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MYCOFLORA, TOXICITY AND NUTRITIVE VALUE OF MOULDY MAIZE*

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ABSTRACT

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Large populations of fungi developed in naturally infested mouldy maize stored under conditions that led to self-heating. Mould counts as high as 50×10^8 propagules per gram of meal were recorded in mouldy maize meal with a moisture content of 30% stored at 10°C. The fungal population of this meal included several known toxigenic species. Pure cultures on autoclaved maize of some of these fungi isolated from the mouldy meal (*Aspergillus candidus* Link., *A. clavatus* Desm., *A. fumigatus* Fres., *Fusarium moniliforme* (Sheld.) Snyd. et Hans., *F. tricinctum* (Corda) Snyd. et Hans. and *Trichothecium roseum* Link.) were extremely toxic to chickens and rats.

The natural mouldy maize meal caused significant reductions in mass gain and feed efficiency of chickens and pigs without causing mortality or significant pathological changes. Chemical analyses of the meal for aflatoxins (*Aspergillus flavus* Link.) and T-2 toxin (*Fusarium tricinctum*) were negative. In some cases chickens fed mouldy diets consumed 1,3 times more feed than the controls per gram of mass gain.

INTRODUCTION

"Mouldy corn toxicosis" is probably the most complex and least understood of the group of animal diseases known as mycotoxicoses. It is an irregularly occurring problem of long standing importance in the United States and elsewhere (Sippel, Burnside & Atwood, 1953; Burnside, Sippel, Forgacs, Carll, Atwood & Doll, 1957; Forgacs & Carll, 1962; Aust, Albright, Olsen, Beyers & Broquist, 1963; Albright, Aust, Beyers, Fritz, Brodie, Olsen, Link, Simon, Rhoades & Brewer, 1964; Forgacs, 1965; Smalley, Marasas, Strong, Bamburg, Nichols & Kosuri, 1970).

The factors which lead to epizootics of "mouldy corn toxicosis" are not well understood and in each outbreak of the disease different fungi and mycotoxins may be involved. The problem is further complicated by the multitude of clinical signs which develop and the great variation in response between different animal species to the ingestion of toxic mouldy maize. The major pathological sign of "mouldy corn toxicosis" in pigs, poultry, cattle and dogs is massive haemorrhage in many tissues (Bailey & Groth, 1959; Forgacs & Carll, 1962; Albright *et al.*, 1964; Forgacs, 1965). A pathologic condition of horses, characterized by gross liquefaction necrosis in the brain (leukoencephalomalacia), has been reproduced experimentally by feeding mouldy maize (Schwarte, 1938; Biester, Schwarte & Reddy, 1940; Badiali, Abou-Youssef, Radwan, Hamdy & Hildebrandt, 1968).

Severe outbreaks of "mouldy corn toxicosis" in cattle and pigs occurred in association with the 1965 Wisconsin maize crop (Smalley *et al.*, 1970). Similar cases of poisoning had previously been observed in 1963 from the maize crop harvested in 1962. In view of these sporadic field outbreaks of "mouldy corn toxicosis" in Wisconsin, an experimental study of the factors leading to such epizootics and of the specific microorganisms involved was considered important.

Weather conditions and the stage of maturity of the maize crop during the autumn of 1967 were sufficiently similar to those occurring in 1962 and 1965 to begin a model experimental system to study the probable development of toxic mouldy maize. To this end a maize field was selected near Campbellsport, Wisconsin during November, 1967. This field, planted to 100-day, yellow field maize (*Zea mays* L.) was located in a low area (kettle) in the Kettle Moraine region of Fond du Lac County, Wisconsin. Most of the ears in this field were immature, high in moisture and extremely mouldy. Preliminary isolations from these ears revealed heavy infestations with *Fusarium tricinctum* (Corda) Snyd. et Hans. and many other fungi. The high incidence of *F. trincinctum* on the ears was of particular interest since this fungus is known to produce several toxic metabolites in pure culture (Gilgan, Smalley & Strong, 1966; Bamburg, 1968; Bamburg, Riggs & Strong, 1968; Bamburg, Marasas, Riggs, Smalley & Strong, 1968; Yates, Tookey, Ellis & Burkhardt, 1968) but the importance of these toxins under natural conditions is unknown.

The experiments in the model system were designed to determine the effect of length of field crib storage on the changes in mycoflora, toxicity and nutritive value of the mouldy maize after grinding. Four feeding experiments were conducted between February and July, 1968. The effects of storage temperature and moisture content of the ground mouldy maize was studied at each sampling date. One experiment was performed to compare the mycoflora and nutritive value of mouldy shelled and ear maize meals because several cases of "mouldy corn toxicosis" observed on Wisconsin farms had been associated with the feeding of maize

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ground on the cob (Smalley & Marasas, unpublished data). Qualitative or quantitative changes in the mycoflora of maize meal stored under various conditions were correlated with changes in the concentration of known mycotoxins and the development of toxicoses in feeding trials to experimental animals (Marasas, Smalley, Bamburg & Strong, 1969).

Materials and Methods

1. *Source, shelling and grinding of maize samples*

During January, 1968 12 tons of mouldy maize were harvested from the Campbellsport farm and stored until used in a wire crib at the Arlington Experimental Farm of the University of Wisconsin. Mouldy maize from this crib for all feeding experiments was shelled in a hand sheller and ground at the University feed mill. Corn meal samples were placed in 45 l galvanized refuse cans and stored either in a walk-in freezer at —25 or 0°C or in a room with temperature control at 10°C (± 2°C). The low storage temperatures were selected to simulate conditions on Wisconsin farms during the winter and early spring.

High quality, yellow field maize grown at the University of Wisconsin Arlington Farm and ground at the University feed mill was used in diets fed to control animals in most of the feeding experiments. In one trial high quality ear maize for feeding to control animals was obtained from a farm near Lodi, Wisconsin and ground by a commercial mill at Waunakee, Wisconsin.

Since many cases of "mouldy corn toxicosis" in Wisconsin have been associated with high moisture maize (Smalley *et al.*, 1970), the moisture content of some maize meal samples was adjusted to approximately 30% by the addition of water and thorough mixing in an electric batch mixer.

2. *Moisture content and temperature of maize meal*

Moisture content determinations were made by drying triplicated 5 g maize meal samples in a forced air oven at 100°C for 12 hours. The moisture content of each large sample was expressed as the average percentage of the wet mass of the three samples.

Changes in the temperature of maize meal during storage were determined for all samples with an electronic temperature recording device (Scanning Tele-Thermometer, Model 47, Yellow Springs Instrument Co.). The thermistor probes were inserted in the central layers of the maize meal samples and the gross temperature changes of the meal measured periodically. The temperature recorder measured gross temperature changes above 15°C.

3. *Isolation media*

The following agar culture media were used in the qualitative and quantitative determination of the mycoflora of the mouldy maize: potato dextrose agar (PDA) containing sodium novobiocin (Butler & Hine, 1958), malt salt agar (MSA) (Christensen, 1946), acidified Czapek-Dox agar (ACA) containing tergitol (Steiner & Watson, 1965) and nutrient dextrose agar (NDA) (Riker & Riker, 1936).

Inoculated plates were incubated at either 24, 36 or 50°C and colony counts made after 1 to 7 days of incubation.

4. *Mycoflora of maize meal*

Maize meal was removed from the refuse cans once a week to prepare animal diets. At the same time a random sample (1 kg) was taken from each large maize meal sample and qualitative and quantitative determinations of the mycoflora done according to the method described by Christensen (1946). The actual colony counts were corrected mathematically for moisture content of the meal so that the mould counts are expressed on a dry mass basis.

5. *Standard chicken feeding experiment*

The chickens used in all the feeding trials were New Hampshire-Leghorn crossbreds which were hatched and housed at the Poultry Research Laboratory of the University of Wisconsin. Groups of 15 one-day-old chicks were fed each diet for 4 weeks unless otherwise stated. Each chick was mass-measured at the beginning of each experiment and at weekly intervals thereafter.

The chickens received a standard diet which contained 69% maize meal (Table 1). This minimal basal diet was selected to obtain the maximum expression of any deleterious effects of the mouldy maize meal while still maintaining a nutritionally balanced ration.

A basal diet containing all the ingredients except the maize meal was prepared in sufficient quantities for the duration of each trial. Appropriate quantities of basal diet and either the mouldy maize meal or the control maize meal were then thoroughly mixed in an electric batch mixer. The complete diets were prepared in quantities sufficient for consumption in one week. Feed left over after a one-week feeding period was mass-measured and discarded. Feed consumption values are expressed on a dry mass basis after correction for moisture content of the maize meal.

At the conclusion of each experiment some of the chickens in each group were slaughtered and submitted to the Wisconsin Animal Health Laboratory, Madison, for *post mortem* examination.

Table 1 *Composition of standard chicken diet containing 69% maize meal*

Ingredient	g/kg	%
Vitamin and Antibiotic Premix*	5	0,5
Salt	5	0,5
Calcium carbonate	10	1,0
Dicalcium phosphate	10	1,0
Alfalfa meal	20	2,0
Fish meal	20	2,0
Soybean oil meal (44%)	150	15,0
Isolated soybean protein†	90	9,0
Maize meal	690	69,0

Vitamin and Antibiotic Premix	g/100 kg
Riboflavin (R–20)	16,0
Calcium Pantothenate	2,2
Niacin	3,5
Vitamin B_{12} (132 mg/kg)	7,5
Vitamin C (10 000 units/g)	10,0
Vitamin D_3 (8 800 000 units/kg)	11,0
Penicillin (44 g/kg)	10,0
Manganese oxide	30,0
dl Methionine	100,0
Menadione bisulphite	0,08
Vitamin E (44 000 units/kg)	10,0
Sucrose	303,0

†ADM assay protein C–1, obtained from Archer-Daniel-Midland Co., Cincinnati, Ohio

6. *Pig feeding experiments*

The pigs used in the feeding experiments were weanling, specific pathogen free, Poland-China pigs purchased from the Vitaplus Company, Madison, Wisconsin.

The pigs were kept in the semi-isolation rooms of the Veterinary Science Department, University of Wisconsin.

Pairs of pigs were fed a balanced diet (Table 2) containing 77% of either uninfested or mouldy maize meal for 4 weeks. Fresh diets were prepared weekly.

At the conclusion of each experiment one pig of each group was slaughtered and submitted to the Wisconsin Animal Health Laboratory, Madison, for *post mortem* examination.

TABLE 2 *Composition of pig diet containing 77% maize meal*

Ingredient	g/kg	%
Maize meal	770	77,0
Soybean oil meal (44%)	200	20,0
Salt	5	0,5
Vitamin and mineral concentrate*	25	2,5

*Vitaplus Hog-N-Rich "B", Vitaplus Corp., Madison, Wisconsin

7. *Toxicity tests of fungi isolated from mouldy maize meal*

The 16 species of fungi marked with an asterisk in Table 3 were cultured in a medium consisting of 125 g shelled yellow maize and 165 ml water in 500 ml Erlenmeyer flasks (Rabie, De Klerk & Terblanche, 1964). Inoculum consisted of spore suspensions prepared from slant cultures of the various fungi on potato dextrose agar. Thirty flasks inoculated with each isolate were incubated at 10°C and thirty at 25°C for 21 days. The cultures of *Aspergillus* and *Penicillium* spp. were incubated only at 25°C. After the required incubation period the contents of the flasks were dried at 40°C for 4 days in wire baskets lined with cheesecloth. The dry material was ground in a Wiley mill and the meal stored at 1°C.

Toxicity of the various fungal cultures was determined by means of chicken and rat oral feeding tests. Groups of 10 one-day-old White Leghorn cockerels (Matthews Hatcheries, Madison, Wisconsin) were fed a balanced ration containing 69% maize meal (Table 1) *ad libitum* for 14 days. The control diet contained high quality maize meal obtained from the University feed mill. The mouldy diets contained meal prepared from maize on which the various fungi had grown. Pairs of 21-day-old, female, albino Sprague-Dawley rats (Holtzman Strain) were fed high quality maize meal and maize on which the various fungi had grown, respectively, as their sole diet for 14 days. The chickens and rats were mass-measured individually at the beginning and at the conclusion of the test. Mortality was recorded twice daily and *post mortem* examination performed by the staff of the Department of Veterinary Science, University of Wisconsin and the Wisconsin State Animal Health Laboratory, Madison.

8. *Chemical analyses of maize meal for aflatoxin and T-2 toxin*

Maize meal samples were analysed for aflatoxin content according to the standard ASAC-procedure (Pons, Cucullu, Lee, Robertson, Frantz & Goldblatt, 1966) modified for fluorometric determination on a Turner Fluorometer (G.K. Turner Associates, Palo Alto, California) and for T-2 toxin content according to the gas chromatographic technique described by Bamburg (1968).

RESULTS

1. *Mycoflora of mouldy maize meal*

A total of 42 different species of fungi and Actinomycetes were isolated from the mouldy maize meal (Table 3). *Cladosporium herbarum* (Pers.) Link was the dominant fungus in the freshly ground mouldy meal. Other fungi which were present in large numbers in the freshly ground meal were *Fusarium moniliforme* (Sheld.) Snyd. et Hans., *Cephalosporium acremonium* Corda, *Penicillium* spp. and members of the Mucorales.

TABLE 3 *Fungi and Actinomycetes isolated from mouldy maize meal*

Absidia corymbifera (Cohn) Sacc. et Trotter
Alternaria tenuis Nees
**Aspergillus amstelodami* (Mangin) Thom et Church
**Aspergillus candidus* Link.
Aspergillus chevalieri (Mangin) Thom et Church
**Aspergillus clavatus* Desm.
**Aspergillus flavus* Link.
**Aspergillus fumigatus* Fres.
Aspergillus niger van Tieghem
Aspergillus terreus Thom
Aspergillus versicolor (Vuill.) Tiraboschi
Candida albicans (Robin) Berkh.
**Cephalosporium acremonium* Corda
**Cladosporium herbarum* (Pers.) Link.
Epicoccum nigrum Link.
**Fusarium moniliforme* (Sheld.) Snyd. et Hans.
**Fusarium roseum* (Link.) Snyd. et Hans.
**Fusarium tricinctum* (Corda) Snyd. et Hans.
**Geotrichum candidum* Link. et Pers.
Gliocladium roseum (Link.) Bain.
Humicola sp.
Monascus ruber van Tieghem
Mucor alternans van Tieghem
Mucor fragilis Bain.
Mucor hiemalis Wehm.
Mucor pusillus Lindt.
Paecilomyces varioti Bain.
Papularia sp.
Penicillium canescens Sopp
**Penicillium chrysogenum* Thom
**Penicillium cyclopium* Westl.
Penicillium duponti Griffon et Maublanc
Penicillium oxalicum Currie
**Penicillium rubrum* Stoll
Penicillium rugulosum Thom
Phoma sp.
Rhizopus arrhizus Fisch.
Rhizopus rhizopodiformis (Cohn apud Licht.) Zopf
Sporotrichum sp.
Thermoactinomyces vulgaris Tsilinsky
**Trichoderma viride* Pers.
**Trichothecium roseum* Link.

2. *Microbial successions associated with self-heating of maize meal*

The temperature of mouldy maize meal with moisture content adjusted to approximately 30% and stored at 10°C increased rapidly to a maximum of 50 to 52°C which was generally reached after 8 days (Fig. 1). No temperature increases occurred in maize meal with a moisture content below 20% stored at 10°C or in high moisture content meal stored at 0°C.

Rapid temperature increases in the meal were always associated with large increases in the numbers of the two thermotolerant Mucorales, *Mucor pusillus* Lindt and *Rhizopus rhizopodiformis* (Cohn apud Licht.) Zopf, which could be isolated at 50°C (Table 4). The peak of the relative frequency of these two Mucorales agreed well with the temperature peak in the meal and they were apparently responsible for the rapid temperature increases.

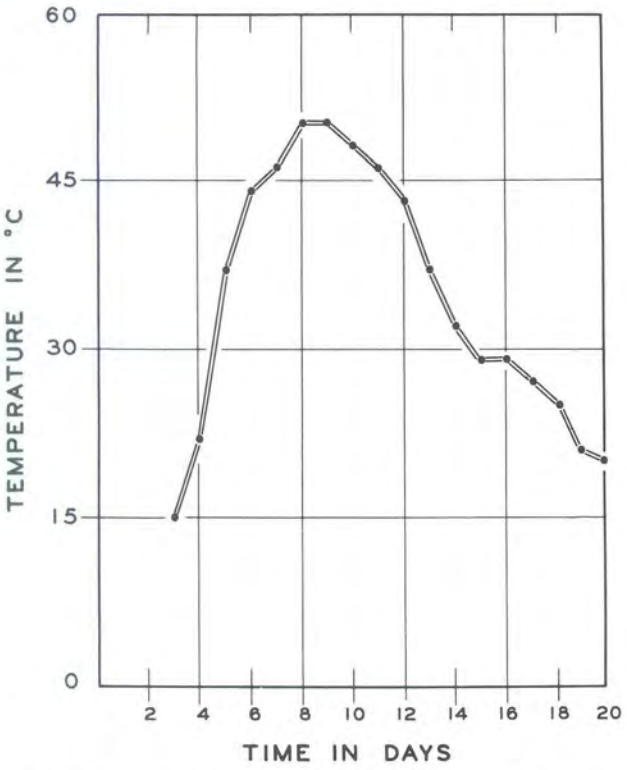

FIG. 1 Temperature changes in the central layers of mouldy maize meal with an initial moisture content of 29,2% stored at 10°C

As the temperature in the meal decreased, the relative frequency of the thermotolerant Mucorales also decreased and *Aspergillus fumigatus* Fres. became the dominant thermotolerant fungus which could be isolated at 50°C (Table 4; Fig. 2). Further decreases in the temperature of the meal were associated with increases in the relative frequencies of other fungi with high temperature optima. The result was that the original fungal population of the meal, dominated by *C. herbarum,* was completely replaced after heating had occurred by a population dominated by *A. fumigatus, A. flavus* Link, *Penicillium* spp. (particularly *P. cyclopium* West., *P. chrysogenum* Thom. and *P. rubrum* Stoll) and certain Mucorales (Tables 5, 6; Fig. 3). This succession

TABLE 4 *Changes in the numbers of thermotolerant microorganisms isolated from mouldy maize meal with a high initial moisture content stored at 10°C*

Organisms	Relative incidence (percentage of total number of colonies on three plates of NDA incubated at 50°C)			
	Storage time (weeks)			
	0	1	2	3
Mucorales*	0,0	78,9	90,2	22,8
Aspergillus fumigatus . .	0,0	0,0	8,3	70,7
Actinomycetes and Bacteria†	100,00	21,1	1,5	6,5
Total Propagules/g × 10^3	2,3	103,4	1 684,0	7 520,0

**Mucor pusillus* and *Rhizopus rhizopodiformis*
†*Thermoactinomyces vulgaris* and *Bacillus licheniformis*

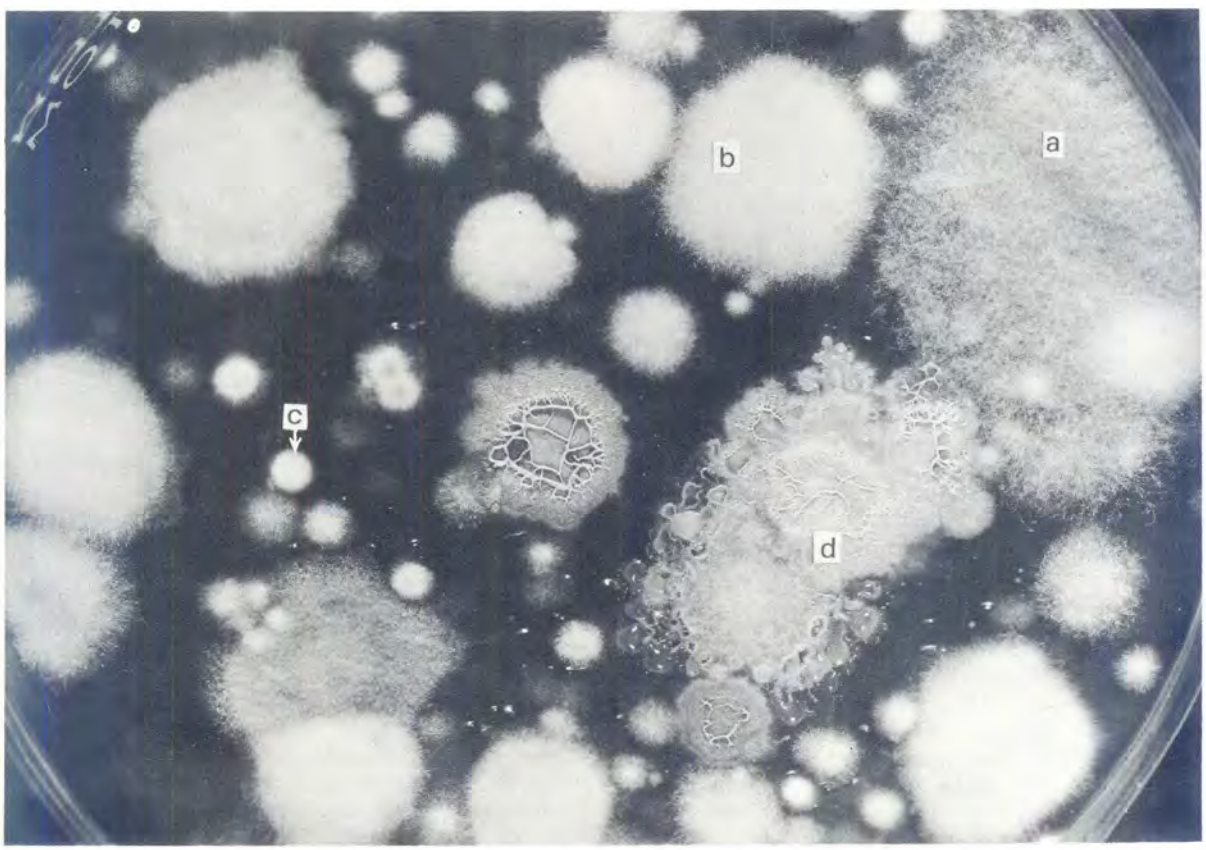

FIG. 2 Close-up photograph of a dilution plate (1:10^5) of mouldy maize meal with a moisture content of 29,2% after two weeks storage at 10°C. Plates prepared on NDA and incubated at 50°C for three days. a = *Rhizopus rhizopodiformis* b = *Mucor pusillus* c = *Aspergillus fumigatus* d = *Bacillus licheniformis*

did not take place in the uninfested meal or in the mouldy meal with a moisture content below 20%. The mould counts and relative frequencies of the various components of the mycoflora of these meals stayed relatively constant during 3 weeks of storage (Tables 5, 6).

TABLE 5 *Changes in the mycoflora isolated at 24°C from uninfested and mouldy maize meal samples stored at 10°C*

Fungi	Culture medium	Relative incidence of fungi (percentage of total number of colonies on three plates)					
		Uninfested maize meal		Mouldy maize meal			
		Moisture content 11,2%		Moisture content 13,5%		Moisture content 29,2%	
		Start	3 weeks	Start	3 weeks	Start	3 weeks
Mucorales	Potato dextrose agar	86,6	66,7	25,8	25,8	4,4	27,5
Cladosporium herbarum		0,0	1,8	40,8	58,5	76,7	0,0
Fusarium moniliforme		10,4	3,6	2,9	1,3	3,2	0,0
Penicillium spp.		0,0	19,3	22,9	6,9	0,0	1,5
Geotrichum candidum		0,0	0,0	2,9	3,1	5,0	61,7
Other		3,0	9,0	4,6	4,4	10,7	9,3
Total Propagules/g × 10^3		25,1	21,5	92,6	61,9	1 498,0	17 400,0
Mucorales	Malt salts agar	52,9	33,3	9,2	10,8	5,1	38,8
Cladosporium herbarum		0,0	3,0	55,4	43,4	84,1	0,0
Fusarium moniliforme		14,7	6,0	2,0	1,9	2,6	0,0
Penicillium spp.		13,5	21,7	30,2	36,5	0,0	12,4
Aspergillus amstelodami		19,1	21,7	2,6	6,8	0,0	0,0
Aspergillus flavus		0,0	3,0	0,3	0,3	0,0	35,0
Other		0,0	11,5	0,3	0,6	8,2	14,0
Total Propagules/g × 10^3		25,5	26,0	235,3	166,2	1 662,0	19 970,0
Mucorales	Acidified Czapek's agar	51,4	6,0	3,6	2,1	11,8	7,1
Cladosporium herbarum		0,0	0,0	23,5	22,8	42,4	0,0
Fusarium moniliforme		35,1	17,9	4,1	3,2	10,6	0,4
Penicillium spp.		13,5	70,2	67,7	68,1	14,1	43,0
Aspergillus flavus		0,0	6,8	0,0	0,4	0,0	44,8
Geotrichum candidum		0,0	0,0	0,0	0,6	20,0	4,7
Other		0,0	0,0	1,2	2,7	0,5	0,0
Total Propagules/g × 10^3		27,7	31,7	323,6	188,0	80,0	34 820,0

TABLE 6 *Changes in the mycoflora isolated at 36°C from uninfested and mouldy maize meal samples stored at 10°C*

Fungi	Culture medium	Relative incidence of fungi (percentage of total number of colonies on three plates)					
		Uninfested maize meal		Mouldy maize meal			
		Moisture content 11,2%		Moisture content 13,5%		Moisture content 29,2%	
		Start	3 weeks	Start	3 weeks	Start	3 weeks
Mucorales	Potato dextrose agar	81,3	76,4	68,8	55,6	80,9	27,7
Fusarium moniliforme		6,3	0,0	10,9	0,0	4,3	0,0
Penicillium spp.		0,0	0,0	18,8	0,0	9,6	0,0
Aspergillus flavus		0,0	23,1	0,0	11,1	0,0	33,9
Aspergillus fumigatus		9,4	0,0	0,0	33,3	0,0	39,3
Other		3,1	0,0	1,5	0,0	5,2	0,0
Total Propagules/g × 10^3		11,9	4,9	24,6	3,5	44,2	50 500,0
Mucorales	Malt salts agar	41,4	55,5	29,4	25,0	15,7	32,3
Fusarium moniliforme		47,4	0,0	20,6	0,0	3,0	0,0
Aspergillus amstelodami		0,0	0,0	38,2	50,0	0,0	0,0
Aspergillus flavus		0,0	44,5	0,0	8,3	0,0	40,0
Aspergillus fumigatus		5,2	0,0	2,9	8,3	0,0	26,2
Candida albicans		0,0	0,0	0,0	0,0	75,3	0,0
Other		0,0	0,0	8,7	8,3	6,0	1,5
Total Propagules/g × 10^3		7,1	3,4	13,1	4,7	78,2	29 200,0
Mucorales	Acidified Czapek's agar	50,0	0,0	0,0	0,0	0,0	0,0
Penicillium spp.		50,0	33,3	96,5	94,4	69,7	13,9
Aspergillus flavus		0,0	66,6	0,0	1,9	0,0	37,3
Aspergillus fumigatus		0,0	0,0	3,5	3,7	0,0	47,9
Candida albicans		0,0	0,0	0,0	0,0	30,3	0,0
Other		0,0	0,0	0,0	0,0	0,0	1,0
Total Propagules/g × 10^3		1,5	1,1	33,1	21,0	15,5	25 980,0

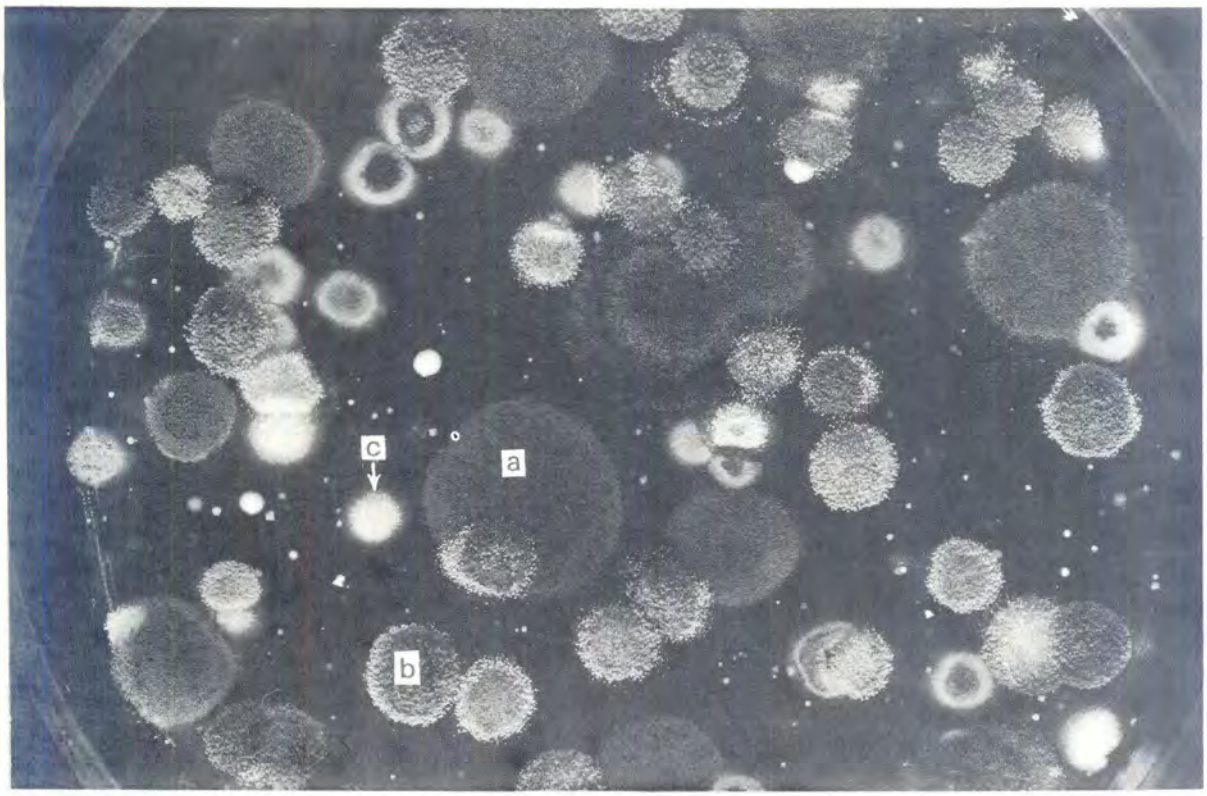

FIG. 3 Close-up photograph of a dilution plate (1:10^3) of mouldy maize meal with a moisture content of 29,2% after three weeks storage at 10°C. Plates prepared on ACA plus tergitol and incubated at 36°C for seven days. a = *Aspergillus fumigatus* b = *Aspergillus flavus* c = *Penicillium* spp.

3. *Effect of milling date on the initial mycoflora of mouldy maize meal*

The initial moisture content of the freshly ground mouldy maize meal decreased while the initial mould count increased as the season progressed from February to July (Fig. 4). The major changes in the composition of the mycoflora of the freshly ground mouldy meal were a decrease in the relative incidence of *C. herbarum* and an increase in the relative frequency of *Penicillium* spp., particularly *P. rubrum, P. cyclopium* and *P. chrysogenum* (Fig. 5).

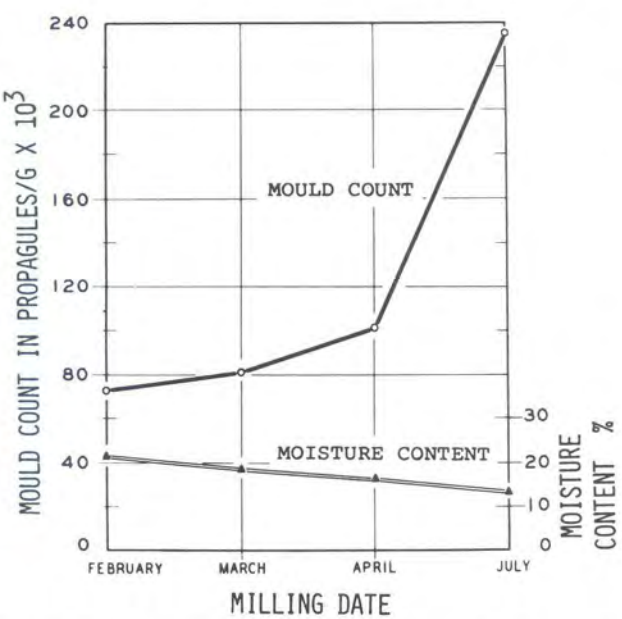

FIG. 4 Effect of milling date on initial mould count and moisture content of mouldy maize meal. Mould counts were made on MSA at 24°C

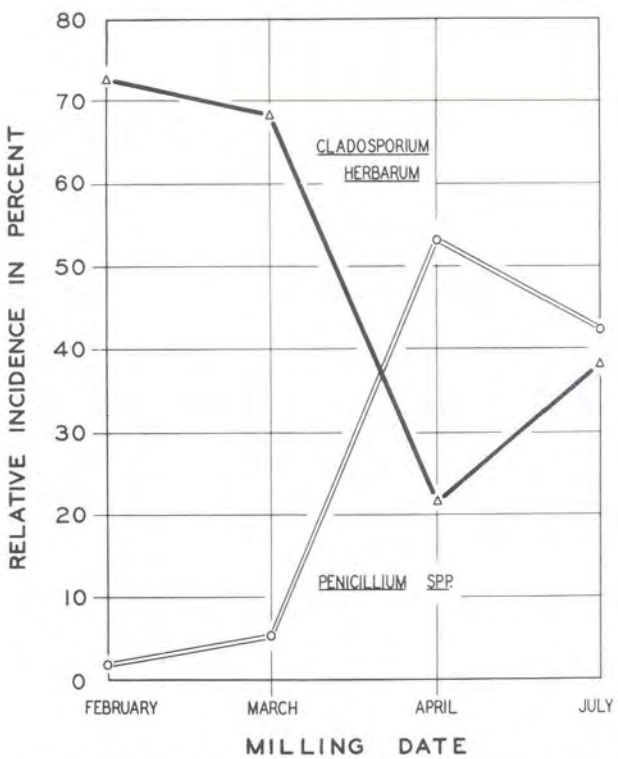

FIG. 5 Effect of milling date on the initial composition of the mycoflora of mouldy maize meal. Mould counts were made on MSA at 24°C

4. *Nutritive value of naturally infested mouldy maize meal*

The primary effect of fungal development in maize meal, under the conditions used in these experiments, was a reduction in the nutritive value of the meal. This was evidenced by the reduction in mass gain of chickens and pigs fed mouldy diets (Table 7; Fig. 6), but the reduction was statistically significant ($P < 0,01$) only in the case of chickens fed during July (Table 7).

The reduced growth-promoting value of the mouldy maize meal was particularly evident in the reduced feed

TABLE 7 *Effects of mouldy maize meal on mass gain of pigs and mass gain, feed consumption and feed efficiency of chickens*

Sampling date	Maize meal treatment	Moisture content (%)	Storage temperature (°C)	Pigs[1] Total mass gain (kg)	Total mass gain (kg)	Chickens Total feed consumption (kg)	Feed efficiency $\left(\frac{\text{gain}}{\text{consumption}}\right)$
February . . .	Control	12,7	—25	—	7,509[2]	13,056[2]	0,57
	Mouldy	21,6	—25	—	7,286	15,066	0,48
	Mouldy	21,6	10	—	7,588	17,259	0,43
March . . .	Control	12,4	0	—	3,891[3]	7,368[3]	0,52
	Mouldy	18,6	0	—	3,877	7,973	0,48
	Mouldy	26,5	0	—	3,936	7,975	0,49
	Mouldy	18,6	10	—	3,860	8,608	0,44
	Mouldy	27,7	10	—	3,671	8,545	0,42
April	Control, shelled	14,2	10	13,2	2,577[4]	5,618[4]	0,45
	Control, ear	13,6	10	10,8	2,444	5,742	0,43
	Mouldy, shelled	16,4	10	9,6	2,543	5,478	0,46
	Mouldy, ear	14,6	10	8,6	2,185	6,084	0,36
July	Control	11,2	10	17,6	4,645[5]	8,383[5]	0,55
	Mouldy	13,5	10	16,2	4,245*	7,763	0,55
	Mouldy	29,2	10	9,2	3,960*	10,300	0,38

* Significantly different from the control ($P < 0,01$)

[1] Each figure represents the total mass gain of two weanling pigs after 4 weeks. Pig feeding experiments were conducted during April and July only

[2] Total mass gain and feed consumption of 45 one-day-old chickens after 3 weeks

[3] Total mass gain and feed consumption of 15 one-day-old chickens after 4 weeks

[4] Total mass gain and feed consumption of 10 one-day-old chickens after 4 weeks. The moisture content of all maize meal samples was adjusted to approximately 30% after 19 days of storage

[5] Total mass gain and feed consumption of 15 one-day-old chickens after 4 weeks

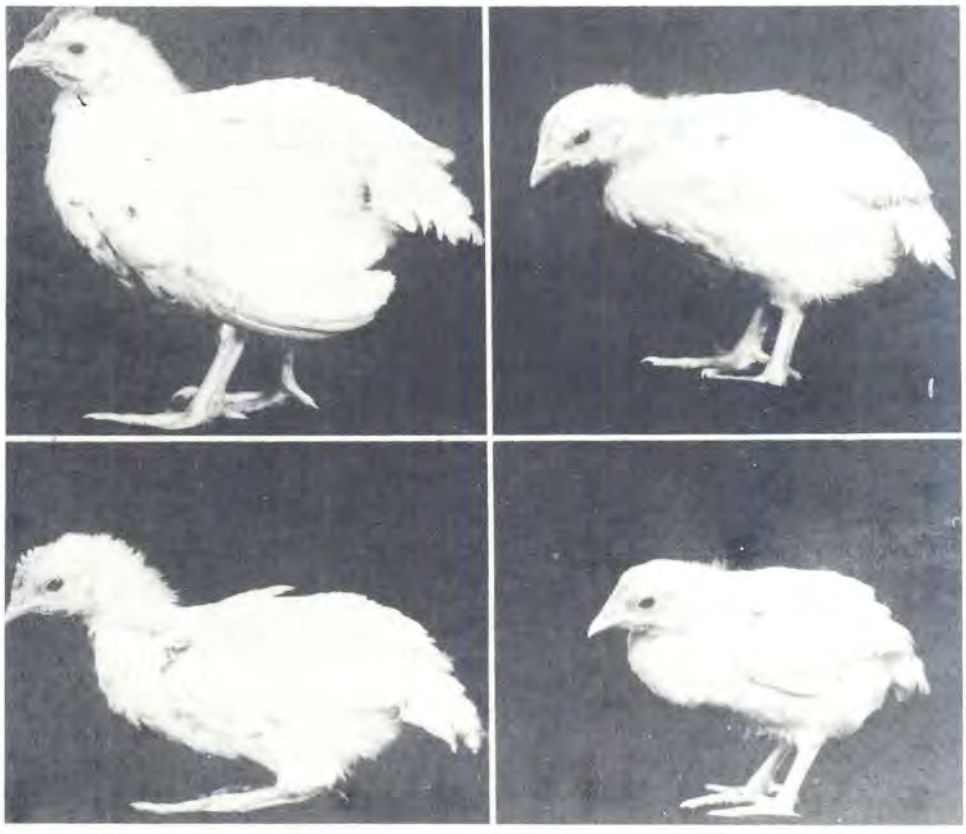

FIG. 6 Growth-stunting effect of mouldy maize on chickens. A male (left) and female (right) fed control maize meal are shown at the top. A male (left) and female (right) fed high moisture mouldy maize stored at 10°C, are shown at the bottom

efficiency of chickens fed mouldy diets (Table 7). The feed efficiency, or ratio between mass gain and feed consumption, gives a good indication of the nutritive value of the feed. The feed efficiency of birds fed mouldy diets was lower than that of the controls in almost all cases. Chickens fed high moisture content mouldy maize consumed up to 1,3 times more feed than the controls per gram of mass gained (Table 7).

No statistically significant differences were found between the average mass of pigs fed uninfested and mouldy maize meal (Table 7). The mass gains of pigs fed ear maize meal were lower than those of pigs fed shelled maize meal in the case of mouldy as well as uninfested maize. The high moisture content mouldy meal which was ground during July caused a 45,5% reduction in mass gain of pigs fed diets containing this meal compared to the controls (Table 7). The pigs fed this mouldy maize meal did not consume their feed readily, appeared distressed and unsteady on their feet and their stools were abnormally soft and darkly coloured.

5. *Toxicity of naturally infested mouldy maize meal*

No mortality occurred amongst the 295 chickens and 14 pigs used in these feeding experiments. Although the mouldy maize had a slight to severe growth stunting effect on chickens as well as pigs (Table 7; Fig. 6). no gross pathological changes were evident in the organs of any of these animals upon *post mortem* examination. Chemical analyses of the various maize meal samples for aflatoxin and T-2 toxin were also negative.

6. *Toxicity of pure cultures of fungi isolated from mouldy maize*

Sixteen species of fungi isolated from mouldy maize meal were cultured on autoclaved maize and tested for toxicity (Table 3). Six of these species of fungi (*Aspergillus candidus* Link, *Aspergillus clavatus* Desm., *A. fumigatus, Fusarium moniliforme, F. tricinctum* and *Trichothecium roseum* Link) caused 90 to 100% mortality within 14 days in chickens (Table 8) as well as rats (Table 9).

The cause(s) of death could not be established by *post mortem* examination of the chickens and rats. Uterine hypertrophy was not evident in any of the rats examined. Most of the chickens showed a generalized anaemia but no other gross pathological changes. The birds fed maize inoculated with *A. clavatus* had greatly distended gall bladders and histopathological examination of their livers revealed diffuse fatty change, congested blood vessels and oedema, but no haemorrhage or necrosis.

Maize inoculated with *A. flavus* killed both of the test rats (Table 9) but caused only 40% mortality in chickens within 14 days (Table 8). This isolate produced no aflatoxin which could be detected with the method of Pons *et al.* (1966).

Maize inoculated with *P. rubrum* caused 50% mortality of chickens (Table 8) as well as rats (Table 9). The toxin produced by this isolate was characterized as rubratoxin A, which comprised 59,2% of the crude toxic extract from cultures on malt extract enriched Raulin - Thom medium (Dr. M. O. Moss, Tropical Products Institute, London, 1968, personal communication).

Four species of fungi [*Aspergillus amstelodami* (Mangin) Thom et Church, *Penicillium cyclopium, P. chrysogenum* and *Geotrichum candidum* Link ex Persoon] caused no mortality in either chickens (Table 8) or rates (Table 9). With the exception of *G. candidum,* these fungi did, however, cause marked reductions in mass gain and feed efficiency of chickens and rats. The average mass of chickens fed maize inoculated with *A. amstelodami* was half of that of the controls after 14 days and these chickens consumed twice as much feed as the controls per gram of mass gain (Table 8).

TABLE 8 *Toxicity to chickens of maize inoculated with various fungi*[1]

Fungi	Incubation temp. (°C)	Mortality (%)	Days to 100% mortality	Average day of death[2]	Average mass (g)	Total mass gain (g)	Total feed consumption (g)	Feed efficiency $\left(\frac{\text{Gain}}{\text{Consumption}}\right)$
Control	—	0	> 14	0	130,6	927	1711	0,54
Aspergillus amstelodami	25	0	> 14	0	66,2	273	1000	0,27
Aspergillus candidus	25	90	> 14	7,7	36,0	—	275	—
Aspergillus clavatus	25	100	6	4,9	—	—	230	—
Aspergillus flavus	25	40	> 14	11,2	47,5	—	740	—
Aspergillus fumigatus	25	100	5	4,4	—	—	135	—
Cephalosporium acremonium	10	50	> 14	9,8	58,0	—	660	—
	25	60	> 14	8,3	49,5	—	555	—
Cladosporium herbarum	10	0	> 14	0	101,4	670	1545	0,43
	25	0	> 14	0	84,2	412	1325	0,31
Fusarium moniliforme	10	10	> 14	5,0	112,1	—	1515	—
	25	90	> 14	8,8	38,0	—	340	—
Fusarium roseum	10	80	> 14	7,3	53,0	—	330	—
	25	70	> 14	7,2	37,6	—	375	—
Fusarium tricinctum	10	100	9	7,2	—	—	195	—
	25	100	11	8,3	—	—	220	—
Geotrichum candidum	10	0	> 14	0	128,5	935	1760	0,53
	25	0	> 14	0	109,0	748	1550	0,48
Penicillium chrysogenum	25	0	> 14	0	102,7	622	1310	0,47
Penicillium cyclopium	25	0	> 14	0	82,2	410	1135	0,36
Penicillium rubrum	25	50	> 14	9,8	42,2	—	405	—
Trichoderma viride	10	10	> 14	14,0	87,8	—	1280	—
	25	30	> 14	10,3	55,0	—	750	—
Trichothecium roseum	10	100	8	6,1	—	—	145	—
	25	90	> 14	8,1	38,0	—	240	—

[1] Each group contained 10 one-day-old White Leghorn cockerels which were fed for 14 days

[2] Number of chicks dying on a given day multiplied by the respective day equals total days to death. Sum of values of the days to death of chicks in the respective group divided by total number of chicks that died in the group equals average day of death. See Forgacs *et al.* (1962)

TABLE 9 *Toxicity to rats of maize inoculated with various fungi*[1]

Fungi	Incubation temp. (°C)	Mortality (%)	Days to 100% mortality	Average day of death[2]	Average mass (g)	Total mass gain (g)	Total feed consumption (g)	Feed efficiency $\left(\frac{\text{Gain}}{\text{Consumption}}\right)$
Control	—	0	> 14	0	60,8	11	271	0,040
Aspergillus amstelodami	25	0	> 14	0	45,5	—	138	—
Aspergillus candidus	25	100	10	10,0	—	—	2	—
Aspergillus clavatus	25	100	9	8,5	—	—	10	—
Aspergillus flavus	25	100	13	12,0	—	—	79	—
Aspergillus fumigatus	25	100	6	6,0	—	—	15	—
Cephalosporium acremonium	10	0	> 14	0	54,0	—	179	—
	25	0	> 14	0	60,0	2	263	0,007
Cladosporium herbarum	10	50	> 14	9,0	56,0	—	131	—
	25	50	> 14	11,0	40,0	—	157	—
Fusarium moniliforme	10	0	> 14	0	50,0	—	195	—
	25	100	7	7,0	—	—	95	—
Fusarium roseum	10	50	> 14	11,0	42,0	—	127	—
	25	100	14	13,5	—	—	133	—
Fusarium tricinctum	10	100	9	8,0	—	—	20	—
	25	100	10	8,5	—	—	27	—
Geotrichum candidum	10	0	> 14	0	65,0	14	435	0,032
	25	0	> 14	0	60,0	8	307	0,026
Penicillium chrysogenum	25	0	> 14	0	55,0	—	169	—
Penicillium cyclopium	25	0	> 14	0	54,0	—	262	—
Penicillium rubrum	25	50	> 14	6,0	40,0	—	60	—
Trichoderma viride	10	0	> 14	0	62,5	5	209	0,023
	25	0	> 14	0	61,0	8	215	0,037
Trichothecium roseum	10	100	9	8,5	—	—	53	—
	25	100	10	9,0	—	—	47	—

[1] Each group contained two female, 21-day-old, albino rats which were fed for 14 days
[2] Number of rats dying on a given day multiplied by the respective day equals total days to death. Sum of values of the days to death of rats in the respective groups divided by total number of rats that died in the group equals average day of death. See Forgacs *et al.* (1962)

DISCUSSION

The changes which occurred in the composition of the mycoflora of mouldy maize meal as the season progressed from winter to summer and following heating of high moisture content mouldy meal, resulted in the establishment of large populations of several known toxigenic species including *A. flavus, A. fumigatus* and *P. rubrum*. Pure cultures of these three species of fungi and of several others isolated from the mouldy maize meal were shown to be extremely toxic to experimental animals. It is realized that the free feeding toxicity assay of these fungi in pure culture was not very satisfactory and that complicating factors such as unpalatability and starvation may have been involved. The toxicity assay was, however, designed only as a rapid screening method for potentially toxic species present in the mouldy meal.

Despite the large population of *A. flavus* in the mouldy meal under certain conditions, chemical analyses of the meal for aflatoxins were negative. The extremely mouldy meal also caused no mortalities or significant pathological changes in either chickens or pigs. Similar results were reported by Van Warmelo (1967), who showed that the presence of a toxigenic species in a feed sample is not necessarily indicative of the toxicity of that sample. It is clear that suitable conditions for the development of large fungal populations, which may include several known toxigenic species, are not necessarily conducive to the production of mycotoxins and the development of "mouldy corn toxicosis" in animals.

The absence of acutely toxic levels of mycotoxins in naturally infested mouldy maize meal which contained several toxigenic species of fungi may have been due to microbial competition and/or degradation of the toxins by other micro-organisms. It was first suggested by Steyn (1933) that complex interactions between microorganisms may affect the toxicity of mouldy feedstuffs. Forgacs, Koch, Carll & White-Stevens (1962) found that the toxicity of chick broiler mash decreased as a *Scopulariopsis* sp. increased in the mash. Ashworth, Shroeder & Langley (1965) found that microbial competition limits the aflatoxin content of groundnut kernels and that aflatoxin is subject to fungal breakdown. Microbial interactions of this nature are probably very important in the development of toxic mouldy maize and should be further investigated.

The primary effect of fungal development in maize meal under the conditions used in these experiments was a reduction in the nutritive value of the meal. Similar results have been reported in previous feeding experiments with naturally contaminated mouldy diets (Ronk & Carrick, 1931; Steyn, 1933; Zeleny, 1948; Richardson & Webb, 1962; Thomke 1967). In all these cases the feeding of mouldy diets to chickens and pigs resulted in no mortalities and no deleterious effects other than a slight reduction in the growth rate and either a slight or no increase in the amount of feed consumed per gram of gain in body mass.

The reduction in growth-promoting value of the naturally infested mouldy maize may have been due to one or more of the following factors: decrease in feed consumption resulting from decreased palatability; toxic fungal metabolites, or deterioration of the nutritive value resulting from fungal metabolic activity.

Decreased palatability can be discounted as the primary cause of the reduction in mass gain of chickens fed mouldy maize because these chickens consumed more feed than the controls. It is not clear whether toxic fungal metabolites played any role because chemical analyses of the mouldy maize meal for aflatoxins and T-2 toxin were negative. If any other mycotoxins were involved their effects must have been very subtle because they caused no mortalities or

significant pathological changes in experimental animals. It seems likely that biochemical changes brought about by the metabolic activity of fungi resulted in the reduced nutritive value of the mouldy maize meal. Richardson, Wilkes, Godwin & Pierce (1962) and Richardson, Hayes & Rigdon (1967) found that the reduction in growth rate of poults fed mouldy soybean meal was due primarily to a reduction in the availability of lysine and that this effect could be completely overcome by supplementing the mouldy diet with 0,8% of lysine. They found that the level of arginine was also reduced in mouldy soybean meal and that poults actually had a higher growth rate on mouldy meal supplemented with 0,8% lysine and 1,0% arginine than on supplemented control meal.

The nutritional deficiencies of mouldy maize, particularly with respect to the essential amino acids, certainly warrant further investigation. This information may be applied in the development of dietary supplements for use in rations containing mouldy maize known to cause no acute toxicity.

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