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GROSS CHEMICAL COMPOSITION OF THE TWO SEXES AND JUVENILES OF TRICHURIS OVIS

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Abstract

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Gross chemical composition (glycogen; lipid - total body, phospholipid, unsaponifiable; protein and nucleic acids - DNA and RNA) of males, females and free-living infective juvenile stages of Trichuris ovis has been worked out on dry weight basis. Marked quantitative differences were found to exist in per cent of the biochemical constituents in between the two sexes as well as between the adult and the juvenile stages.

Glycogen, lipid, protein, nucleic acids, RNA, DNA, Trichuris ovis.

During a survey of nematode parasites of Meerut region, Trichuris ovis, the whipworm, was found to be one of the most prevalent nematodes in sheep and goat. Total ash content and complete elemental make up of the nematode species have already been worked out (Lal, Kumar 1982). Studies carried out on gross chemical composition of nematodes have been reviewed by von Brand (1973). In addition references hitherto reported and a careful screening of available literature (Kumar 1984) reveal that no work has been done on gross chemical composition of T. ovis. Present investigations were, therefore, undertaken.

Materials and methods

Motile whipworms were recovered from the intestines of sheep and goats slaughtered freshly in a local abattoir. The worms were washed in cold phosphate buffered saline (PBS), pH 7.4. The two sexes were separated, dried at 100 °C until a constant weight was achieved and weights were recorded.

For eggs containing infective L_2 stages female whipworms were incubated at 37 °C in physiological saline. They laid large number

Table 1

Gross chemical composition of adult male and female and free-living L_2 infective stage of Trichuris ovis

Sex/life-cycle stage	Glycogen	Lipid			Protein	Nucleic acids	
		Total body	Phospho-lipid *	Unsaponifiable *		DNA	RNA
	Mean value (N = 5) ± S.D.				(% dry wt)		
Male	5.39±0.18	9.49±0.91	51.11±2.24	7.73±1.07	73.75±2.32	0.24±0.002	1.81±0.003
Female	9.41±1.12	7.34±1.21	60.09±1.76	18.54±1.84	76.59±0.71	0.23±0.001	1.90±0.012
L_2	8.67±1.27	12.77±1.70	67.09±1.88	18.67±2.07	68.91±2.11	0.23±0.001	1.99±0.024

* In % of total body lipid

of eggs which were cultured in the filtered cow faeces-charcoal extract at 25 ± 1°C. This temperature was found best for the development of egg into L_2 stage. The eggs containing L_2 stages were separated by centrifugation at 2000 r.p.m. for 15 min, dried as above and weighed.

For glycogen dry tissue was homogenized in 10% (w/v) trichloroacetic acid and centrifuged at 4000 r.p.m. for 15 min. Glycogen was precipitated from supernatant with ethanol and estimated according to Roe et al. (1961). Optical Density (O.D.) was measured at 490 nm using Sicospec-100 spectrophotometer.

Total lipid content was estimated gravimetrically. Dry tissue was extracted in chloroform-methanol (2:1) using Soxhlet apparatus according to Folch et al. (1957). Phospholipids were estimated by Youngberg's method modified by Oser (1976) using lecithin as standard. Phosphate was estimated according to Fiske, Subba Row (1925) at 673 nm. Unsaponifiable lipid content was determined according to Rendina (1971).

For proteins dry tissue was homogenized and extracted with cold PBS in the ratio of 3 ml PBS/g of tissue at 4°C. After centrifugation (2h at 10,000 r.p.m. at 4°C) protein concentration was estimated using Folin Reagent (Lowry et al. 1951) at 540 nm.

For nucleic acids dry tissue was homogenized in 0.25 M sucrose and 0.003 M calcium chloride solution. Nucleic acids were extracted according to Schneider (1945). Spectrophotometric procedures (Orcinol method for RNA and Dische's diphenylamine reaction for DNA) specific for pentose sugars or for deoxysugars, as have been described by Dische (1955), were employed. O.D. was measured at 640 nm and 600 nm for RNA and DNA respectively.

Statistical calculations were done according to Fisher, Yates (1948). All chemicals (E. Merck, Germany/ B.D.H., England) used were of analytical grade. Each experiment was repeated at least thrice and the results presented in Table 1 are based on three values obtained for each sample (male/female/L_2).

Results

Results obtained have been summarised in Table 1. L_2 stages were found to contain highest lipid (total, phospholipid and unsaponifiable) and RNA contents, females highest glycogen and lipid contents and males highest DNA content. Though glycogen, phospho- and unsaponifiable lipid and RNA contents were lowest in males, total lipid and DNA contents were lowest in females. Protein contents were lowest in L_2 stages.

Discussion

Biochemical analyses of adult and larval stages of various species of nematodes have revealed different normal ranges of glycogen (3.3. in Nippostrongylus brasiliensis adult to 55 in Porrocaecum decipiens larva), total lipid (3.5 in P. decipiens adult to 37 in Tylenchorhynchus claytoni adult), phospholipid (16 in N. brasiliensis larva to 72.2 in Trichinella spiralis larva, in per cent of total lipid) and unsaponifiable lipid matter

(2.2 in N. brasiliensis adult to 10 in T. spiralis larva, in per cent of total lipid) in per cent of dry weight of tissue (von Brand 1973). The average per cent glycogen (7.82), total lipid (9.87), phospholipid (59.43) and unsaponifiable lipid matter (14.98) in present studies are well within the limits of these ranges except unsaponifiable lipid content which was exceptionally high. From present observations it is apparent that lipid as well as glycogen constitute the major reserve source of energy in the L_2 stage probably being related to its higher energy requirements due to high growth rate. The amount of glycogen in the tissues of animal-parasitic nematodes has been stated to vary from species to species and also from stage to stage within species but it is usually present in considerable amounts and provides the major reserve store of energy (Lee, Atkinson 1976). Present findings support this view. Though lipid is the chief food reserve in the free-living stages of many animal-parasitic nematodes, glycogen is the chief food reserve in most adult animal-parasitic nematodes but they have also been reported to contain large amounts of lipid (von Brand 1973; Lee, Atkinson 1976). The large amount of lipid present in L_2 stage of T. ovis seems related to its aerobic existence. The free-living, non-feeding, infective juveniles of several animal-parasitic nematodes (Nippostrongylus, Haemonchus, Ancylostoma, Necator) have been found to use their stored lipids as an energy reserve and the infectivity of these juveniles is related to the amount of their stored fat since juveniles that had depleted their reserves of lipid were observed not to be as infective nor as motile as juveniles that contained large amounts of lipid (Rogers 1962; Croll 1972). Lipid has been stated to be the primary source of energy in Tylenchulus semipenetrans, Aphelenchus avenae, Caenorhabditis sp., Panagrellus sp., Thelastoma bulhoesi and second-stage juveniles of Meloidogyne javanica (Lee, Atkinson 1976). Most of the phospholipids are structural components (Lee, Atkinson 1976). Their high percentage in L_2 stages of T. ovis seems related to the high rate of growth. Detailed studies on the nature of the lipids of nematodes have been carried out on relatively few species (von Brand 1973; Krusberg 1971) and Fairbairn (1969) states that Ascaris lumbricoides is the only species in which detailed studies on lipids have been made. Glycogen (1.39, male; 1.79, female; 2.81, L_3, in per cent of fresh weight), total lipid (8.73, male; 12.27, female; 10.91, L_3, in per cent of dry weight), phospholipid (54.67, male; 48,27, female; 67.17, L_3, in per cent of total lipid) and unsaponifiable lipid (6.3, male; 13.53, female; 8.7, L_3, in per cent of total lipid) contents have been reported to differ in the two sexes and in the adult and larval stages of Bunostomum trigonocephalum (Kumar 1984).

The total protein content is known for relatively few nematodes, but it varies between about 50 and 80% of the dry weight of tissues (Lee, Atkinson 1976). The average per cent protein in present study, 73.08%, falls within above-mentioned range. The highest glycogen and protein contents of the females may be accounted for due to the high fecundity and their additional storage in the eggs and ovaries which has been confirmed histochemically (Bhatnagar 1985). Lee, Atkinson (1976) have,

however, described differences in the percentage protein content of nematodes due partly to variation in the amounts of carbohydrate and lipid in the tissues and have pointed out that ovary of nematodes contains a significant amount of protein which contributes in the formation of egg shell. Difference in protein content in male (64.73), female (62.46) and L_3 (47.71), in per cent of dry weight have been reported (Kumar 1984) in B. trigonocephalum. The intestine of several nematodes has been found to contain globules of protein but there is little evidence that they are stored as energy reserve. Granules of protein in the intestine of juvenile Meloidogyne disappear during development and may be incorporated into structural proteins (Lee, Atkinson 1976). Freshly hatched juveniles of M. javanica and of T. semipenetrans and infective juveniles of N. brasiliensis and Cooperia punctata, metabolise some of their proteins during periods of starvation (Krusberg 1971; von Brand 1973; Nicholas 1975).

Relatively higher RNA content in females and L_2 seems related to a higher rate of protein synthesis in the two required chiefly for the egg production and their subsequent development in females and for a high rate of growth in L_2 which obviously requires high rate of protein metabolism. Differences in RNA contents have been reported between male (1.256), female (1.307) and L_3 (1.27) in per cent of fresh weight, of B. trigonocephalum (Kumar 1984). He (1984) has also reported differences in DNA contents of male (0.056), female (0.061) and L_3 (0.058) of B. trigonocephalum, in per cent of fresh weight. The distribution of DNA and RNA in the tissues of nematodes has been examined by histochemical methods in a variety of nematodes and biochemical methods have, rarely been used (Rogers 1969). Available literature reveals that most of the work on quantitative aspects of nucleic acids have remained restricted only to such large nematode species as Ascaris (Samoilenko, Slyusarev 1975; Sulimov 1976; Kuhn, Tobler 1978; Moritz, Roth 1978; Davis et al. 1979; Dubinsky, Rybos 1980) and Ascaridia (Vovchenko 1978).

Základní chemické složení juvenilních forem a obou pohlaví Trichuris ovis

Základní chemické složení (glykogen, celkové tuky v těle, fosfolipidy, bílkoviny a nukleové kyseliny DNK a RNK) bylo stanovováno v sušině těla dospělých samečků, samiček a volně žijících infekčních stadií parazita. V procentuálním zastoupení jednotlivých biochemických komponent byly nalezeny velké kvantitativní rozdíly mezi pohlavím samčím a samičím a též mezi juvenilními a dospělými stadii.

Основной химический состав ювенильных форм и обоих полов Trichuris ovis

Основной химический состав (гликоген, общие жипы в теле, фосфолипиды, белки, нуклеиновые кислоты ДНК и РНК) определяли в сухом веществе тела взрослых самцев, самок и свободно живущих инфекционных стадий паразита. В процентном выражении отдельных биохимических компонентов были установлены существенные количественные расхождения между самцами и самками, а также между ювенильной и взрослой стадиями.

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