

A Study of Free Amino Acids in Rice Moth Larvae During Mycotoxicosis

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SUMMARY

Free amino acid pattern has been studied in rice moth larvae during aflatoxicosis and compared with normal in ground nut meal and wheat bran. It was observed that there is an increase in free amino acids in intoxicated larvae and this was directly proportional to the degree of toxicity as indicated by decrease in growth of these larvae. The study of excretory pattern of amino acids shows that intoxicated larvae excrete less amino acids than the normal larvae. The results have been discussed.

Aflatoxin, a toxic metabolite of certain strains of *Aspergillus flavus* is a hepatotoxin (1, 2, 3, 4) and a carcinogen (5, 6, 7). Investigations carried out in our laboratory have indicated that insects are also susceptible to aflatoxin (8). Insect blood is known to have an unusually high amino acid concentration. Fluctuations of free amino acids may be related to protein synthesis during development. As there was reduction in growth of these larvae during aflatoxicosis it was of interest to study the changes in free amino acid contents in these larvae during aflatoxicosis.

Experimental

A toxic strain of *A. flavus* was used for contaminating the diets and for the preparation of aflatoxin. Groundnut meal and wheat bran were contaminated with *A. flavus* and the toxin prepared as described earlier (8).

Methods

About ten days old larvae were transferred to control groundnut meal, groundnut meal contaminated with *A. flavus*, control wheat bran, wheat bran contaminated with *A. flavus* and wheat bran containing crude aflatoxin (1 mg/gm diet). These larvae were picked from the experimental diet on the eighth day and taken for analysis of free amino acids. Larvae were homogenized with 80% alcohol and the homogenate was left overnight at room temperature for complete precipitation of proteins. The supernatant was dried *in vacuo* and taken in a known volume of 50% alcohol.

The excreta of the larvae was collected from the diet after picking the larvae. A known weight of excreta was taken in 0.1 N HCl. It was then treated with ion exchange resin (Dowex 50) to remove salts. The amino acids were eluted from the resin by treating it with 2 N NH₄OH. Ammonia was completely removed by drying *in vacuo* and the residue was taken in 50% alcohol.

The amino acids were separated and identified by two dimensional paper chromatography. The chromatogram was first developed with butanol: acetic acid: water (4:1:5) and 80% phenol was used for the second run. Amino acids were estimated by the method of Giri and coworkers (9).

The nitrogen was estimated by micro Kjeldhal method.

Results and Discussion

While studying the free amino acid pat-

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TABLE I. — Total Nitrogen Present in mg/gm of Diet

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Groundnut meal Control	Groundnut meal contaminated with <i>A. flavus</i>	Wheat bran control	Wheat bran contaminated with <i>A. flavus</i>
300	300	100	100

tern, the available nitrogen source in different diets has to be taken into consideration (Table I). The free amino acids of rice moth larvae during toxicosis has been presented in Table II and the excretory pattern in Table III.

As presented in Table II, free amino acid pattern of larvae of the same age changes to a great extent when they are reared in different diets. Groundnut meal has a higher nitrogen content than wheat bran (Table I). The number of amino acids as well as the concentration of amino acids are higher in larvae reared in groundnut meal than in wheat bran. The amino acids found in larvae reared in control groundnut meal and not present in larvae reared in control wheat bran are glutamine, asparagine, glycine, leucine, hydroxyproline and phenylalanine.

In larvae reared in groundnut meal contaminated with *A. flavus*, there is an increase in glutamic acid, aspartic acid, asparagine, alanine, threonine, lysine, arginine, histidine, proline and cystine. Al-

though the reduction in growth of larvae is not much in groundnut meal after eight days, there is about 18% increase in total free amino acids.

When the free amino acid pattern of rice moth larvae reared in wheat bran is considered, we find an increase in the concentration of alanine, arginine, histidine, valine and tyrosine and the total increase in amino acids is about 35%. From the growth studies (8) it was observed that the reduction in growth of larvae was highly significant in contaminated wheat bran. Present studies indicate that the increase in free amino acid content is highest in the group where there is maximum reduction in weight. This increase in free amino acid seems to be directly proportional to the toxicity of the diet.

The results on the analysis of free amino acids in the excreta of rice moth larvae (Table III) indicate that the excretion of free amino acids and other ninhydrin positive substances are less in intoxicated larvae. This reduction in the excretion of free amino acids may also help in the increase of free amino acid content in intoxicated larvae.

Variations in free amino acids must be related to protein synthesis during development of rice moth larvae (10). Reduced growth of intoxicated larvae indicates that tissue proteins are synthesised at a slower rate than in normal larvae. This may directly or indirectly due to the interference of aflatoxin in protein synthesis. Aflatoxin

TABLE II. — Free Amino Acids in the Homogenate of Rice Moth Larvae ($\mu\text{g/gm}$ of wet tissue) Results are an Average of Replicates

Amino Acids	Control Groundnut meal	Contaminated Groundnut meal	Control Wheat Meal	Contaminated Wheat bran	Wheat bran + Aflatoxin
Glutamic Acid . . .	600	688	550	525	338
Aspartic Acid . . .	25	100	13	13	—
Glutamine	269	63	—	—	200
Asparagine	63	558	—	44	10
Alanine	250	313	31	138	259
Glycine	425	110	—	152	25
Threonine	69	163	69	50	8
Serine	—	—	—	—	45
Lysine	275	325	425	44	138
Arginine	250	394	25	142	63
Histidine	488	512	75	445	445
Leucine	106	106	—	69	20
Valine	110	163	25	93	16
Proline	63	138	100	50	—
Hydroxyproline . .	163	13	—	—	—
Tyrosine	75	63	44	75	8
Phenylalanine . . .	25	35	—	—	35
Cystine	44	75	25	25	13
Unknown	1	1	—	3	0
(No. of Spots)					

TABLE III. — Free Amino Acids in the Excreta of Larvae Raared in Different Diets ($\mu\text{g}/\text{gm}$ of excreta)

Amino Acids	Control Groundnut meal	Contaminated Groundnut meal	Control wheat bran	Contaminated Wheat Bran	Wheat Bran + aflatoxin
Glutamic Acid ..	263	150	30	45	—
Glutamine.....	90	60	+	—	—
Alanine.....	15	—	—	—	—
Glycine.....	180	60	+	—	—
Threonine.....	15	—	—	—	—
Serine.....	143	38	+	+	+
Lysine.....	263	105	30	—	—
Arginine.....	150	225	38	—	—
Histidine.....	333	173	15	—	—
Leucines.....	75	—	—	—	—
Valine.....	+	—	+	—	—
Tyrosine.....	105	—	—	—	—
Cystine.....	143	43	43	—	—
Unknown.....	7	3	6	2	2

+ Very low concentration present.
 — Absent.

is reported to inhibit protein biosynthesis *in vitro* in liver slices (11) and yeast (12). Hence, the increase in certain amino acids may be due to the reduced growth rate which might be due to inhibition of protein synthesis.

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