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EFFECT OF NUCLEOPOLYHEDROSIS VIRUS ON NITROGEN, URIC ACID AND PROTEIN CONTENTS OF GROUNDNUT RED-HAIRY CATERPILLAR, *AMSACTA ALBISTRIGA*

NUCLEOPOLYHEDROSES have been reported to cause considerable derangement in the physiology of infected insect[1]. Though the occurrence, pathogenicity and field efficacy of a nucleopolyhedrosis virus (NPV) in groundnut red hairy caterpillar, *Amsacta albistriga* (W.) have been reported in India[2–4], its effect on the metabolic disturbances in the insect has not been well studied. In the present study observations on the changes in total nitrogen, uric acid and total protein contents during the course of virus infection in *A. albistriga* are reported.

The polyhedral inclusion bodies (PIBs) were processed from the diseased final instar larvae of *A. albistriga* and purified by differential centrifugation. Freshly moulted fifth instar larvae were infected by leaf spot feeding technique[5] with 10 µl virus suspension containing $8 \cdot 05 \times 10^6$ PIBs/larva. Those larvae which did not consume completely the entire leaf bit within 2–4 h were discarded. The larvae which fed with the normal leaf bit not spotted with virus suspension served as control. Samples were taken at 24 hr intervals for a period of 144 hr after treatment. Total nitrogen was estimated using the whole body dry homogenates by micro-kjeldahl method[6]. Uric acid was determined following the method of Brown[7] after precipitating the protein by the addition of tungstic acid in dry sample and expressed as percentage of dry matter. Total protein was extracted from the perchloric acid insoluble residue in the whole body homogenates of fresh material[8] and estimated following the method of Lowry *et al.*[9], and expressed in mg/g protein.

The results presented in Table I show that the average nitrogen content in diseased larvae (9·21%) was significantly higher than that of healthy larvae (8·67%). Similar increase in total nitrogen content has been observed in *Bombyx mori*[10] and *Spodoptera litura*[11] larvae infected with NPV. The increased nitrogen content of the diseased larvae may be due to the preservation of large quantities of nitrogen in the form of polyhedral protein since polyhedra consist of 95% protein[12].

Though there was a slight increase in uric acid content in the case of diseased larvae, the difference was not statistically significant. Since uric acid is one of the characteristic catabolites of the insect, the slight increase at the end of the catabolism may probably be due to the reflection of the metabolic disturbances induced by a viral infection as suggested by Smirnoff and Loisella[13]. The finding that NPV-infected larvae of *A. albistriga*, having comparatively higher levels of total protein, though not statistically significant may be due to increased protein leading to the formation of millions of polyhedra. It has been observed that

TABLE I

Effect of nuclear polyhedrosis virus on nitrogen, uric acid and protein content of A. albistriga

Period after infection (hrs.)	Nitrogen (% dry weight)		Uric acid (% dry weight)		Protein (mg/g of wet weight)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
24	..	..	1·22	1·24	..	..
48	8·51	8·91	1·39	1·36	45·73	46·51
72	8·91	9·31	1·34	1·38	48·74	49·33
96	9·81	10·11	1·32	1·39	54·11	56·80
120	8·11	8·71	1·22	1·47	42·59	47·64
144	8·01	9·01	1·35	1·47	41·88	48·40
Mean	8·67	9·21	1·29	1·39	46·61	49·73
C.D. (P = 0·05)	0·347		N.S.		N.S.	

N.S. Not significant.
C.D. (P = 0·05): The critical difference at 5% probability level.

a single larva weighing 0·7337 mg has yielded as much as $5·80 \times 10^6$ PIBs[14]. Thus the levels of nitrogen, uric acid and protein show the physiological development of healthy insects and have an important diagnostic value of viral infections.

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CONCOMITANT ISOLATION OF *BRUCELLA ABORTUS* AND *CAMPYLOBACTER FETUS* FROM AN ABORTED BOVINE FETUS

Brucella abortus and *Campylobacter fetus* (*Vibrio fetus*) are recognised pathogens of the genital tract of cattle, the former causing abortions and the latter mostly involved in early embryonic death and repeat breeding. Occasionally, *C. fetus* also causes abortions[4]. The isolation of *B. abortus* and *C. fetus* from aborted fetuses, infected cows and bulls is well documented. However, there is no record of the isolation of these two genital pathogens from a single aborted bovine fetus. This report records the concomitant isolation of *B. abortus* and *C. fetus* from an aborted bovine fetus.

During an investigation of infectious abortions amongst bovines in Karnataka State, an aborted bovine fetus, aborted around six months of gestation, was received in this laboratory from a brucella infected farm. The stomach contents and heart blood of the fetus were aseptically removed and cultured for possible isolation of Brucella, Campylobacter and any other pathogenic bacteria. A few drops of the stomach contents and heart blood were streaked on the following media: Bacto Tryptose (Difco Laboratories, USA) for Brucella, modified Florent medium[2] for Campylobacter and blood agar plates for any other pathogens. The blood agar and tryptose agar plates were incubated in 10% CO_2 tension in an anaerobic jar. Modified Florent medium plates were incubated in an atmosphere of 40% nitrogen, 10% CO_2 and 50% air in a locally manufactured anaerobic incubator. The plates were incubated at 37° C for 4–6 days prior to examination.

B. abortus was isolated in pure culture from the stomach contents in tryptose agar. The heart blood yielded a mixed culture of *B. abortus* and *C. fetus* in this medium. Modified Florent medium showed pure colonies of *C. fetus* only from the heart blood. Stomach contents of the fetus did not yield any growth in this medium. The colonies of *B. abortus* on tryptose agar, when examined on the fourth day, appeared round, with regular margin measuring about 2 mm in diameter. They were transparent and straw yellow coloured. On Grams staining, the organisms appeared as Gram negative coccobacilli arranged singly. When a loopful of the colonies was mixed with a drop of anti *Brucella abortus* serum, visible agglutination was observed in the cavity slide. The isolate conformed to the tests for *B. abortus* as described by Alton and Jones[1]. The isolate was further confirmed as *B. abortus* (biotype 1) by Brinley Morgan of the Central Veterinary Laboratories, Weybridge, U.K.

The colonies of *C. fetus* on the modified Florent Medium were circular about 1 mm in diameter, greyish white, transluscent and glistening in appearance. Under the stereoscopic microscope with a magnification of about 50 diameters, the older colonies had a greyish white opaque central area with a thinning out transparent periphery. Microscopic examination of a Grams stained smear from a young colony revealed Gram variable curved bacilli. Some seagull forms also were seen. Smear from an old culture grown in Bacto-Thiol (Difco Laboratories, USA) showed long spiral forms. Growth in this medium was in the form of a white opalescent ring about 5–6 mm from the surface. The isolate was catalase positive, did