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Changes in Protein, Free Aminoacids, Nucleic acids, Total Lipids and Free Fatty Acids in Larvae of *Tribolium castaneum* (Herbst) due to *Farinocystis tribolii* Weiser Infection

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ABSTRACT

Farinocystis tribolii Weiser-infected larvae of *Tribolium castaneum* (Herbst) had significantly lower amounts of protein. The diseased larvae had lower total free aminoacids content when compared to uninfected larvae. The DNA levels were enhanced due to infection, whereas RNA levels did not vary significantly. Total lipids were depleted in diseased larvae, but the free fatty acids were more in diseased than in healthy larvae.

The reduction in the levels of protein, free aminoacids and lipids in diseased larvae is primarily due to the destruction and depletion of the fat bodies by the sporozoans. The increased levels of DNA and free fatty acids in infected insects could have been contributed by the parasites themselves.

Key words: *Farinocystis tribolii*, *Tribolium castaneum* larvae, protein, aminoacids, nucleic acids, total lipids, free fatty acids, changes

The pathological effects of the schizogregarine, *Farinocystis tribolii* Weiser in the larvae of *Tribolium castaneum* (Herbst) have been described previously (Rabindra *et al.*, 1981). The present communication deals with changes in protein, free aminoacids, nucleic acids, total lipids, and free fatty acids in *F. tribolii* - infected larvae of *T. castaneum*.

METHODS AND MATERIALS

Fourth instar larvae of *T. castaneum* of uniform age, reared on whole wheat flour fortified with 5% brewer's yeast were starved for 8 h and inoculated with *F. tribolii* spores by allowing the larvae to feed for 24 h on wheat flour containing 10^8 spores g following methods described by Rabindra *et al* (1981). Three samples of 500 mg of larvae were drawn from each treated and untreated groups at 48 h interval upto 10 days and the biochemical estimations were made using fresh whole larval homogenates. The methods of Orr (1964) and Price (1969) were used to separate the nucleic acids and proteins from other fractions and the DNA was estimated following the method of Burton (1956) using the sodium salt of calf thymus gland DNA (BDH) as the standard. RNA was determined by the Orcinol method of Schneider (1955) with yeast RNA (BDH) as the standard. Protein was estimated using Folin-ciocalteu

reagent (Lowry *et al.*, 1951) and compared with standards prepared with bovine serum albumin.

Total free aminoacids from larval samples were extracted by homogenizing in 80% hot ethyl alcohol and estimated colorimetrically (Moore and Stein, 1948) using leucine as the standard. The total lipids were extracted from fresh larvae with chloroform-methanol (2 : 1 V/V) by the method of Folch *et al.* (1957) and the total free fatty acids were estimated in the total lipid residue by the rapid colorimetric determination of Lowry and Tinsley (1976). Oleic acid was used as the free fatty acid standard

All data were subjected to analysis of variance and the means compared with Least Significant Difference.

RESULTS AND DISCUSSION

The protein content in diseased larvae was significantly lower than in healthy larvae at all periods (Table 1). As the disease progressed, protein was depleted from the sixth day onwards in contrast to the accumulation of protein in healthy larvae. Histopathological studies (Rabindra *et al*, 1981) have revealed that on the sixth day of infection, the protozoan parasites were at the peak of multiplication and that the albuminoid granules in the fat body steadily decreased as the disease progressed On the eighth day, the albuminoid granules disappeared completely. Thus, the disappearance of albuminoid granules should have contributed to the depletion of protein.

The total free aminoacids were lower in diseased than in healthy insects at all periods and the reduction was more pronounced on the second and fourth days (Table 2). Studies on the respiratory behaviour of healthy and *F. tribolii* - infected larvae of *T. castaneum* have shown that the diseased larvae had lower RQ than the healthy ones (Rabindra *et al.*, 1984). Taking into consideration the lower amount of free aminoacids found in diseased larvae than in healthy ones, it is possible that the diseased larvae were utilizing the free aminoacids (Wigglesworth, 1972) along with the available carbohydrates for the respiratory metabolism leading to the lowering of the RQ. Wang and Moeller (1970) found that *Nosema* infected honeybees had lesser amounts of free aminoacids when

TABLE 1. Protein in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	Protein (mg/g wet wt.) Mean* (N=3) ± S. E.		% decrease over healthy
	Healthy	Diseased	
2	188.0 ± 2.3	163.3 ± 1.8	13.3
4	193.3 ± 0.6	174.7 ± 1.3	9.6
6	190.7 ± 1.3	162.0 ± 1.1	15.1
8	204.0 ± 2.3	156.0 ± 2.3	23.5
10	232.0 ± 1.1	138.0 ± 1.1	40.5
Mean* *	201.6 ± 8.1	158.8 ± 6.0	21.2

* Differences between the means of healthy and diseased significant (P=0.05) on all days.

* * Differences between the means significant (P=0.05).

TABLE 2. Free aminoacids in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	Free aminoacids (Leucine equivalent - mg/g wet wt.) Mean* * (N=3) ± S. E.		% decrease over healthy
	Healthy	Diseased	
2	6.08 ± 0.22	4.92 ± 0.22	19.1
4	7.42 ± 0.22	6.00 ± 0.38	19.1
6	6.33 ± 0.03	6.08 ± 0.03	3.9
8	7.08 ± 0.60	5.75 ± 0.03	18.8
10	7.75 ± 0.29	6.42 ± 0.17	17.2
Mean*	6.93 ± 0.33	5.83 ± 0.28	15.9

* Differences between the means significant (P=0.05).

* * Interaction not significant (P>0.05)

TABLE 3. DNA content in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	DNA (µg/g wet wt.) Mean* (N=3) ± S. E.		% increase over healthy
	Healthy	Diseased	
2	61.0 ± 0.58	99.0 ± 0.43	60.0
4	50.0 ± 0.58	80.0 + 2.10	60.0
6	61.0 ± 2.10	85.0 ± 1.20	66.7
8	70.0 ± 0.41	162.0 ± 4.10	131.4
10	82.0 ± 0.50	140.0 ± 1.20	70.3
Mean* *	64.8 ± 5.3	113.2 ± 16.1	74.7

* Differences between the means of healthy and diseased significant (P=0.05) on all days.

* * Differences between the means significant (P=0.05).

compared to the uninfected bees and that the hypoaminoacidemia was the result of the pathogen's need for aminoacids. Protozoa can take up aminoacids either through the surface or from food vacuoles (von Brand, 1966).

The amount of DNA in diseased larvae was significantly more when compared to healthy larvae (Table 3). The increase in the DNA levels in the infected larvae was not followed by a concomittant increase in the RNA levels (Table 4). There were no significant differences in the RNA content of healthy and diseased insects. Hartwig and Przelecka (1971) observed active DNA replication in the cells of *Nosema apis* Zander multiplying in the intestine of *Apis mellifera* L. and this active DNA replication in the parasite cells resulted in the depression of RNA synthesis in the host cells. Hence, the increased level of DNA in diseased larvae could have been contributed by the proliferating protozoans themselves.

Total lipids were significantly reduced from the fourth day of infec-

TABLE 4. RNA content in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	RNA (µg/mg wet wt.) Mean* (N=3) ± S. E.		% decrease (−) or increase (+) over healthy
	Healthy	Diseased	
2	7.0 ± 0.03	5.9 ± 0.11	− 15.7
4	11.2 ± 0.12	7.0 ± 0.08	− 37.5
6	6.5 ± 0.15	8.0 ± 0.04	+ 23.1
8	11.5 ± 0.15	12.6 ± 0.08	+ 9.6
10	3.8 ± 0.10	7.5 ± 0.06	+ 97.4
Mean*	8.0 ± 1.5	8.2 ± 1.1	+ 2.5

* Differences between the means not significant (P>0.05).

TABLE 5 Total lipids in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	Total lipids (mg/g wet wt.) Mean* (N=3) ± S. E.		% decrease over healthy
	Healthy	Diseased	
2	144.0 ± 3.4	142.7 ± 7.1	0.9
4	183.3 ± 1.8	132.0 ± 0.0	28.0
6	161.3 ± 2.7	126.7 ± 1.3	21.5
8	156.5 ± 3.1	92.8 ± 2.4	40.7
10	184.7 ± 2.4	81.3 ± 5.7	56.0
Mean* *	166.0 ± 7.9	115.1 ±11.9	30.7

* Differences between the means of healthy and diseased significant (P=0.05) on all days except the second day.

* * Differences between the means significant (P=0.05).

tion in diseased larvae and the rate of depletion increased with time (Table 5). This is understandable, since the pathogen developing in the fat body completely destroyed and depleted the fat reserves as seen in histological sections (Rabindra *et al.*, 1981).

In contrast to the lipids, the total free fatty acids (FFA) content was significantly more in diseased than in healthy larvae from the fourth day onwards of infection (Table 6. The lower levels of FFA in healthy larvae may be the result of esterification by the fat body of the FFA into triglycerides. In diseased larvae, the synthetic processes of the fat body should have been disrupted due to infection. Further, *F. tribolii* infection could have reduced the rate of metabolism and according to Gilbert (1967) the FFA release is normally high in tissues of lower metabolism. The spores of the protozoan themselves could have contributed to the FFA level as in *N. apis* (Roberts, 1968) and *Mattesia grandis* McLaughlin (Thompson and McLaughlin, 1977).

Gilbert (1967) reported that in the isolated fat body of the cockroach *Leucophaea maderae* (Fabricius), JH extract decreased the rate of biosyn-

TABLE 6. Free fatty acids in healthy and *F. tribolii* - infected larvae of *T. castaneum*.

Days after inoculation	Free fatty acids (Oleic acid equivalent - mg/g wet wt.) Mean* (N=3) ± S. E.		% decrease (—) or increase (+) over healthy
	Healthy	Diseased	
2	2.99 ± 0.09	2.78 ± 0.07	— 7.0
4	1.51 ± 0.09	2.82 ± 0.25	+ 86.8
6	0.93 ± 0.08	2.00 ± 0.13	+ 115.1
8	1.09 ± 0.03	1.94 ± 0.03	+ 78.0
10	0.29 ± 0.02	1.37 ± 0.04	+ 372.4
Mean* *	1.36 ± 0.45	2.18 ± 0.28	+ 60.3

* Differences between the means of healthy and diseased significant (P=0.05) on all days except second day.

* * Differences between the means significant (P=0.05).

thesis of lipid and also the oxidation of fatty acids. This should naturally result in the accumulation of fatty acids. In the present instance, there was a rise in the FFA level in infected larvae on the fourth day which was 86.8% more than in healthy larvae. Active multiplication of the pathogen was observed on the fourth day of infection and the juvenile hormonal (JH) action reported (Rabindra *et al.*, 1981) to be induced by the pathogen was probably initiated around that time as evidenced by the inhibition of moulting. This indicates the possibility of the JH action interfering with the fatty acid oxidation leading to the accumulation of fatty acids.

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Combined Efficacy of the Bacterial Spore Parasite, *Pasteuria penetrans* (Thorne, 1940) and Nematicides in the Control of *Meloidogyne javanica* on Tomato

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ABSTRACT

Experiments were carried out under greenhouse conditions to test the efficacy of *Pasteuria penetrans* (Thorne, 1940) Sayre and Starr (1985) in combination with five nematicides viz., aldicarb, carbofuran, miral, phorate and sebufos for control of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 on tomato var. Pusa Ruby. Results revealed that the application of *P. penetrans* in combination with nematicides significantly improved plant growth characteristics and the increase in growth was more than additive when compared with their individual effects. Combined application resulted in additive reduction of *M. javanica* galling. When *P. penetrans* and nematicides were applied together, there was a high degree of nematode control wherein a maximum of 89.37 per cent reduction in final nematode population was recorded with *P. penetrans* and carbofuran combination.

Key words : *Pasteuria penetrans*, *Melojdogyne javanica*, combined effect of bacterium and nematicide.