine. Thus, the difference in incidence of urine contamination between rural and urban populations was found to be marked (Table 11).

Aflatoxins have been recognised to be more harmful to malnourished than well-nourished animals and humans. Thus, malnourished children in less-developed and developing countries are more susceptible to aflatoxin toxicity (Bhat, 1987). In a 1987 study survey of the association between aflatoxin and protein energy malnutrition (P.E.M.) in Zambia, Samuel collected and analyzed urine and serum samples from malnourished children admitted to the U.T.H., Lusaka and St.Francis Hospital in Katete. Urine samples showed a 6% incidence of contamination with aflatoxin metabolites while serum samples showed only 3% aflatoxin metabolite incidence. The level of excretion was lowest in the kwashiorkor group, a possible indication of a decreased capacity to deal with aflatoxin through the liver (Table 12).

1.7.6. AFLATOXIN CONTAMINATION OF GROUNDNUTS.

Groundnut (Arachis hypogaea L.) is an important food and, cash crop in Zambia, especially the Eastern Province where it is grown in large amounts. Most of the groundnuts produced in Zambia are locally consumed, while the quantity exported has been fluctuating. In Zambia, monitoring kernels for aflatoxin contamination has been carried out for export quality control (Kannaiyan et. al., 1987, Njapau, personal comm.) and has been handled by the Eastern Province cooperative union (ECU), Chipata and NCSR, Chilanga.

TABLE 10.

AFLATOXIN ANALYSIS OF URINE SAMPLES COLLECTED FROM JUNE 1979 - SEPTEMBER 1980.

Total	Liver patients	Afl M_1 +ive	Control patients	Afl M_1 +ive
219	50	0	165	6

Total	Male	Afl M_1 +ive	Female	Afl M_1 +ive
219	131	2	88	4

Liver and Control patients.

Age (years) 0-29yrs	AFM_1 +ive	30-39 yrs	Afl M_1 +ive	40-49 Yrs	Afl M_1 +ive	50+	Afl M_1 +ive
56	2	48	3	51	0	64	1

Total	Rainy season (Nov-Mar)	Afl M_1 +ive	Dry season (Apr-Oct)	Afl M_1 +ive
219	97	5	122	1

Lovelace et.al., 1983.

TABLE 11
AFLATOXIN ANALYSIS OF URINE AND LIVER SAMPLES FROM LIVER PATHOLOGY AND CONTROL CASES IN ZAMBIA.

	TOTAL NO. OF SAMPLES	INCIDENCE OF POSITIVE SAMPLES (%)	MEAN AFLATOXIN CONCENTRATION (ng/100ml)
Urine samples from U.T.H., Lusaka.	136	3.7	9.1
Urine samples from St. Francis, Katete.	26	15.4	16.5
Autopsy liver samples from U.T.H., Lusaka.	34	14.7	2.3

Dil (1986).

TABLE 12.

ANALYSIS OF TOTAL URINE SAMPLES FROM KATETE AND LUSAKA.

	MARUSMUS	MARASMIC KWASHIOKOR	KWASHIORKOR	CONTROL	TOTAL
NO. OF SAMPLES	11	17	23	63	114
NO. OF POSITIVE SAMPLES	0	1	2	4	7
% OF POSITIVE SAMPLES	-	5.9	8.7	6.0	6.1
AFLATOXINS DETECTED (ng/ml)		(i) aflatoxicol -0.5	(i) AFB1 - 0.0625 (ii) aflatoxicol -0.125	(i) AFB1 -0.25 Afl 0.63 (ii) AFB1 -0.313 (iii)AFL -0.5 (iv) AFL -0.8	mean AFB1 0.21 AFL 0.45

Samuel, 1987

Research was initiated on infection of groundnuts kernels by A. flavus. From 1977/78 to 1985/86 season, a total of 28,410 groundnut samples were analyzed. These samples were newly harvested groundnuts meant for export that were bought from local farmers across the province . A mean of 6.3% of the samples had aflatoxin contents of more than 5 mg/kg and these were rejected for export (Kannaiyan et al., 1987). Thus,the tolerance levels for groundnuts in Zambia has been set below 5mg/kg (Table 13) for export.

In another survey to study the prevalence of A. flavus in market samples, 8 random samples of the predominant chalimbana cultivars were collected from the market in July 1987. These had been harvested in May the same year. Seeds of Chalimbana from Msekera Research Station were used as controls. After incubation for one week, the seeds were examined for percentage germination and A. flavus colonisation. It was found that both market and control had A.flavus and infection of samples ranged from 0.8 to 8.0% while the controls had 4.5% (Kannaiyan et.al., 1987)
(Table 14).

To study if A.flavus infection differed among common cultivars of groundnuts, 8 cultivars grown during the 1985/86 season at Msekera Research Station were sampled. Of these, three were short-duration types with small seeds, while the other were long-duration types with medium to large seeds. Sampling was repeated in the 1986/87 season when seven of the original eight cultivars were tested. The results showed cultivars differed significantly in seed germination and A. flavus infection.

In addition to the aflatoxins, diplosporin, zearalenone and deoxynivalenol have been detected in Zambian maize and maize-based animal feeds. Lovelace and Nyathi (1977) reported the occurrence of zearalenone in 75% of maize malt and traditional beer samples. Zearalenone alone and together with deoxynivalenol have been isolated from mouldy maize and poultry feeds.The incidence varied from 15 to 30% for zearalenone and was 1.7% for deoxynivalenol.

TABLE 13

FREQUENCY OF AFLATOXIN CONTAMINATION DETECTED IN EXPORT SAMPLES OF GROUNDNUTS KERNELS IN ZAMBIA, MONITORED BY ECU FROM 1977/78 TO 1985/86.

HARVEST	NO. OF SAMPLES TESTED	SAMPLES CONTAINING >5mg/Kg AFLATOXIN (%).
1977/78	9 200	3.3
1978/79	1 050	9.5
1779/80	1 060	9.4
1980/81	*3 300*	*3.0*
1981/82	2 400	8.3
1982/83	4 900	8.2
1983/84	-	-
1984/85	3 900	5.1
1985/86	2 600	3.9
OVERALL	28 410	MEAN 6.3

Kannaiyan et. al., 1987.

TABLE 14.

RESULTS OF LABORATORY SCREENING OF GROUNDNUT VARIETIES FOR ASPERGILLUS FLAVUS INFECTION (%), MSEKERA RESEARCH STATION FROM 1985/86 TO 1986/87.

VARIETY	1986		1987	
	Germination %	A.flavus infection %	germination %	A.flavus Infection %
SHORT DURATION (Small seed)				
- Tifspan	97	1	86	8
- Natal common	95	10	75	5
- Comet	87	1	81	0
LONG DURATION (Medium/large seeds)				
- Makulu Red	94	14	73	3
- Egret	91	5	-	-
- 4a/8/2	90	4	70	0
Chalimbana	79	1	55	4
Mean	88	7	71	5
SE	±1.5	±1.1	± 2.9	± 0.9
MGS -2	69	19	57	17

Kannaiyan et.al., 1987

1.8. DROUGHT AND CONSUMPTION OF MOULDY MAIZE.

For more than a decade now (1982 -1995), Africa has been experiencing drought which has meant declining food production and strategic reserves on the whole continent. Southern Africa has seen two major droughts : one in 1982-1984 and the other in 1991-1993. Before the 1982-1984 drought, Southern Africa had never seen the equivalent for 50 years. 1983 had seen what was called the "killer drought in Africa" because it hit many African countries, ranging from the Sahel belt bordering the sahara to the southern half of the continent. For several countries, it was the second year in succession in which the rains had failed, leaving a spectre of mass hunger.

The dry spells or droughts that Africa has been experiencing since 1982 were expected to continue for least 10 years. Now in 1995, it is clear that this pronouncement was correct. Most of the African countries have experienced the impacts of severe drought crises. The "perpetual droughts of the 1980's" eventually turned into the worst drought disaster Southern Africa has ever recorded.
Zambia has faced hunger and starvation. In 1981 and 1982, Zambia imported 21,450 and 30,000 tonnes of maize, respectively, following the drop in maize production. In 1983, Zambia mounted emergency measures and the United States of America agreed to assist, signing an agreement for 31,000 tonnes of maize. The United States of America Shipped about 20,000 tones of its wheat to Zimbabwe in exchange for white Zimbabwean maize (31,000 tones) that was to be supplied to Zambia (Zambia Daily Mail, March 25, 1983). Some of this (3000 x 90 kg bags) (270 tonnes) was rejected due to bad quality.

The drought continued in most parts of Zambia during the 1984/85 rainfall season. The government in an effort to supplement the serious crop shortfall and to avert serious maize shortages imported 20,000 tonnes of maize from Malawi and 50,000 tonnes from Zimbabwe. Zambia also suffered a serious shortfall of millions of bags of maize which resulted from a prolonged drought in several parts of the country in 1987, resulting in more than 770,000 people being affected by famine. Maize grain had to be imported, and several pledges to pay for the grain were received from donor countries and agencies (Zambia Daily Mail, September 2, 1987).

In 1990, a 6-week partial drought hit 8 of the 9 provinces in Zambia. According to Zambia Daily Mail, March 13 1990, all provinces except North-western had seen poor rains that had devastated crops. A forecast showed a reduced maize yield of between 9 and 10 million bags. This output represented a drop in maize production of between 3 and 4.5 million bags from the bags marketed during the 1989/90 harvest. The Times of Zambia (October 3, 1990) reported that due to the 6-week drought of February-March 1990, villagers in Gwembe Valley were eating boiled roots to survive. The villagers were forced to eat a root called "Cimpama" found in the hills.

The devastating drought of 1991-1993 caused a total crop failure that was declared in the whole Southern African region. This crop failure led to a food crisis that brought an estimated 130 million people and an undisclosed number of domestic and wild animals and poultry to the brink of starvation, hunger and death.

The rural people were most affected and competed with livestock for the little water available. Zambia, Malawi and Mozambique were among the worst-off of the drought-affected countries. Mozambique obtained 80% of its food from international aid.

Inadequate rains have also occurred in most areas in the 1993/94 season. There were reports that the dry spell would reduce national maize yields from the initial 16.5 million to 9.7 million bags signalling fears of possible famine since there were not adequate maize stocks to last up to the next harvest period (March/April 1994). In July 1994, the government declared a national food disaster that needed K32 billion to cushion the impact on the starving and suffering rural population. The food disaster statement followed several reports of villagers suffering from food shortages.

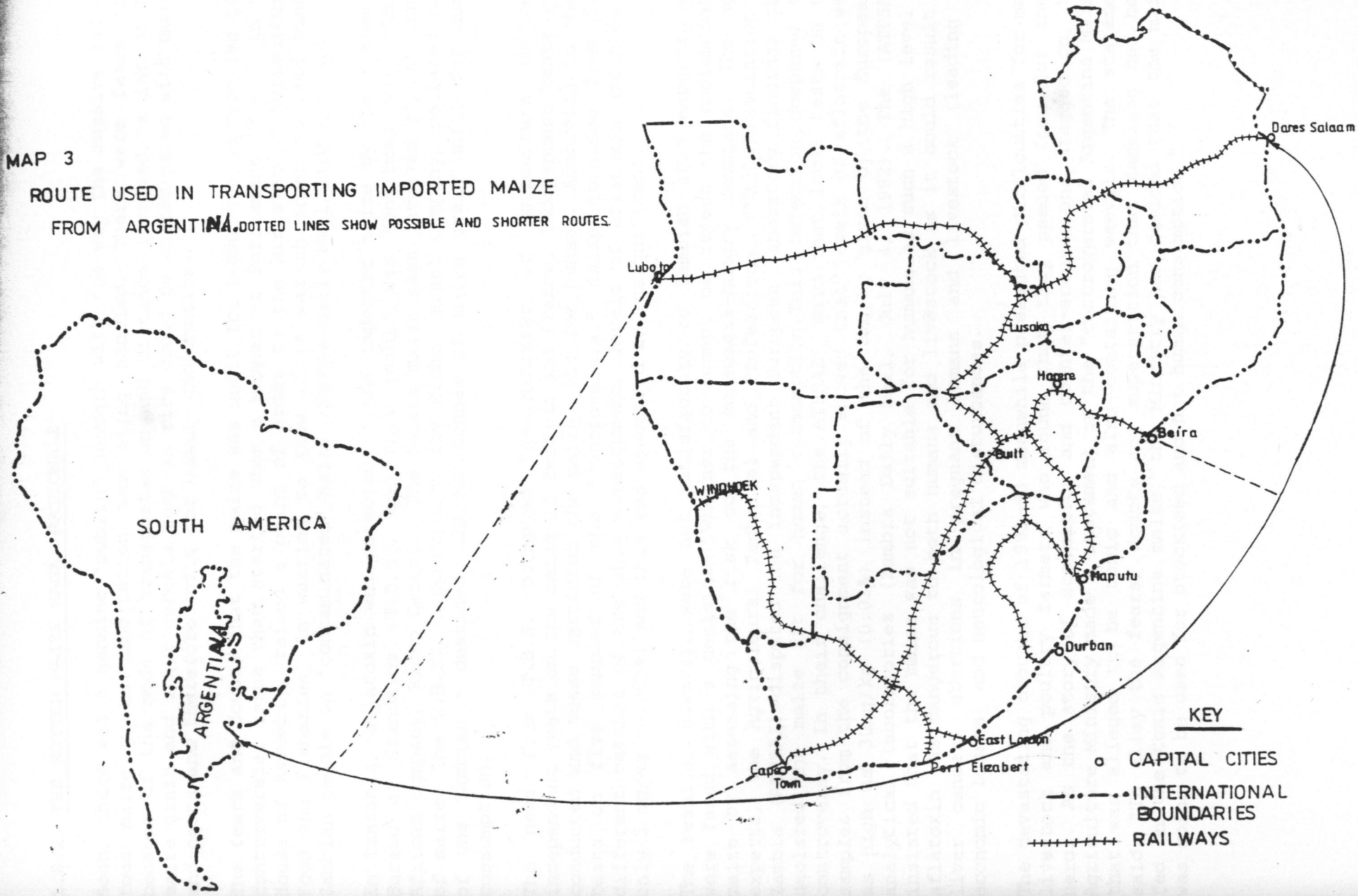
1.8.1. MASSIVE RELIEF-FOOD SHIPMENTS.

Only months after the regional crop failure declaration, in 1992 and the several SOS messages, a number of donor countries met in Geneva and made several pledges and commitments for relief food and monetary aid. A United Nations conference meeting promised nearly 1 billion dollars in relief aid and 665,000 tonnes of food to ten southern African countries that were suffering from the worst drought (Zambia Daily Mail, June 3, 1992). By August 1992, Zambia was expected to "import" 1 million tonnes of maize from as far as South America. About 2 million people were under serious risk of undernutrition if the food was not delivered regularly and in sufficient quantities (U.S. Office of Foreign Disaster Assistance, 1992).

Soon, massive relief food shipment from Argentina and France started. Water transport was used for its low cost and bulk carriage as compared to air, which is very expensive. However, some of the maize imported from Argentina was transported under humid condition in unusually large quantities. Some of the maize was even soaked while in transit to the port of Dar-es-salaam, Tanzania, East Africa. This could have provided the kind of humidity conditions that could enable the growth of moulds and allowed pest infestation. This was compounded by the fact that the transport route ended up in Tanzania, on the eastern coast of Africa, instead of the ports on the western coast (fig 12). The longer transport route was caused by the politically volatile situations in Angola, Mozambique and South Africa.

MAP 3

ROUTE USED IN TRANSPORTING IMPORTED MAIZE FROM ARGENTINA. DOTTED LINES SHOW POSSIBLE AND SHORTER ROUTES.



1.8.2. THE ROTTEN MAIZE SAGA: RATIONALE

Soon, there was a genuine "public" concern with the way the massive relief-food maize grain importation was being handled. There were fears that because of the mode of transportation and distance involved, a lot of the maize grain and other cereals such as rice could be contaminated with moulds and pests and therefore unfit for human consumption.

The fears and doubts that the maize was unfit for human consumption led to a controversial issue that started when a Member of Parliament (M.P.), in the House of Assembly, raised a point of order to the Minister of Agriculture, Food and Fisheries. In wanting to know if it was in order to feed hungry Zambian people on "contaminated" maize (Zambia Daily Mail, July 2, 1992).

In Tanzania, aflatoxin was detected in the imported maize by the Tanzanian Bureau of Standards (T.B.S.) and this result was confirmed by a South African company, Richo Cereals. The rotten maize saga involved 20,000 tonnes of maize. The T.B.S., supported by the Richo Cereal Company, declared part of the Zambian - destined 20,000 tonnes of maize grain unfit for human consumption.

The news from T.B.S. prompted the Minister of Agriculture to seek independent tests on the maize in Belgium and France. Independent tests were conducted and these certified the maize fit for humans. According to these tests on five samples of the "controversial" maize obtained from five different batches of the ship consignment, levels of aflatoxin amounted to only 5 mg/kg (0.005%) and this was described as insignificant.

The results, however, were not satisfactory to Zambian agro experts, who were faced with a decision whether to accept or reject the contaminated maize by assessing the risk to the consumers-hungry Zambians. The agro experts, the Agricultural Technical and Professional Staff Association of Zambia (ATPSAZ) disputed the independent European laboratory findings that declared the maize fit for human consumption.This rejection deepened the controversy. In their challenge, the ATPSAZ said that local tests on the samples from the consignment actually showed that levels of aflatoxin were as high as 30mg/kg (0.03%) instead of the 5mg/kg given by the European analytical laboratories (Zambia Daily Mail, July 4, 1992). The (ATPSAZ) insisted that the maize was not suitable for humans as such a high level of aflatoxin was dangerous to both humans and livestock as it could result in liver cancer, abortions in pregnant humans and livestock (leading to economic losses) and neurological disturbances.

The devastating drought of 1991-93 meanwhile presented difficulties for many livestock and poultry farmers who found that they needed food for their herds. As the story of accusation and counter-accusations dragged on, the Agriculture Ministry made a statement on the "controversial Argentina Maize" that was alleged to be toxic and also containing weevils. The statement said, "To allay the fears, doubts and superstition that Zambians had been fed on the toxic Argentina maize, the Ministry had decided that the maize was going to be used for stockfeed and not human consumption."

The knowledge of difficulties to farmers, caused by the scarcity and high cost of cereal grains and stockfeed, could possibly explain how the Ministry thought they could avoid a total destruction of the maize imported at high cost in foreign exchange.

Our initial interest in "Aflatoxin Residues in Edible Tissue of Poultry in Zambia" was stimulated by the announcement that the controversial toxic maize was going to be diverted into stockfeed. As soon as the Ministry responsible for the importation of the potentially toxic and contaminated maize was thinking of this, it was clear to us (Lovelace, personal comm.) that more attention should be given to a study on aflatoxins in livestock intended for human consumption. It seemed logical to approach the problem of the toxicity caused by aflatoxin as a result of ingestion of contaminated maize and other feed substrates of plant origin, by determining the presence and levels in edible tissue from known dietary levels of aflatoxin in maize. The main theme was to show whether it was a good idea to feed aflatoxin contaminated maize to livestock, especially poultry, that was destined for human consumption itself. At the time of these events, the only source of food was the imported maize and levels of aflatoxin no matter how "insignificant" could prove dangerous and endanger the lives of younger children who need to eat several times a day.

1.9. OBJECTIVES.

The objectives of this investigation were to study:

(1) **Entry into Edible tissue.**

To identify whether aflatoxins contained in poultry feed enter the edible portions of chickens maintained on the feed;

(2) **Quantities not Causing Physiological Malfunction**

To determine the amounts of aflatoxins that could be consumed in a mouldy feed by chickens in a given period;

(3) **Retention**

To determine the amounts of aflatoxins retained in the different edible portions, in particular the liver, the gizzard and the muscle of thigh.

CHAPTER II.

2.0. MATERIALS AND METHODS.

2.1. EQUIPMENT AND CHEMICALS

2.1.1. EQUIPMENT

a. Glass stoppered flasks.
b. Thin-layer glass plates (200 x 200 mm).
c. Measuring cylinders (10, 50, 100, 200 ml).
d. Hamilton Microsyringes (10 ml, 100 ml).
e. Development tanks (Chromatography).
f. Ultraviolet lamp in a viewing box (365 mm wavelength).
g. Wrist-action shaker.
h. High speed blender (7 speed blender, Hamilton Beach, Scovill).
i. Glass minicolumns for chromatography (27 x 4 cm).
j. Filter papers (Whatman type No.113, large type).
k. Glass funnels.
l. R110 Buchi rotary evaporator and vacuum pump (Edwards High vacuum Pump, ED 50, Crawley, England).
m. Glass Vials (5 ml).
n. Glass wool.
o. Face Mask.
p. Disposable or plastic gloves.
q. Labelling pens and masking tape.
r. Parafilm or paper foil.
s. Precoated TLC aluminium sheets Silica gel G60, E.Merck, Darmstadt, Germany (100 x 100 mm), handcut from 200 x 200 mm plates.
t. Oven.

2.1.2. REAGENTS AND CHEMICALS

a. Acetone.
b. Acetonitrile.
c. Benzene.
d. Chloroform (containing £ 0.75% ethanol).
e. Citric acid monohydrate.
f. Diatomaceous Earth (DAE)
g. Dichloromethane.
h. Diethyl ether (containing £ 0.01% ethanol)
i. Glacial acetic acid.
j. Hexane.
k. Isopropanol.
l. Methanol.
m. Silica gel G 60 (E.merck, Darmstadt, Germany).*
n. Sodium chloride.
o. Sodium sulphate (granular, anhydrous).*
p. Trichloroacetic acid.
q. Nitric, Sulphuric and Hydrochloric acids.
r. Aflatoxin standards, Sigma Chemical Co., Poole, England.*
s. Toluene.

All reagents were either General purpose Reagents (GPR) or analaR* quality from BDH chemicals.

2.1.3. WORKING AFLATOXIN STANDARDS.

The aflatoxin standards used in this study were set at 2ng/ml for quantitation purposes. For example to achieve this for aflatoxin B1, a 10.0 mg aflatoxin B1 sample was dissolved in 10 ml benzene-acetonitrile (98: 2 v/v) to give 1 mg/ml aflatoxin B1, which is equivalent to 1 mg/ml. A 10.0 ml aliquot of this was then diluted to 5.00 ml with benzene-acetonitrile to give 2 mg/ml or 2ng/ml.

For the aflatoxin M1 standard, 0.01 mg aflatoxin M1 was dissolved in 5.00 ml acetonitrile-benzene (2:98 v/v) to give 0.002 mg/ml or 2ng/ml.

2.1.4. SOLVENT SYSTEM FOR AFLATOXIN RESOLUTION

Various solvent systems for aflatoxin resolution are available from literature (Getz,1980b) and the following were tested to select the most satisfactory resolution of aflatoxins B1, B2, G1, G2, and M1.

a. Diethyl ether - methanol - water (96:3:1 v/v/v).
b. Chloroform - acetone - water (86.5:12:1.5 v/v/v).
c. Chloroform - acetone - water (86:12:2 v/v/v).
d. Chloroform - acetone - water (86.5:12:2.5 v/v/v).
e. Chloroform - acetone - methanol (87:10:3 v/v/v).
f. Chloroform - acetone - methanol (90:10:2 v/v/v).
g. chloroform - acetone - isopropanol (82:10:8 v/v/v).

In the resolution of aflatoxin B1, B2, G1, and G2, the Rf values were expected to be between 0.4 and 0.7. Trial results using silica gel plates prepared in this laboratory showed Rf values between 0.9 and 1.0. This meant that variations in the treatment of silica gel, or laboratory conditions, or solvent proportions, or solvent would be needed to achieve the desired development. Similar aflatoxin analysis has been done with desired resolutions in Zambia under similar laboratory conditions. Emphasis was therefore placed on the investigation of the silica gel properties by changing solvent proportions and developing aflatoxin on silica gel. Most trials gave very high Rf values.

Use was then made of newly acquired precoated aluminium silica gel TLC plates (E.Merck) which were tested with success. Rf values of the solvent system were between 0.45 and 0.75 in most of the systems, and these were used in all further experiments.

2.2. EXPERIMENTAL POULTRY.

2.2.1. SOURCE OF CHICKENS.

The chickens used in this study were obtained from farms west of Lusaka within 20 kilometres.

2.2.2. MAINTENANCE OF CHICKENS

A group of indigenous village chickens of both sexes, obtained from the same mother, aged between 3-4 weeks, to be used as an experimental flock was housed in metal cages and maintained in the infected animals section of the Samora Machel School of Veterinary Medicine Small Animal Quarters, at the University of Zambia. The chickens were initially equalised on body weight and allocated to the group cages and fed as a group from the metal feeders and drinkers. The metal cages were fixed with a wire mesh at the base, just above the refuse tray. The floor of the tray was covered with a newspaper to serve as a litter material. The feed droppings of each group were trapped in the tray, collected and weighed.

The chickens assigned to group cages and dietary treatments were allowed an ad libitum access to their assigned diets and fresh clean water two - three times a day. The experimental rations were fed in two feeder-full portions at 08:00 hours in the morning and 14:00 hours in the afternoon. Individual body weights and group feed consumption were recorded weekly and daily, respectively, for 21 days. On the 21st day, the experiment was terminated and the chickens collected for sacrifice, post-mortem and total aflatoxin determination. Feed but not water was withdrawn after weighing prior to slaughter.

2.2.3. FEEDING AND WEIGHING

The group, used as control, was fed on clean feed (i.e. feed to which no contamination was made by way of adding mouldy feed grown for experimental purposes). The experimental groups were fed on feed containing aflatoxin-contaminated (mouldy) maize at two different levels ie 25% mouldy maize weight by weight (or 1185 mg/kg total aflatoxin) and 75% mouldy maize w/w (or 3555 mg/kg total aflatoxin). The feed for the controls contained roller meal (whole maize) without contamination as detected by the aflatoxin analytical method. Each of the diets had an equal number of chickens. The chickens used in the trials were not treated against any common poultry disease or internal parasites. There were no replicates per dietary treatment due to shortages of chickens.

At the end of the feeding trials, but before sacrifice, each of the chickens from each diet was number-labelled on one leg with a marker for weighing and identification. The individual weights were recorded. This gave the final (week 3) live weight of each individual chicken.

2.2.4. POST-MORTEM

The chickens were sacrificed by cervical dislocation, and the jugular vein cut to allow bleeding from the neck. The chicken was then sprayed briefly with hot water and set with the anterior side up on the post-mortem table. A deep incision was cut, using a sharp knife, along the anterior side of the chicken to give access to the selected organs and tissues.

The internal organs were removed and their weights were recorded individually. Immediately after removal, sensory evaluation of the liver, kidney, gizzard, heart and spleen for colour, tenderness and overall pathological appearance was carried out by the veterinary pathologists, Dr M.M. Musonda and Dr C. Nyeleti (Department of Paraclinical Studies, Samora Machel School of Veterinary Medicine). The evaluated samples were dropped into an ice-cooled receptacle until all the same organs (e.g livers) from all the chickens in the same group were pooled together and these stored below or at 4 degrees celsius until when required for tissue aflatoxin analysis.

2.3. PREPARATION OF AFLATOXIN CONTAMINATED MAIZE

2.3.1. FUMIGATION OF MAIZE WITH METHYL BROMIDE

The white maize bought from Soweto Public Market in Lusaka was treated by fumigating with methyl bromide for 22 hours at the Chisamba Depot of the Zambia Cooperative Federation (ZCF) Limited. This was done to kill microoganisms and to destroy the germ of the grain to reduce grain germination during the growth of the A.flavus fungus.

Methyl bromide (bromomethane) is a highly toxic gas and is supplied in pressurised tins or gas cylinders. Because it is very poisonous to humans, the bagged maize was first covered by polythene sheets. The cylinder of methyl bromide is then opened under the polythene sheets, and as the gas escapes from the cylinder, it penetrates the grain and inactivated the germs.

2.3.2. GROWTH OF FUNGUS ON EXPERIMENTAL MAIZE.

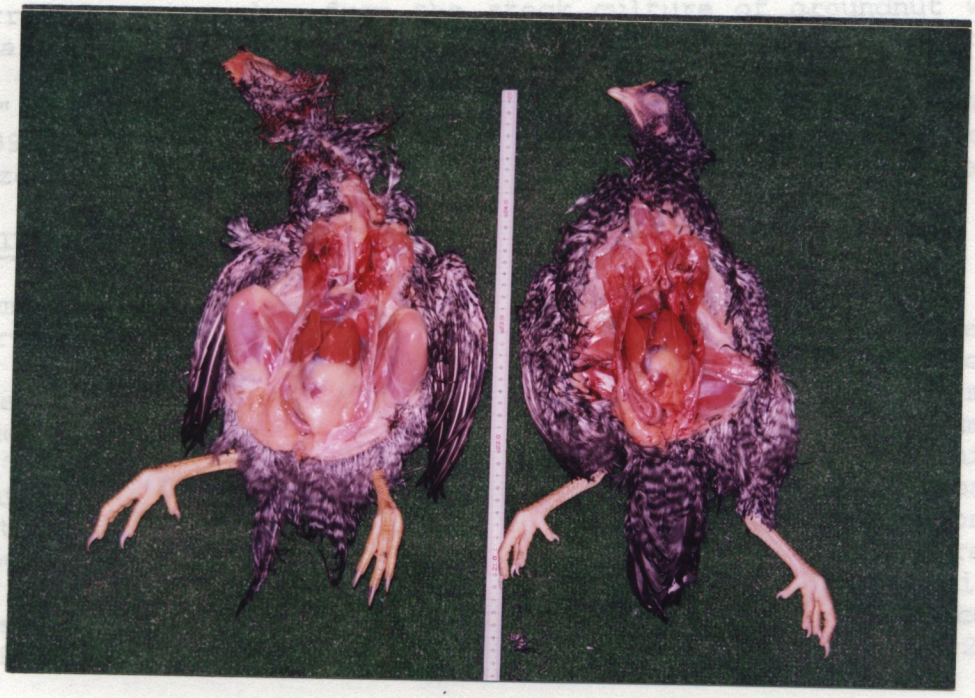
The polished and fumigated maize was used as substrate for aflatoxin production by the A.flavus fungus. The mould was isolated from mouldy groundnuts obtained from the Livestock and Pest Research Centre of Mt Makulu, NCSR, Chilanga. Maize constitutes about 50-65% by weight of a balanced diet in poultry production (Aregheore et.al., 1993).

A.flavus requires certain conditions for growth ie
(a) A temperature ranging from 25 to 32 degrees celsius,
(b) An atmosphere with moisture (about 80-95% relative humidity),
(c) Good air circulation, and
(d) A suitable substrate containing starch.

In Zambia, especially in the dry season or drought, these four essential growth conditions are rarely found together in the open air for any length of time. In order to produce aflatoxin on an experimental basis, it was realised that at least two of the essential conditions should be artificially maintained. This was done in a room.

Fig 13

POST-MORTEM



Chickens with deep incisions to access some selected organs and tissues.

During the fungus growth experiment, climatic room temperatures ranged from 28 to 32 degrees celsius on average. These high temperatures favoured the growth and development of the mould and therefore could maximise production of the metabolite, aflatoxin. An atmosphere saturated with moisture was maintained by covering the maize with grain sacks kept wet by sprinkling water on top. The grain sacks were not allowed to drip with water as this would disturb the growing fungus (Hard,1961). The wet grain sacks on top of the maize also prevented loss of moisture from the maize. Due to the high climatic temperatures, evaporation was inevitable but controlled evaporation was ensured by sprinkling water onto the top grain sacks when they appeared a bit dry. The grain sacks were porous and allowed good air circulation to provide oxygen for the growing fungus.

2.3.3. INOCULATION.

A mouldy groundnut inoculum from the stock culture of groundnut was spread over the maize previously soaked for six hours and laid on wet sacks. After spreading, the inoculated maize was covered with moist sacks. The maize was "incubated" for 24 days at the prevailing room temperatures (December 1993-January 1994). After 24 days, the top grain sacks were removed and the mouldy maize left to dry completely for 7 days, at room temperature.

2.3.4. GRINDING.

The dried maize was mixed thoroughly before being ground to a grit meal in a small, portable Dicken's Mill (serial No.UNL 242259) fabricated by the University of Nebraska. During grinding, the grinder sampled ground maize through one of the spouts. The ground maize was sampled from a mixture of grit blended in an electric rotary mixer (15 minutes) at maximum speed in top gear. To avoid loss of grit and powder during rotation and mixing, the mouth of mixer was covered with plastic and bound with a rubber band. After blending, the representative sample was taken for further reduction from the grit to powder. This was achieved by grinding in a C and N Laboratory Mill size 8" (Christy and Norris Limited, Chelmsford, England) grinder. The powdered sample was then analysed for its total aflatoxin content as aflatoxin B1, B2, G1 and G2 using the A.O.A.C. method outlined in 2.4.

2.4. ESTIMATION OF AFLATOXIN IN EXPERIMENTAL FEED

2.4.1. EXTRACTION WITH 80% V/V METHANOL SOLUTION.

The method used was adapted from AOAC (Stubblefield, R., U.S. Department of Agriculture, U.S.A.). In this method a 50.0g sample of completely ground aflatoxin contaminated material was weighed into a 500ml glass-stoppered conical flask and 250ml of 80% V/V methanol in water added. The glass stopper was then placed and sealed tightly into the flask with masking tape. The flask and its contents were shaken on a wrist-action shaker (Stuart Scientific Model). After 30 minutes, the contents of the flask were filtered through Whatman filter paper (Grade 113, Large type) and the yellow-orange filtrate collected for clean up.

2.4.2. CLEAN-UP OF METHANOL SOLUTION.

In the chromatographic clean-up stage, a 50.0ml yellow-orange filtrate portion was mixed with 50.0ml n-hexane in a 250ml separating funnel. To the mixture, 10.0ml of saturated sodium chloride were added to improve miscibility. The contents of the separating funnel were vigorously hand-shaken for 2 minutes with intermittent pressure releases by opening the spout while the separating funnel was upside down. The two layers were allowed to separate by standing for 5 to 10 minutes, after which the bottom methanol layer was collected for further hexane defatting (2 times). Before each repeat, the separating funnel was rinsed with 80% v/v methanol in water to remove any attached fat. The top hexane layers were discarded after each separation.

2.4.3. EXTRACTION OF AFLATOXIN WITH CHLOROFORM

To extract the aflatoxin from the defatted methanol portion, chloroform was added in three portions of 30, 20 and 10ml. After each addition and vigorous shaking, the bottom chloroform layer was collected. The top layer remaining in the separating funnel was treated with the next chloroform portion, the bottom layer collected in the same flask and then this repeated. The total chloroform extract (about 60ml) was washed twice with 50.0ml portions of distilled water by shaking vigorously in the separating funnel (Njapau, personal comm.). The bottom chloroform layer was collected and the top layer discarded. Before the second washing, the separating funnel was once more rinsed with 80% v/v methanol in water. The water-washed chloroform extract was drained into a conical flask containing 10.0g of anhydrous sodium sulphate for drying. The contents were swirled gently and then filtered through Whatman filter paper to remove the sodium sulphate. A total of 45ml was collected. Because of the suspected high aflatoxin concentration, no prior concentration was done.

2.4.4. THIN LAYER CHROMATOGRAPHY ANALYSIS.

A 100 x 100mm silica gel G thin layer plate was scored and spotted as shown in figure **14**. A 10 ml aliquot of sample of extract was spotted together with aflatoxins B1, B2, G1 and G2 standards for visual and quantitative analysis. Parallel starting spots were made with micro-litre syringes from the chloroform sample extract and the benzene-acetonitrile standards solutions. Spots were left to dry in air for 5 minutes.

The prepared plate was transferred to a chromatographic tank containing 87/10/3 v/v/v chloroform-acetone-isopropanol for development to the scoreline. A suitable volume of solvent mixture was placed in the bottom of the glass tank so that the starting spots on the plate would be above the upper surface of the solvent mixture. The developed plate was removed from the tank and allowed to dry in air for 5 minutes. Spots were viewed under a long wave U.V lamp (365nm). Rf values, colours and intensities of the unknown spots were compared with those of the reference aflatoxins B1, B2, G1 and G2.

This is known as the "Comparison-of-standards" technique, where the amount of aflatoxin in the sample extract was estimated by comparing its intensity of fluorescence with those of the standard aflatoxin solution spot. Interpolation was necessary.
The amount of aflatoxin was given by

$$mg/kg = \frac{\text{vol std spot x std concn x extract vol}}{\text{vol extract spot x mass of sample}}$$

where vol std spot = Volume of standard solution giving fluorescence intensity equal to that of sample spot, in ml.

std concn = Concentration of standard aflatoxin solution in ng/ml

extract vol = Total volume of final extract, in ml.

vol extract spot = Volume of sample extract, in ml.

mass of sample = mass of sample used in extraction, in g.

2.5. DIETS.

Experimental diets used in this study consisted of commercial growers mash manufactured by National Milling Co. Limited, Lusaka and two diets containing different amounts of mouldy maize added to the growers mash. In the control diet, the mouldy maize was replaced by roller meal. The experimental whole maize feed given to the experimental chickens during the study was analysed and found to contain 1650 mg aflatoxin B1 per kg feed and 4750 mg total aflatoxin per kg (Table 15.). In order to achieve the 25% or 75% w/w mouldy maize, roller meal was adjusted as mouldy meal increased. Animal protein and vegetable sources were deliberately omitted from the formulations since one of the objectives was to determine how much mouldy maize would be consumed by the chickens in a time of scarcity as occurred in the 1991-1993 drought of southern Africa.

Diets were also analysed for proximate composition (Table 16).

2.6. FEEDING TRIALS.

2.6.1. PRELIMINARY FEEDING TRIALS AND IDENTIFICATION OF FLATOXIN IN EDIBLE TISSUE.

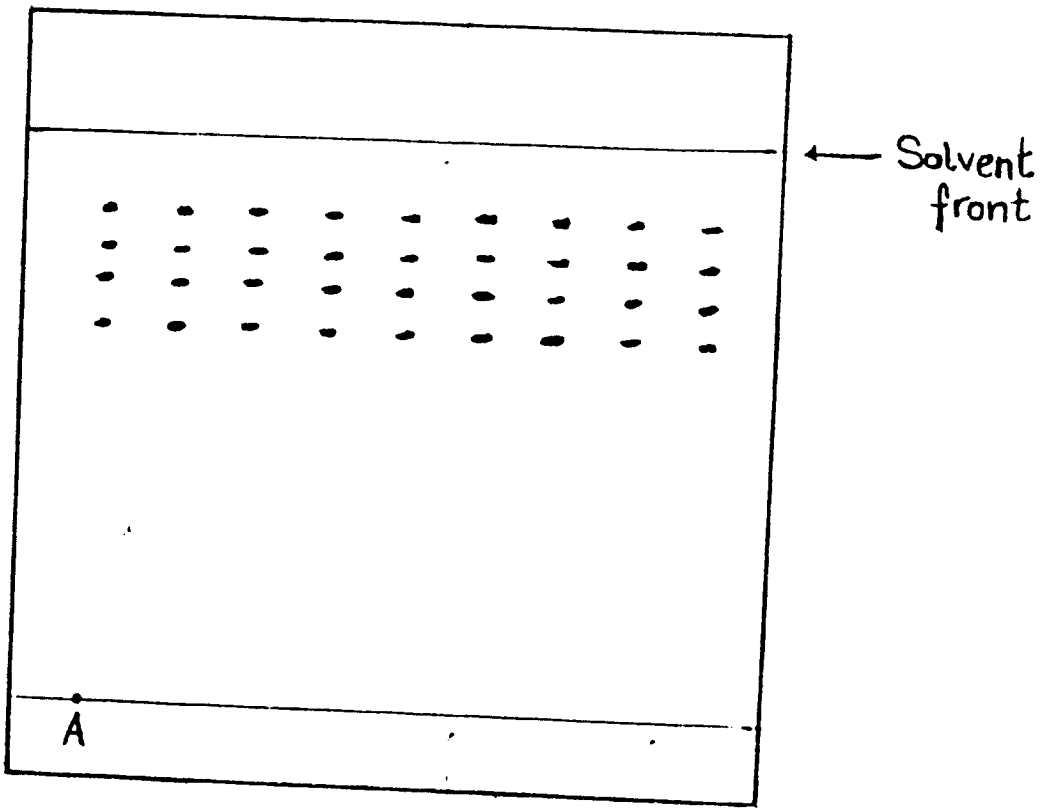
EXPERIMENT 1 AND 2

In these trials, both lasting five days four village chickens of both sexes and aged between 3-4 weeks were used. The trials were designed to show the presence (qualitative identification) of aflatoxin in the proposed tissues. This was going to be the basis of the choice of organs and tissues to be analysed further on in the study.

In Experiment 1, the four village chickens were divided into a control group and an experimental group of two chickens each. The control diet consisted of whole maize meal with no aflatoxin as analysed by the aflatoxin analytical procedure. The experimental diet consisted of the same whole maize meal spiked with standard aflatoxin B1 to give 1200mg/kg fed ad libitum for 5 days. The tissues removed for analysis were the livers (mixed with the spleens), kidneys, gizzards, (mixed with the hearts) and the thigh tissue. The spleens and hearts were mixed with the livers and gizzards, respectively, because of similar texture and because their small amounts could not make enough weight (50.0g) to be analysed separately.

In Experiment 2, like in Experiment 1, four village chickens of both sexes were divided into two groups. The controls were fed on the clean whole maize meal described in experiment 1. The experimental group was fed on the just prepared mouldy feed (1650mg/kg aflatoxin B1) for 5 days. The mouldy feed was provided at 100% total aflatoxin equivalent i.e there was no dilution with the clean whole maize meal. This experiment was also designed to study the feed consumption differences between the control and experimental chickens. On the 5th day, the chickens were sacrificed and samples for pathological examination and aflatoxin analysis removed.

Fig. 14



Tlc Analysis of Experimental Feed

TABLE 15
AFLATOXIN COMPOSITION OF EXPERIMENTAL (MOULDY) FEED

AFLATOXIN	mg/kg
B1	1650
B2	445
G1	1980
G2	675
Total	4750

TABLE 16

PROXIMATE COMPOSITION OF BASIC DIETS USED IN STUDY

GROWERS MASH	
INGREDIENT	%
Crude Protein (min)	16.0
Crude fibre (max)	7.0
Calcium	1.0 - 1.2
Total phosphorous (min)	0.75
Enriched with amino acids, vitamins and trace elements as per requirement.	

Namfeeds, National Milling Co. Ltd.

MOULDY WHOLE MAIZE	
INGREDIENT	%
Crude Protein (min)	17.54
Crude fibre (max)	8.40
Calcium	0.02
Total phosphorus	0.28

Consultancy Services, Animal Science Dept, Agriculture Sciences, UNZA.

TABLE 17a EXPERIMENTAL DESIGN
EXPERIMENTS 1 and 2

	EXPERIMENT	1	EXPERIMENT	2
	CONTROL	EXPTAL	CONTROL	EXPTAL
Number of chickens	2	2	2	2
Aflatoxin (ug/kg AF B1)	0	1200	0	1650
Duration of feeding (days)	5	5	5	5

PURPOSE: Qualitative check for aflatoxin transmission into tissues.

2.6.2. INVESTIGATION OF THE CONSUMPTION BY CHICKS OF MEAL CONTAINING AFLATOXIN

(EXPERIMENT 3 AND 4).

These experiments were the major trials and lasted for 21 days each. They were aimed at studying the consumption by the chickens of meal containing aflatoxin at two different levels.

In experiment 3, fourteen (14) chickens from one batch averaging about 157g in live body weight and aged 3 weeks were equalised for body weight and allocated to three dietary treatments (Table 17). After equalising on body weight, the mean weights were 156g (controls), 157g(low level) and 158g (high level) groups. The initial (before commencement of treatment), intermittent and final (just before slaughter) body weights were taken at 0, 7, 14 and 21 days. Feed intake records were kept on a daily basis and feed refusals were weighed everyday.

In experiment 4, eleven (11) village chickens from one batch of both sexes, about 4 weeks of age, were used. The chickens were divided into 4 groups and assigned to the four dietary treatment groups given in Table 18. These chickens were also used in excretion trials, consisting of four collection periods. Faeces from each group were collected on the 2nd, 7th, 14th and 21st days. The faeces were sun-dried on a concrete floor for 3-4 days. These were stored in polythene bags at room temperature. Faecal samples were analysed for aflatoxin using the A.O.A.C.
method outlined for estimation of aflatoxin in the experimental feed (section 2.4.5.).

TABLE 17b
PERCENTAGE COMPOSITION OF AFLATOXIN DIETS USED IN:

Experiment 3

INGREDIENT (% W/W)	TREATMENT GROUP		
DIET (No OF CHICKENS)	CONTROL DIET (4)	LOW LEVEL AF (5)	HIGH LEVEL AF(5)
MOULDY MAIZE	0	50	100
Growers mash and whole maize meal (clean) 50:50	100	50	0
TOTAL	100	100	100

EXPERIMENTAL DESIGN
EXPERIMENT 3.

	TREATMENT 1	TREATMENT 2	TREATMENT 3
Number of chickens	4	4	4
Aflatoxin (ug/kg AF B1)	0	825	1650
Duration of feeding (days	21	21	21

PURPOSE: Measure growth rates and feed intake.

TABLE 18a PERCENTAGE COMPOSITION OF AFLATOXIN DIETS
Experiment 4

INGREDIENTS (%W/W) (No of Chickens)	TREATMENT GROUP			
	2A (3)	2B (2)	3A (3)	3B (3)
Mouldy Maize	75	75	25	25
Growers Mash	0	25	75	0
Whole Maize Meal	25	0	0	75
TOTAL	100	100	100	100

TABLE 18b

EXPERIMENTAL DESIGN
EXPERIMENT 4

	TREATMENT 2A	**TREATMENT 2B**	**TREATMENT 3A**	**TREATMENT 3B**
Number of chickens	3	2	3	3
Aflatoxin (ug/kg AF B1)	1237	1237	412	412
Duration of feeding (days	21	21	21	21

PURPOSE: Measure feacal content (excretion) on selected days -1, 7, 14 and 21.

2.6.3. INVESTIGATION OF THE LEVELS OF AFLATOXINS IN EDIBLE TISSUE OF CHICKENS
EXPERIMENT 5

Finally, A flock of 10 village chicks of average live weight 351g and 3 weeks of age from the same batch were fed two levels of aflatoxin for 21 days (Table 19). Each group of treatment had 5 chickens. This experiment was designed to investigate the levels of aflatoxin deposited in the proposed edible tissue of the chickens. Similar tissues and organs from the same group were pooled together and analysed. Analyses were done of the following organs in each group,livers and spleens, gizzards and hearts and thigh tissue.

2.7. ESTIMATION OF AFLATOXIN RESIDUES FROM POULTRY TISSUE

In the determination of aflatoxin residues in animal tissue, several methods have been applied successfully to liver and other tissues. In this study, the method of Stubblefield and Shotwell (1981) and modified by the World Health Organisation (1983) was adopted. The volumes given are for a 50.0g tissue sample although sample weights varied from 25.0 to 50.0g and all volumes were adjusted accordingly.

2.7.1. EXTRACTION WITH DICHLOROMETHANE

The poultry tissue to be analysed was blended in a homogeniser to form a paste. A 50.0g tissue paste was transferred into a glass-stoppered flask and a 20% w/v citric acid monohydrate solution (5ml) was added and mixed thoroughly with a glass rod.After 5 minutes, 10.0g of diatomaceous earth was added, followed by 100.0ml dichloromethane. The flask was tightly sealed with masking tape and shaken vigorously for 30 minutes on a wrist-action shaker and the mixture subsequently filtered through Whatman filter No 113 paper into a conical flask containing 10.0g of anhydrous sodium sulphate. The filter paper top was closed and the residue tissue compressed against the glass funnel to expel a maximum volume of filtrate. The conical flask was gently swirled intermittently for 2 minutes and then re-filtered into a 100ml graduated cylinder and the total filtrate volume recorded. This was then evaporated to near dryness in a round-bottomed flask under vacuum. After cooling to room temperature, the residue was dissolved in 25 ml of dichloromethane, ready for column clean-up.

Table 19a

PERCENTAGE COMPOSITION OF AFLATOXIN DIETS USED IN:

Experiment 5

INGREDIENT (%W/W) (No OF CHICKENS)	TREATMENT GROUP	
	LOW LEVEL AF (5)	HIGH LEVEL AF (5)
MOULDY MAIZE	25	75
WHOLE MAIZE MEAL	75	25
TOTAL	100	100

TABLE 19b

EXPERIMENTAL DESIGN
EXPERIMENT 5

	TREATMENT 1	**TREATMENT 2**
Numbers of chickens	5	5
Aflatoxin (ug/kg AF B1)	412	1237
Duration of feeding (days)	21	21

PURPOSE: Investigation of the levels of aflatoxins in edible tissue of chickens.

2.7.2. CLEAN-UP WITH COLUMN CHROMATOGRAPHY.

The clean-up step employed a silica gel G packed column (100ml in size). The chromatographic column (27x4cm) was set up by plugging it with glasswool at the bottom, packing with anhydrous sodium sulphate (4cm), pre-activated silica gel G (8cm) and anhydrous sodium sulphate (4cm) into a glass column that was half-filled with dichloromethane. Each packing was stirred with a glass rod to remove any air bubbles. The tissue extract was quantitatively transferred to the packed column with a medicine dropper and solvent was allowed to drain through the column. The column was washed with 25ml glacial acetic acid-toluene (1:9 v/v), 25ml hexane and 25ml acetonitrile-diethyl ether-hexane (1:3:6 v/v/v) and the total washings discarded. Aflatoxin were eluted with 60ml of chloroform-acetone (4:1 v/v) and the eluate evaporated to near dryness under vacuum in a rotavapor. The extract was then quantitatively transferred to a glass vial with chloroform, evaporated to dryness under nitrogen, and the residue dissolved in 50ml acetonitrile-benzene (2:98 v/v) for two dimensional TLC analysis.

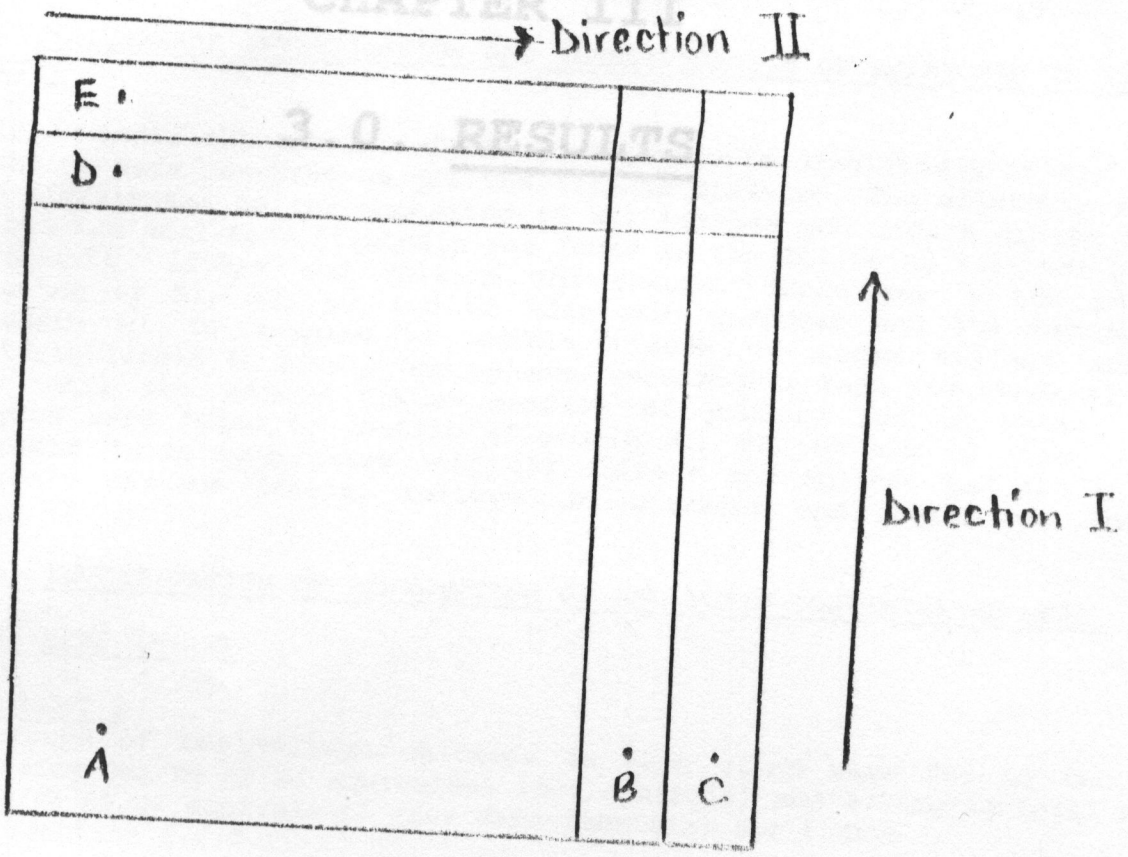
2.7.3. TWO-DIMENSIONAL TLC ANALYSIS.

A 100 x 100mm silica gel G thin layer plate (precoated) was scored and spotted (fig 15). A 10ml aliquot of the tissue extract was spotted together with aflatoxins B1, B2, G1, G2 and M1 standards for visual analysis. Parallel starting spots were made with microlitre syringes from the acetonitrile-benzene (2:98 v/v)sample extracts and standard solutions. Spots were left to dry in the air (5 minutes) and developed in two dimensions; first in chloroform-acetone-isopropanol (87:10:3 v/v/v) and then chloroform-acetone-water (86.5:12:2.5 v/v/v). After developing in the first direction, the plate was air-dried for about 10 minutes before developing in the second direction. The fully developed plate was removed from the chromatographic tank and allowed to dry in air for 5 minutes. Spots were viewed under long wave UV lamp (365nm).

The determination of the amount of the aflatoxin was done as described in the estimation of aflatoxin in the experimental feed (section 2.4.4). Care was taken to ensure that no sample spot fluorescence intensity exceeded that of the most concentrated standard spot by appropriate dilution. Interpolation was necessary as before.

Fig.15

TWO-DIMENSIONAL TLC FROM TISSUE EXTRACT.



Type 2
A = aliquot from tissue.
B & D = M_1 and B_1 standards.
C & E = B_1, B_2, G_1 and G_2 standards.

CHAPTER III

3.0. RESULTS

3.1. TEMPERATURES DURING GROWTH OF FUNGUS

From fig 16, it can be seen that generally the floor temperatures, taken from within the layer of maize on which the fungus was growing, were higher than wall temperatures reflecting the day's natural climate. This confirms the findings by Christensen (1975) and others that when the fungus A. Flavus grows on a substrate, it produces heat. The maximum maize temperature occurred on day 9 and this was 37.5 degrees celsius while the maximum wall temperature was 31.5 degrees celsius and occurred on days 15 -17.

3.2. PRELIMINARY INVESTIGATION AND IDENTIFICATION OF AFLATOXIN IN TISSUE OF POULTRY

Table 20 shows the results of the preliminary feeding trials AF B1, B2, G1, G2 and M1 were observed in all the tissues analysed. The aflatoxin residues were distributed to and deposited in all tissues and organs of the body of the chicken analysed. Aflatoxin was found in the following tissues: gizzards and hearts, livers and spleens and thighs. There was a 100 per cent incidence of B1, B2, G1 and G2 aflatoxin residues and 75% incidence of aflatoxin M1 in samples of edible tissue of these village chickens. Aflatoxin levels in livers and spleens were higher than any other tissue or organ. All the edible tissue samples of poultry fed on mouldy maize, analysed were found to contain aflatoxin B1, B2, G1 and G2 (fig. 17) and aflatoxin M1 in some cases (fig 18). Livers and spleens had the highest aflatoxin residue levels, followed by gizzards and heart, and then the thighs (Table 20).

3.2.1. INVESTIGATION OF CONSUMPTION OF AFLATOXIN-CONTAMINATED MEAL.

3.2.2. FEED INTAKE

EXPERIMENT 2

Two groups of two village chickens in each group were fed on two diets comprising 0mg/kg AF B1 equivalent (for controls) and 4750mg/kg total AF (or 1650mg/kg AF B1 equivalent) (for experimentals) for 5 days (section 2.6.2.).

EXPERIMENT 3

Three groups of village chickens, four (4) on the control diet, five (5) in each of the low AF diets and high AF diets were fed for 21 days. The controls were fed on clean maize (0mg/kg AF), the low AF diet contained 2370mg/kg AF and the high AF diet contained 4750mg/kg AF. (section 2.6.2).

EXPERIMENT 4: MEASUREMENT OF FAECAL AFLATOXIN

The two groups of chickens on the high level of Aflatoxin (2A and 2B) were not producing solid faecal matter after one week. Serious diarrhoea was observed. It was not possible to collect these faeces quantitatively, and therefore the experiment was abandoned.

FIG. 16

ROOM TEMPERATURE DURING GROWTH OF FUNGUS ON MAIZE

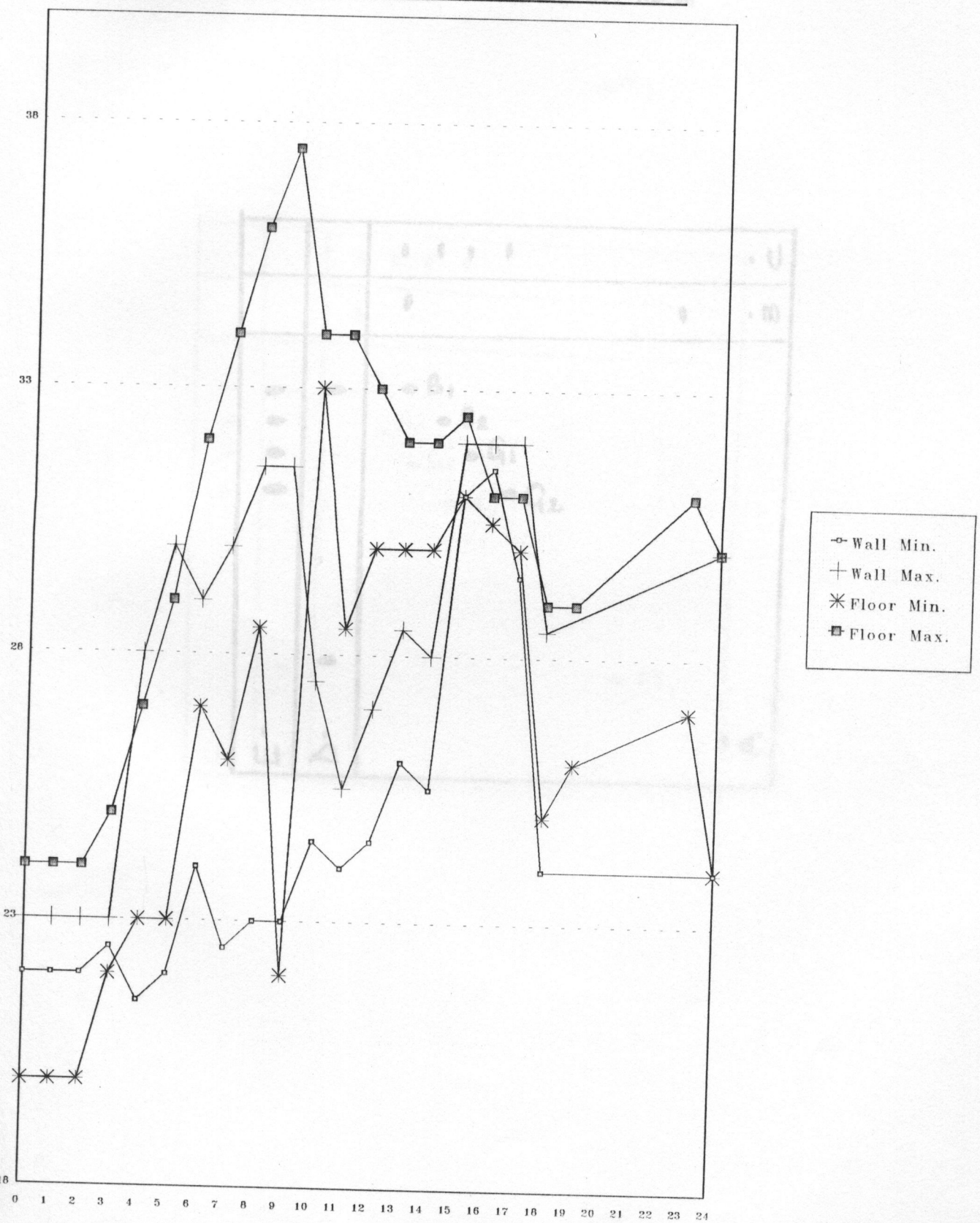


FIGURE 17

TISSUE SHOWING AFLATOXIN B_1, B_2, G_1, G_2, BUT NO M_1

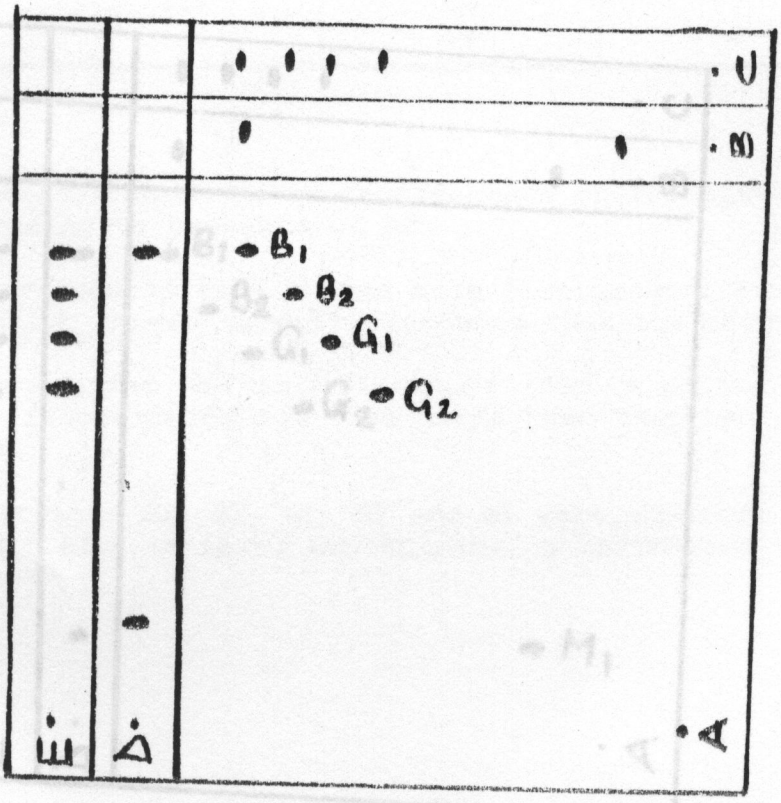


FIG. 18

TISSUE SHOWING AFLATOXIN B_1, B_2, G_1, G_2, AND M_1.

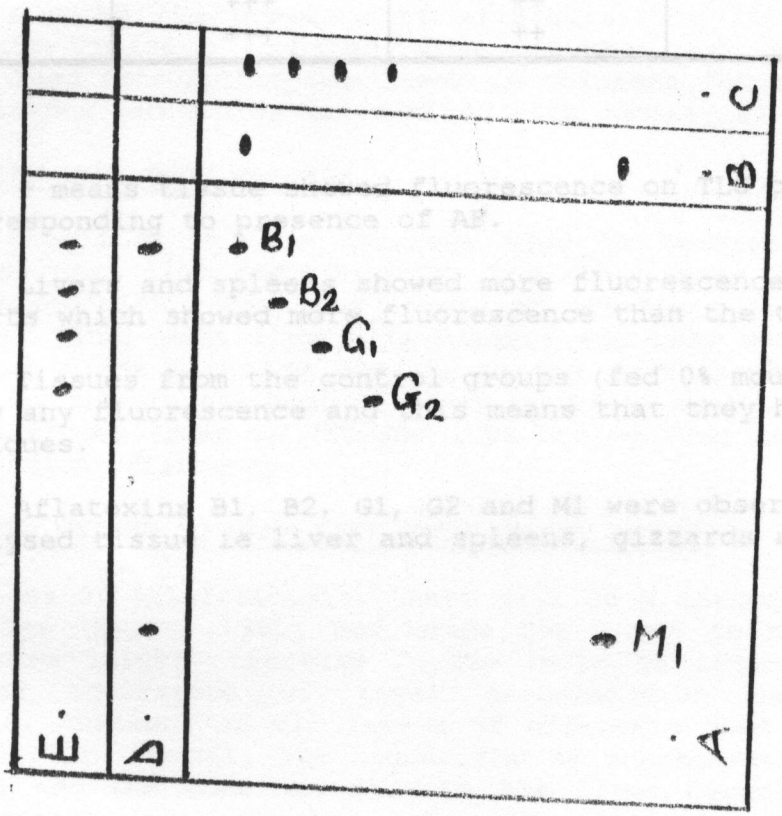


TABLE 20

FEEDING TRIALS.

INVESTIGATION AND IDENTIFICATION OF AFLATOXIN IN EDIBLE TISSUE

EXPERIMENT	TISSUE ANALYSED		
	LIVERS AND SPLEENS	GIZZARDS AND HEARTS	THIGHS
1	+++	++	+
2	+++	++	+

Note (1). + means tissue showed fluorescence on TLC plate corresponding to presence of AF.

(2). Livers and spleens showed more fluorescence than gizzard and hearts which showed more fluorescence than the thighs.

(3). Tissues from the control groups (fed 0% mouldy maize) did not show any fluorescence and this means that they had no detectable AF residues.

(4) Aflatoxins B1, B2, G1, G2 and M1 were observed in all the analysed tissue ie liver and spleens, gizzards and thighs.

EXPERIMENT 5: INVESTIGATION OF GROWTH RATES

Two groups of five (5) chickens were fed on two diets, the low AF diet at 1185 mg/kg and the high AF diet at 3555 mg/kg AF.

Figs 19, 20, 21 and 22 show the consumption results of chickens. In all the cases (figs 19, 20, 21, and 22 section 2.6.3.), the feed intake was reduced at both levels of aflatoxin inclusion as compared to the controls. Growth of the chicks were progressively retarded by each increment of aflatoxin in the diets as evidenced by the mean live body weights. This means that the tolerable amount of mouldy maize (the amount of maize showing no effect) was below the concentrations used in this study.

The intake of chickens fed diets containing whole mouldy maize only were more affected by the presence of aflatoxin (fig.19) than those fed with whole mouldy maize combined with growers mash or growers mash only (fig.19). Feed consumption was lower in chickens fed only mouldy maize than in the chickens fed mouldy maize mixed with commercial feed (fig.20)

3.3.1. MEAN LIVE BODY WEIGHTS.

The same groups of village chickens used in section 3.3.1. were used to measure the mean live body weights. The final body weight (mean) and body gain decreased with increasing levels of aflatoxin in the diets (figs. 23, 24 and 25). Final mean live body weights and body weight gain for all the experimental birds were low compare to the corresponding controls. The low live body weights in the high AF diets could be associated with reduced feed intake, diarrhoea (seen in 75% and 100% mouldy feed diets only) and reduced feed conversion efficiency.

3.3.2. LIVER WEIGHT TO MEAN LIVE BODY WEIGHT RATIO

In conditions of aflatoxicosis, there will be a marked increase in the size of the liver (Wyatt, 1991) and hence the liver to mean live body weight ratio becomes larger. Increase in the ratio of liver weight to mean live weight and occurrence of liver discoloration was recorded in the experimental chickens at all levels of aflatoxin used in the study (Tables 21; 22; 23). In general, the higher the AF concentration, the heavier were the livers and the more severe were the liver discolorations. (Table 23) These increased liver weights and histopathological liver discolorations also point to the fact that tolerable amounts of mouldy maize in poultry feed is below the levels used in these experiments.(Tables 21; 22; 23).

At the end of aflatoxin feeding, only the experimental livers were pale (figs 26 and 27) and enlarged in some cases. Though the livers were pale discoloured and enlarged, their surfaces had no irregular lesions or granulations that were reported by other researchers in similar studies on other species of chickens and poultry.

FIG.19 EXPERIMENT 2

Investigation of Consumption by Chickens of Meal Containing Aflatoxin

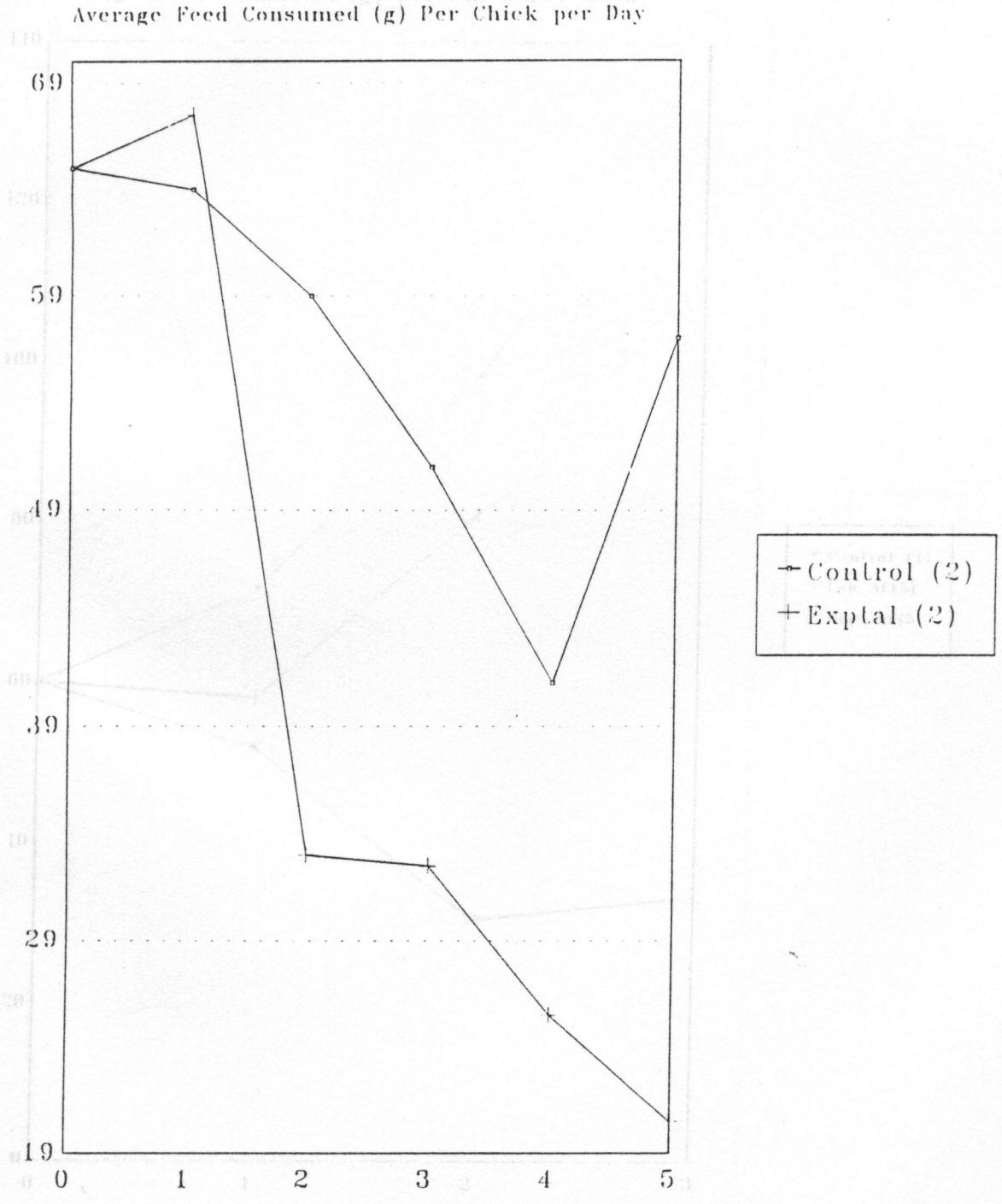


FIG. 20 EXPERIMENT 3

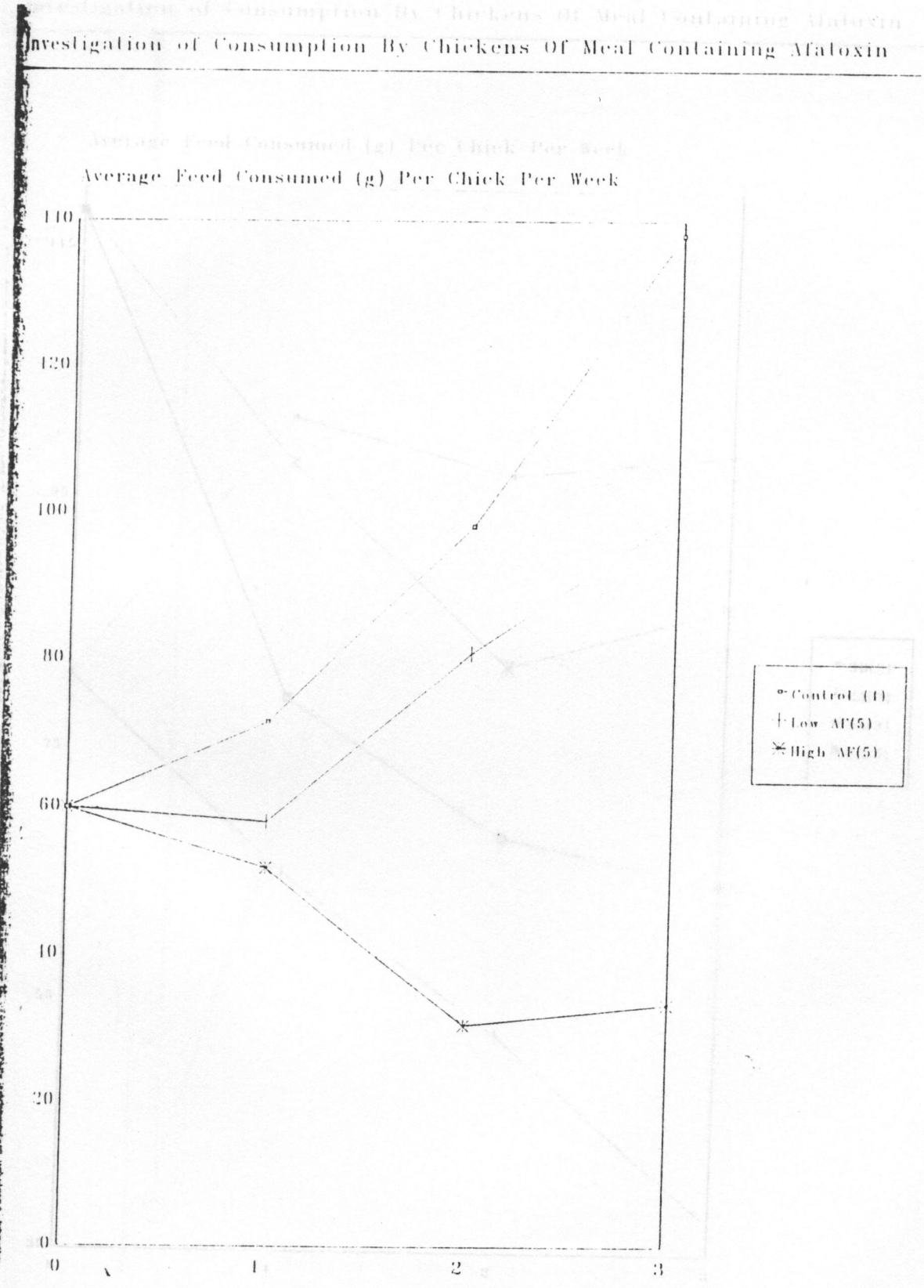


FIG. 21 EXPERIMENT 4

Investigation of Consumption By Chickens Of Meal Containing Mafoxin

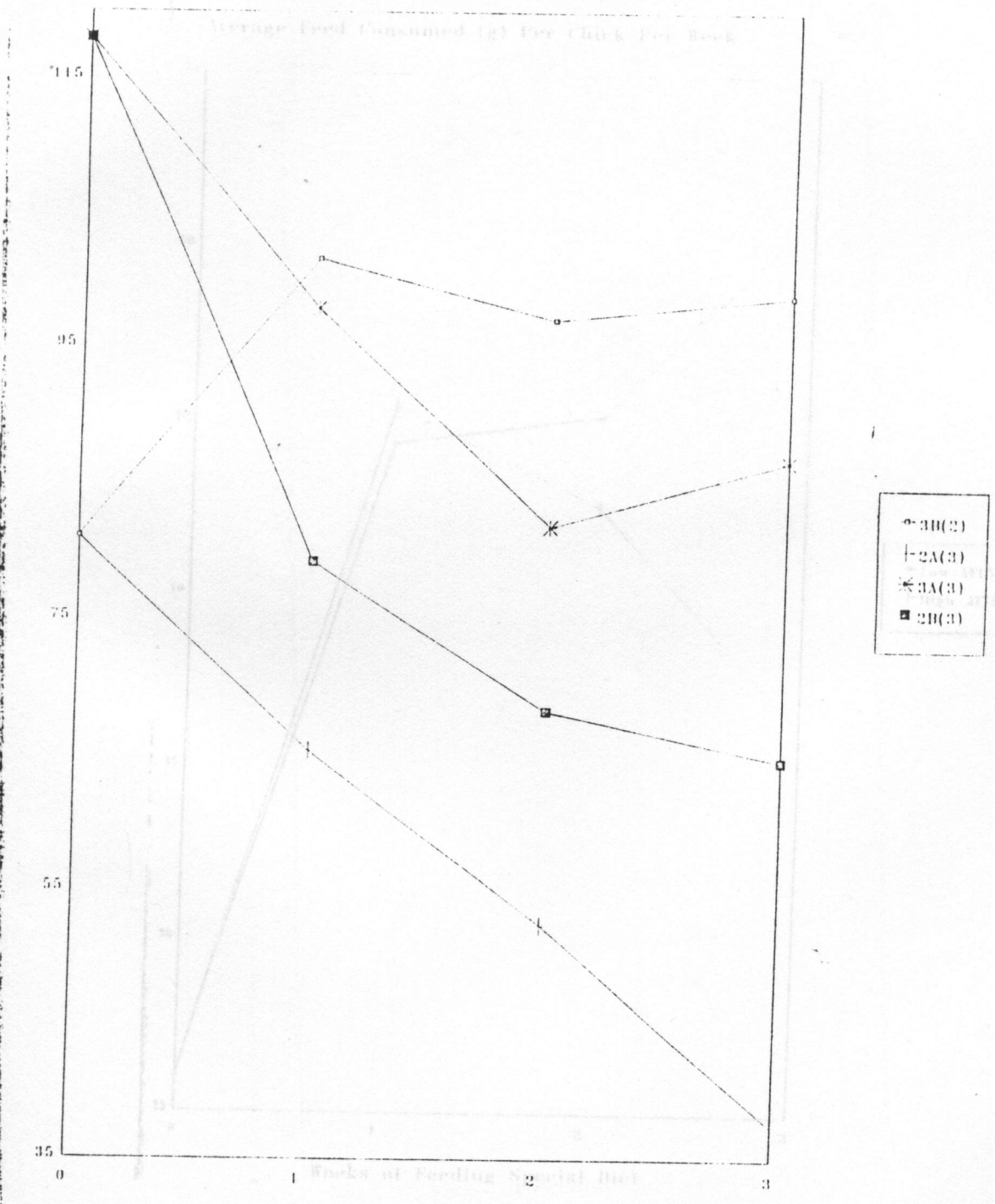


FIG. 22 EXPERIMENT 5

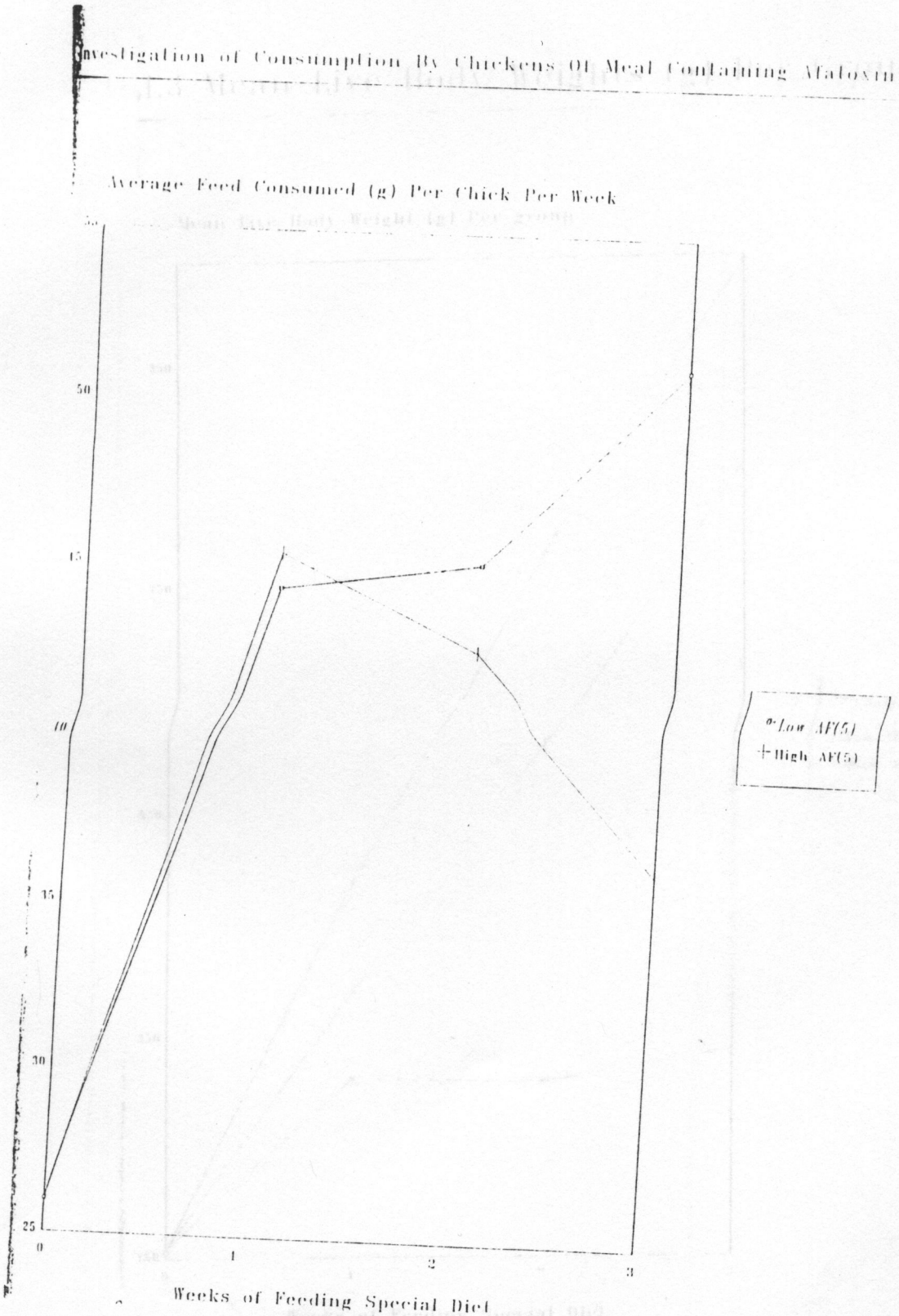


FIG.23 EXPERIMENT 3

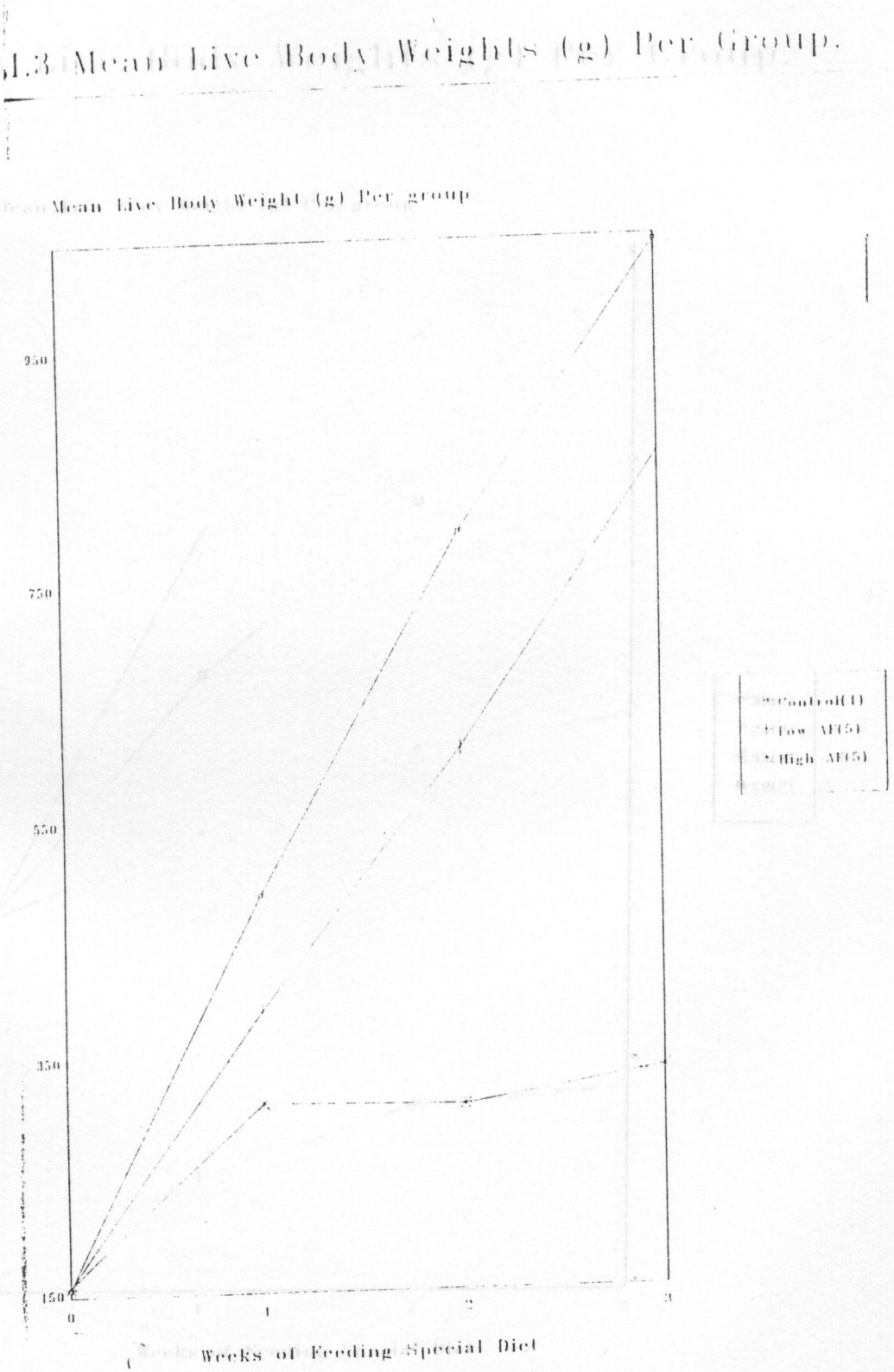


FIG. 24 EXPERIMENT 4

Mean Live Body Weights (g) Per Group.

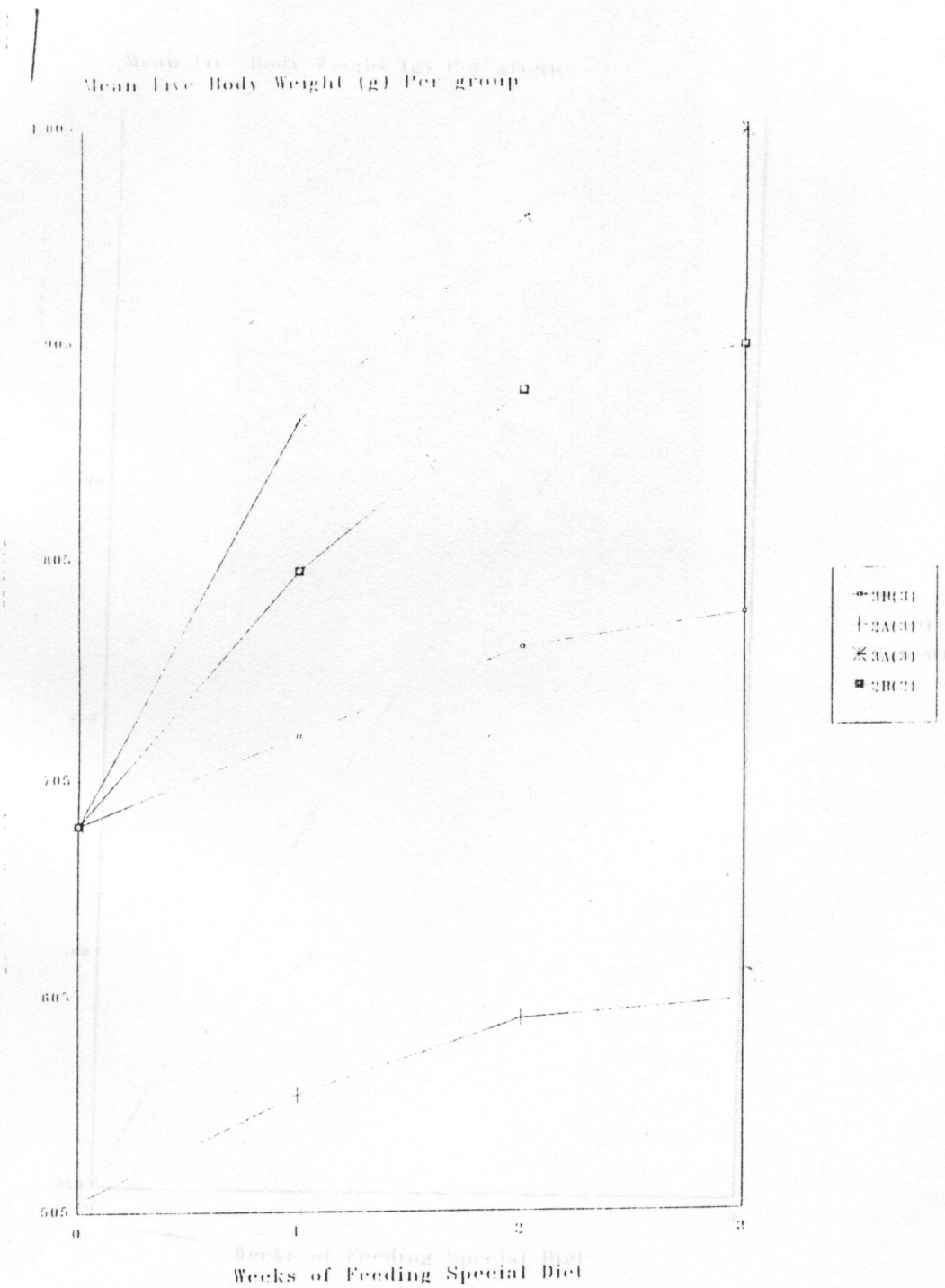


FIG. 25 EXPERIMENT 5

Mean Live Body Weights (g) Per Group.

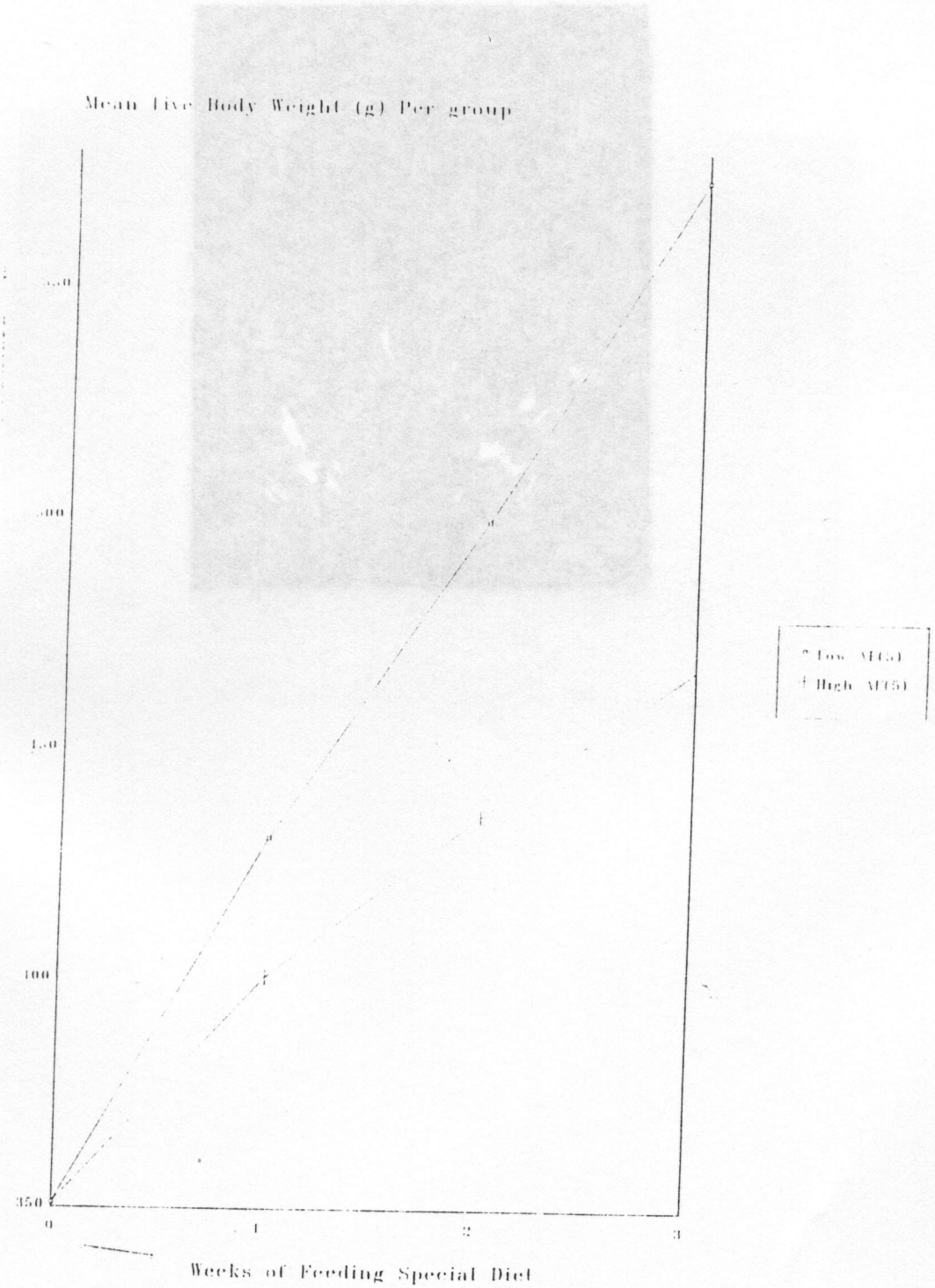


FIG. 26 LIVER FROM CONTROL GROUP:- NORMAL BROWN COLOUR

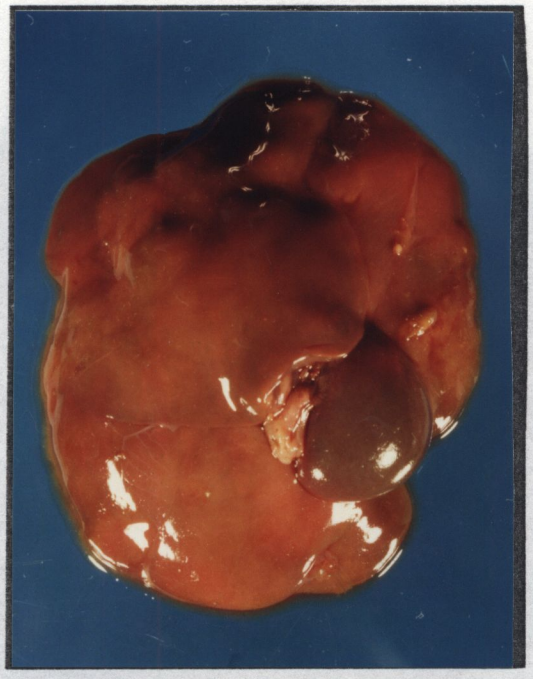


FIG. 27 LIVERS FROM EXPERIMENTAL GROUPS

LEFT: LOW AF GROUP, PALE BROWN COLOUR

RIGHT: HIGH AF GROUP, DISCOLOURED

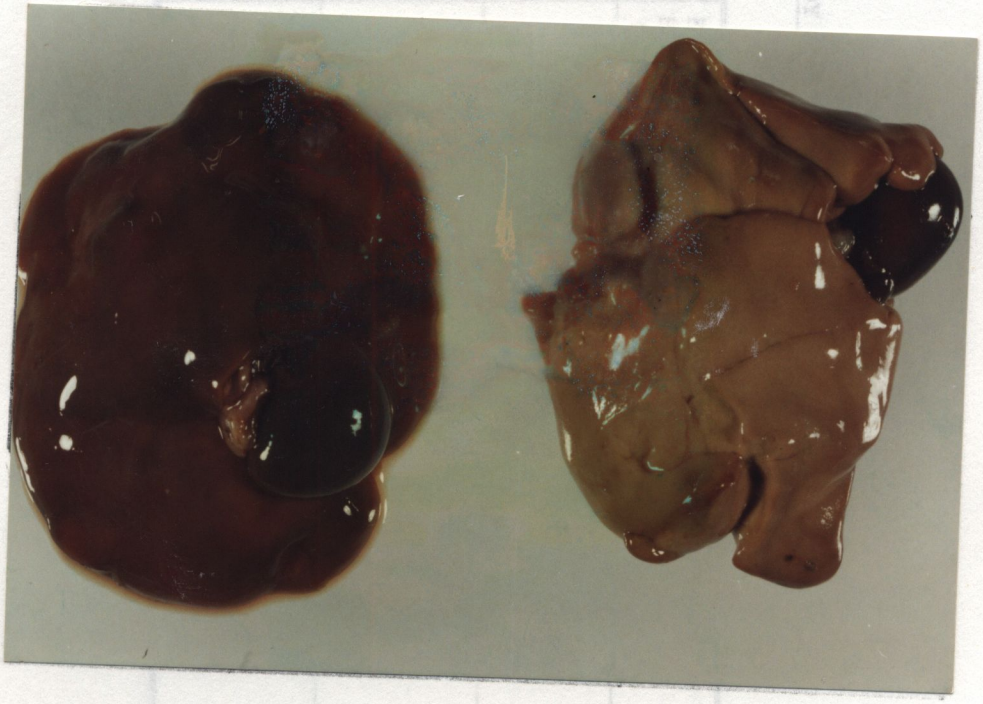


TABLE 21.

LIVER WEIGHT TO LIVE BODY WEIGHT RATIO.

EXPERIMENT 3.

TREATMENT GROUP	CHICKEN #	FINAL LIVE BODY WEIGHT (g)	LIVER WEIGHT (g)	LIVER TO LIVE BODY WEIGHT RATIO	MEAN RATIO	DESCRIPTION OF LIVER
CONTROL (4) A (0mg AF)	A1	1040	28.94	0.0278	0.0302 ±0.002	NORMAL BROWN
	A2	1040	32.83	0.0316		
	A3	1040	30.60	0.0294		
	A4	1040	33.16	0.0319		
LOW AF(5) B (2370mg AF)	B1	1168	30.25	0.0259	0.0333 ±0.004	PALE BROWN
	B2	790	29.34	0.0371		
	B3	864	29.93	0.0345		
	B4	750	25.57	0.0341		
	B5	720	25.03	0.0348		
HIGH AF (5) C (4750mg AF)	C1	220	5.42	0.0246	0.0403 ±0.01	WHITISH DISCOLORATION AND ENLARGED
	C2	220	5.99	0.0272		
	C3	470	23.33	0.0496		
	C4	480	22.73	0.0474		
	C5	300	15.85	0.0528		

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TABLE 22

LIVER WEIGHT TO LIVE WEIGHT (BODY) RATIO

EXPERIMENT 4

TREATMENT GROUP	CHICKENS #	FINAL LIVE BODY WEIGHT (g)	LIVER WEIGHT (g)	LIVER WEIGHT TO LIVE BODY WEIGHT RATIO	MEAN RATIO	DESCRIPTION OF LIVER
3B (2)	3B-1	950	26.38	0.0278	0.0284 ±0.0009	NORMAL BROWN
	3B-2	610	17.66	0.0290		
2A (3)	2A-1	560	19.25	0.0344	0.0321 ±0.003	PALE BROWN, ENLARGED
	2A-2	600	17.14	0.0286		
	2A-3	650	21.70	0.0334		
3A (3)	3A-1	1025	27.20	0.0265	0.0238 ±0.003	NORMAL BROWN
	3A-2	970	21.00	0.0216		
	3A-3	1010	23.52	0.0233		
2B (3)	2B-1	710	30.68	0.0432	0.0373 ±0.006	PALE BROWN, ENLARGED
	2B-2	990	30.23	0.0305		
	2B-3	1000	37.32	0.0373		

Note: 3B: 25% Mouldy Maize + 75% Whole Maize Meal
2A: 75% Mouldy Maize + 25% Whole Maize Meal
3A: 25% Mouldy Maize + 75% Growers Mash
2B: 75% Mouldy Maize + 25% Growers Mash

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TABLE 23

LIVER WEIGHT TO LIVE BODY WEIGHT RATIO

EXPERIMENT 5.

TREATMENT GROUP	CHICKEN #	FINAL LIVE BODY WEIGHT (G)	LIVER WEIGHT (G)	LIVER TO LIVE BODY WEIGHT RATIO	MEAN RATIO	DESCRIPTION OF LIVER
LOW AF (5) A (1190MG AF)	A1	900	20.31	0.023	0.028 ±0.003	PALE BROWN
	A2	480	13.48	0.028		
	A3	530	14.90	0.028		
	A4	420	13.20	0.031		
	A5	540	15.08	0.028		
HIGH AF (5) B (3560MG AF)	B1	440	12.77	0.029	0.033 ±0.02	PALE BROWN
	B2	510	15.50	0.030		PALE BROWN
	B3	400	15.10	0.038		PALE BROWN AND ENLARGED
	B4	470	13.10	0.028		PALE BROWN AND ENLARGED
	B5	520	19.77	0.038		PALE BROWN AND ENLARGED

TABLE 24

LEVELS OF AF IN EDIBLE TISSUE OF CHICKENS

TISSUE		AFLATOXIN (µg/kg)					
		B1	B2	G1	G2	M1	TOTAL AF
FEED	Low AF	411	111	495	169	-	1186
	High AF	1234	333	1485	506	-	3558
GIZZARDS AND HEARTS	Low AF	0.5	0.4	1.6	0.6	neg	3.1
	High AF	3.2	1.3	2.4	1.0	2.0	9.9
LIVERS AND SPLEENS	Low AF	2.2	0.8	1.8	0.8	2.4	8.0
	High AF	3.9	3.7	3.0	1.2	3.6	15.4
THIGHS	Low AF	0.5	0.2	0.6	0.3	neg	1.6
	High AF	3.2	1.0	1.4	1.0	2.8	9.4

Note (1). Neg (negligible) means less than quantifiable level or lowest detectable.

(2). Results from pooled tissues in treatment groups.

(3). Five samples were pooled in each treatment group.

TABLE 25

PERCENTAGES OF AF RESIDUES RETAINED IN EACH TISSUE

TISSUE		AFLATOXIN				
		B1	B2	G1	G2	M1
FEED	Low AFmg/Kg	411	111	495	169	-
	High AFmg/Kg	1234	333	1485	506	-
GIZZARD AND HEARTS	Low AF %	0.12	0.36	0.32	0.36	-
	High AF %	0.26	0.39	0.16	0.20	0.16
LIVERS AND SPLEENS	Low AF %	0.54	0.72	0.36	0.47	0.58
	High AF %	0.32	1.10	0.20	0.24	0.29
THIGHS	Low AF %	0.12	0.18	0.12	0.18	-
	High AF %	0.26	0.30	0.09	0.20	0.23

Note %

$$\frac{\text{mg/Kg AF in tissue}}{\text{mg/Kg AF in feed eaten}} \times 100$$

The hearts, spleens, gizzards and thighs appeared to be unaffected and were apparently normal. In experiment 3, two chickens in the high AF group had a much reduced growth rate (C1 and C2) and this affected the livers.

3.3.4. LEVELS OF AFLATOXIN IN EDIBLE TISSUE (EXPERIMENT 5).

Aflatoxin levels in the tissues and organs of the chickens at the end of feeding with aflatoxin meals are presented in Table 24 . As shown in this table, measurable amounts of AF's B1, B2, G1 and G2 were carried over to all tissue samples analysed. AF MI was also detected in some tissues in low amounts. The highest levels of total AF were detected in the livers and spleens with concentrations of 8.0 mg/kg (low AF group) and 15.4 mg/kg (high AF group). The lowest AF residue levels were detected in the thighs, with the thighs having a total concentration of 1.6 mg/kg (low AF group) and 9.4 mg/kg (high AF group). The recovery percentages ranged from 65 to 79% depending on the tissue under analysis (65% for hard muscular tissues and 79% for soft tissues).

$$\% \text{ AF retained} = \frac{\text{Concentration of AF in tissue}}{\text{Concentration of AF in diet}} \times 100\%$$

Approximately 0.3 to 0.5% of the total dose of AF ingested in 21 days was retained by the livers and spleens, 0.1 to 0.3% by the gizzards and hearts and 0.1 to 0.2% by the thighs (Table 24). As seen too from Table 25, there were generally lower AF residue concentrations in tissues from the low AF group than in corresponding tissues from the high AF level group. All comparisons were made between poultry exposed for the same period of feeding.

CHAPTER IV

4.0. DISCUSSION AND CONCLUSION.

4.1 FEEDING TRIALS.

Aflatoxin affects the growth of animals and poultry in experimental studies, although no field effects have been reported in Zambia. Aflatoxin B1, in particular, has been reported to suppress feed intake or feed consumption and feed conversion efficiency, growth rate and to cause an increase in the mortality of animals and poultry (Ostrowski-Meissner, 1983).

The transfer of AF residues from feed into the tissue of food- producing animals and poultry is known to occur (Rodricks and Stoloff, 1977; Chen, et.al., 1984). Aflatoxin residues have been found in edible tissue of exposed animals and poultry, including liver, heart, kidney and muscle (Chen, Pearson, Coleman et.al., 1984).

This study has confirmed that Zambian village chickens fed a diet based on mouldy maize could accumulate AF residues in edible portions.

Increased levels of aflatoxin meal resulted in dietary energy reduction which causes depression in feed utilization and hence growth rate (Aregheore et.al. 1993). The decline in the live body weight obtained in this study agree with Chen, Pearson, Coleman et.al. (1984) and Wolzak et.al. (1986) who reported significant growth depression of chicken fed on aflatoxin diets.

The experimental model used in this study may be criticised because the diets/rations were not completely balanced and sometimes composed wholly of mouldy maize and no supplements that might occur in a village, for a chicken that is scratching from the ground.

The chickens were not reared on space-free range as occurs in a village. It was decided that attempting space-free range to make a balanced ration would have introduced other confounding variables in amounts of ingredients just as exist in this intensive model employed. It may also be questioned whether the model reproduced the same degree of aflatoxicosis as in the field. The aim was to show that a village chicken eating mouldy maize could accumulate aflatoxin residues or metabolites that could be transmitted to humans in the food chain.

The primary effects of aflatoxicosis in poultry can be used to assist in accurate clinical diagnosis of the toxication. Changes associated with the visceral organs in response to aflatoxin can assist in the initial recognition of aflatoxin in poultry. Many disease patterns are reflected by the alteration in the live body weight and the relative proportions of the liver (Wyatt, 1991).

These results are in agreement with those of other researchers that the livers are the most affected during aflatoxin feeding. Although the liver is the main target organ, AF B1 is known to cause damage to other tissues and organs. The differences in disease patterns in the tissues and organs are probably due to variation in the strains (breeds) of birds and in levels and/or purity of aflatoxins fed as well as to the duration of feeding (Wolzak et.al. 1986).

The amount of AF may be reduced at high level as food intake is reduced if the food is mouldy. Also impaired liver function may reduce appetite.

Wolzak et.al. (1986) also reported that while livers were enlarged, ovaries and hearts were reduced. These results are in agreement with those of Trucksess et.al.(1984) and Wyatt (1991) who reported that the livers and ovaries of laying hens were most affected during aflatoxicosis and that the livers, spleens and kidneys are increased in size, respectively. In addition to size, the livers of birds with aflatoxicosis will be characteristically yellow in colour, with a friable texture (Wyatt, 1991).

Similar results were also reported by Chen, Pearson, Coleman et.al. (1984) and Wolzak et.al. (1986) in chickens. The high capacity of the livers to concentrate toxic compounds in comparison to other organs is probably related to the important role they play in eliminating xenobiotics (cited by Wolzak et.al., 1986).

The tissue level of mycotoxin metabolites is usually less than 1% of the level of parent mycotoxin in feed (Hsieh, 1983). Because the mature animal or bird is an effective toxin modifier, detoxifier and eliminator (Stoloff, 1979), the metabolism and transmission process acts like a filter allowing only a small portion to pass through (Hsieh, 1983).

It is suggested that the effects in the field (on the farm) would be much reduced because of the alternative dietary constituents and low levels of contamination by aflatoxin as compared to the high total AF feed given to caged poultry. The levels of contamination used (4740 mg/Kg total AF) were based on levels used in comparable experiments in the literature. They do not represent a realistic common level of aflatoxin residues in poultry in Zambia. According to surveys in Zambia (Lovelace et.al., 1982) the range of AF contamination in foods and feeds varies up to 57.6 mg/kg. It should be noted, however, that currently there are no regulations for aflatoxin levels in feeds in Zambia.

The relatively high AF concentrations used were also necessary to produce some symptoms of poisoning within a reasonable time period. Levels of aflatoxin up to 100 mg/Kg may not affect the growth of mature chickens.

Ostrowski-Meissner (1983) also reported that the detrimental effects of AF on animals can be markedly influenced by the protein nutrition because mycotoxins usually increase the protein requirements of animals. However, even relatively low levels of AF would be expected to markedly reduce the productive performance of chickens.

There is limited statistical analysis to support the disease patterns observed in the results of the study due to limited numbers of chicks in each experiment. The funding for the project was not adequate.

4.2. CLEARANCE OF AFLATOXIN

The beauty of nature endows the mature chickens and other poultry species with the capacity to eliminate much of the ingested toxin. Urinary excretion ordinarily accounts for 95-99% of the xenobiotic excretion. With the small levels of residues (0.1 to 0.5%)(Table 25) reported in this study, it is likely that the major way of mycotoxin poisoning is via the direct/primary aflatoxicosis.

4.3. FEED TISSUE CONVERSION RATIOS.

By comparing the $LD_{50's}$ (mg/kg body weight), the total AF toxicity in terms of only AF B1 can be estimated.

Table 7 shows that the sum of the LD_{50} of aflatoxins G1 + G2 + B2 $\cong$ 0.82 of the LD_{50} of AFB1. In order to express the AF as B1 equivalent, the concentrations of G1+G2+B2 was multiplied by 0.82 and added to the concentration of the AF B1. This allows the calculation of the feed-tissue conversion ratios for the village chickens (Table 27).

The quoted ratio for broiler chickens is 1200 (Wyllie and Morehouse, 1980) and all the calculated ratios for experiment five are below this in the high aflatoxin diet group. Therefore, Village chickens are at least as sensitive as broilers to AF.

4.4. AFLATOXIN LIMITS ALLOWABLE IN POULTRY FEEDS-SAFE LEVELS OF EXPOSURE.

In many countries in the world, measures have been and are being taken and limits being set to safeguard the animals, poultry and humans from mycotoxin poisoning and ill effects. Because of its implications for animal, poultry and human health, a total ban of highly contaminated feed to animals and poultry is a must. Maize highly contaminated with aflatoxin (or any other mycotoxin) should be destroyed, with compensation where necessary, to remove it from the human food chain.

New legislation to reduce consumption of mycotoxins should be passed on expert advice for health matters to make sure no public health hazard is endorsed. Although we have indications of what happens during aflatoxicosis, the local research could prove the only answer because aflatoxicosis depends among other things on the breed of the chicken and the enviroment. Legislation could prove difficult and expensive in Zambia but these precautionally measures have been adopted in our neighbours e.g Tanzania, Malawi and South Africa to protect health. Pronouncing that the risk of contamination with AF of maize and other food and feed materials is not appreciable or insignificant without testing is itself very risky and not justifiable.

TABLE 27.

CALCULATED FEED - TISSUE CONVERSION RATIOS.

Total AF B1 equiv.in feed(µg/Kg).	Level (µg/Kg) in					
	Livers and Spleens		Gizzards and Hearts		Thighs	
	B1	M1	B1	M1	B1	M1
750 (Low Group) 25% of 3000	2.2 (341)	2.4 (313)	0.5 (1500)	- -	0.5 (1500)	- -
2252 (High Group) 75% of 3000	3.9 (578)	3.6 (626)	3.2 (704)	2.0 (1126)	3.2 (704)	2.8 (804)

NB. Feed - tissue conversion Ratios

$$\text{Conversion ratio} = \frac{\text{concentration of AF in feed}}{\text{concentration of AF in tissue}}$$

(Ratios in brackets).

Table 28

FEED-TISSUE CONVERSION RATIOS FOR AFLATOXIN

ANIMAL	CONVERSION RATIO
Beef cattle	14000 feed B_1 / Liver B_1
Dairy cattle	100 feed B_1 / Milk M_1
Pigs	800 feed B_1 / Liver B_1
Layer hens	2200 feed B_1 / Egg B_1
Broiler chickens	1200 feed B_1 / Liver B_1

Wyllie and Morehouse, 1980.

Total AF residues in the tissue of chicken after feeding AF for 21 days were all below 16 mg/kg and thus were all less than the action level of 20mg/kg, set by the United States of America for aflatoxin levels permited in foods and food ingredients intended for human consumption. However, histopathological changes in liver indicate that a restriction of AF level to reduce the ill effects should be considered. In this regard, this study has shown that safe levels of allowable intake of AF should be set below levels used in the study. The EEC set this at 200mg/kg AF B1 (Wyllie and Morehouse, 1980).

One basis for establishing a safe level of exposure may be that finite exposure to a chemical agent at which the likelihood of a molecule getting to a critical site is so remote as to be immeasurable. More acceptable bases for establishing safe levels of exposure are that (1) the body can easily detoxify the dose through normal physiological mechanisms; (2) the lesions produced are easily repaired by body mechanisms with no resultant unwanted effect; and (3) the lesions produced are so inconsequential that they do not influence the reserve capacity for the maintenance of normal bodily functions (Lee,1972).

Although the word "safe" has been used, Lee (1972) argues that there really is no such thing as a guarantee of absolute safety. Thus to say that all chickens may be exposed safely for 21 days to a given amount of AF B1 is proven absolutely true only after most chickens have tolerated this exposure, and even then the question remains,what about 42 or 63 days of exposure?

In the 1992 rotten maize saga, there was great pressure on the Ministry of Agriculture, Food and Fisheries about the importation of the alleged toxic maize grain. Though the press is usually naive about such academic issues as aflatoxicosis, it should be commended for alerting a lot of people about the possible and appreciable risks to humans of consuming toxic food products. It should be noted also that this was not the first time poor grade maize had been rejected. Interestingly however, in Zambia, poor grade maize has always been used in making opaque beer.

4.5. APPROACHES TO MINIMISE EFFECTS OF MYCOTOXINS IN POULTRY.

Contamination of agricultural food and feed products by AF is a problem not only because the products themselves are lost, but also because disposal is costly. Consequently, the development of processes by which AF-contaminated agricultural products can be reclaimed or salvaged for animal or poultry feed has been a major research priority (Bothast, 1991). Various approaches to minimize AF in maize have been addressed.

4.5.1. BLENDING WITH GOOD MAIZE.

A direct route is to blend the AF-contaminated maize with AF-free, clean maize. When the level of AF in contaminated maize is high, a very large amount of good and clean maize is needed to reduce the contamination of the blend (Bothast,1993).

4.5.2. PREVENTION.

Faced with an established case of aflatoxicosis, the very first measure must obviously be to change the feed so as to remove the primary source of illness. The easiest way to prevent the formation of aflatoxin is to eliminate contaminated feed material. Efforts ought therefore to be primarily aimed at preventing contamination of grain on one hand, and the destruction of A. flavus as soon as detected (Goldblatt and Dollear, 1977). Early detection and diversion of small consignments of contaminated material may prevent contamination of larger supplies.

Another approach to the AF problem is to harvest, handle, store, transport and process the high-AF hazard products so that they can not become invaded by A. flavus (Christensen, 1975). This includes use of good harvesting and shelling practises that avoid damaging the grain (Goldblatt and Dollear, 1977). In Zambia, good harvesting and shelling practises are possible on commercial farms with modern farm equipments. In rural areas and on small subsistence farms, grain entering the commercial market is usually shelled by beating the maize cobs with sticks and metal bars. This causes breakages and cracks that encourage fungal growth once contamination has occurred.

4.5.3. DECONTAMINATION.

The discovery of AF in the various tissues gave rise to more extensive studies of how to reduce the entrance of this dangerous compound in food chains leading ultimately to man.

4.5.3.1. CHEMICAL DESTRUCTION.

When a cottonseed meal initially containing about 500 mg/kg AF was treated with ammonia (48 pound pressure, 118 C, 30 minutes), the AF content was reduced to below 5 mg/kg. From a 2-year feeding test in which this treated meal was fed to rats as 20% of the diet, it was concluded that ammoniation effectively detoxified the meal (Goldblatt and Dollear, 1977).

4.5.3.2. CHEMICAL ADSORPTION METHODS.

Studies by Swedish scientists indicate that the levels of AF in tissue of poultry dropped dramatically after introduction of sodium sulphate as feed agents or constituents. The treated feed was consumed by rodents, which in turn, were an important source of foods for the birds (Lee, 1972). Recently, American scientists have shown that AF adsorption is also possible with Calcium aluminium Silicate (Kubena et.al, 1991).

4.6. COMMERCIAL MAIZE STORAGE IN ZAMBIA.

In the rural areas, maize is stored in traditional storage facilities (Section 1.4.). It is interesting to compare these less expensive and less secure facilities with the large metal/plastic/cement "bins" that have been introduced and built in Zambia (fig.28) by a number of various donor agencies e.g Canadian International Development Agency (CIDA), Japanese International Cooperation Agency (JICA), the EEC, the Australian, the Saudi Arabian, the Czechoslovakian and the Germany maize Storage Programmes.

For a long time, the conditions of storage in Zambia have not generally been carefully controlled. The development of improved storage facilities has been very welcome. Improvements in grain storage in the rural areas are urgently needed throughout Zambia.

The need for drying facilities at the site of production is essential if post-production food losses due to fungi and biodeterioration are to be kept to a minimum. Systems are needed to develop efficient village level food drying techiniques that are economically attractive and acceptable to the small-scale farmers in the rural areas in Zambia. Inadequate drying of harvested maize presents a serious health hazard through mycotoxin contamination. Little attention has been given to post-production drying technologies for the small-scale farmers, although a variety of small-farm facilities for drying crops are available.

4.7. GRAIN DRYER MODELS.

There are many driers being developed. Much of the development, however, is being carried out for use in large-scale drying operations. The newer drying methods described by Lindblad and Druben (1974) use heated air to dry grain. They dry the grain quickly and well. Most of them require the burning of fuel to heat the air. This fact, together with the cost of building the machine often limits the usefulness of drying machines for use by small farmers.

Fig 28

COMMERCIAL MAIZE STORAGE

Large, metal storage bins built by donor agencies. Picture: Choma, Southern Province, Zambia.

4.7.1. THE OIL-BARREL DRYER.

The two dryers made out of oil barrels and hand-rammed earth or mudblocks have only one part which may be expensive - the oil barrel itself. In the pit Oil Barrel Dryer, the barrels are sunk into a pit. The Simple Oil Barrel Dryer is built above the ground. They each require mostly simple labour and would be good projects for a group of small-scale farmers.

4.7.2. THE SOLAR DRYER.

The Solar Dryer provides faster drying and requires no fuel. By enclosing the drying grain, the solar dryer retains the heat of the sun better than just spreading the grain out in the sun to dry. It requires little or no maintenance. Except possibly for plastic sheets or corrugated roofing, all the materials should be available everywhere in Zambia.

After drying, it is important that the maize is stored properly in some structure that keeps the maize dry (Wynne and Wanga, 1993). In the rural areas, the store should be completely plastered with mud and it should have a good thatched roof.

4.8. CONCLUSION.

Taken together, the data from this study suggest that the levels of AF residues in poultry tissue should not pose a threat to public health up to the concentration investigated. The possibility of anyone in Zambia taking in enough AF chicken-edible tissue to be injurious seems to be pretty remote. The presence of AFs B1 and M1 in poultry tissue, however, is a matter for concern.

It can be concluded further that the transmission of AF from feed to humans via village chickens can be ignored. If the results of Lovelace et.al.(1982) showing 24mg/kg AF natural occurrence in Zambia are used as a measuring stick of the AF situation in the village, it may be concluded that the decision to use contaminated maize for poultry and animal feed taken by the Ministry of Agriculture, Food and Fisheries was acceptable. Beef cattle would have also been used to degrade the AF-contaminated maize because they have a higher feed/tissue conversion ratio. However, use in dairy cattle should be limited.

We still need more information about the occurrence of AF (mycotoxin) residues in the various edible parts of the village poultry since we have little information on the levels of AF. This dissertation cannot be complete but it is hoped that it will stimulate interest for further research and legislation so currently needed in Zambia.

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