Comparative Calorie Values of Nematodes[1]

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Abstract: Calorie values for a wide biological selection of nematodes, determined with a microbomb calorimeter, ranged from 3.86 to 6.85 Kcal/g. The mean of 5.095 Kcal from 16 species was lower than means recorded in three previous studies of other invertebrate groups. The nematode values were skewed to the lowest limit. Larvae of *Ditylenchus dipsaci* showed lower calorie values after storage, and the calorie values of separate tissues of *Ascaris lumbricoides* were highest for eggs and the intestine and lowest for cuticle and body-wall musculature. No clear calorie distinction exists between nematodes with a parasitic or free-living habit or between large and small nematodes. *Key Words:* Energetics, calorimetry, comparative energetics, parasitism.

In the last few decades parasitism has been examined with methods other than the classical morphological and life-cycle approaches. With each, there has been a further narrowing of the gulf between the "bizarre" parasite and the more "normal" free-living forms. Thus, metabolism, ultrastructure, behaviour and population studies have established much common ground between parasitic and free-living organisms and have demonstrated the subtlety of their adaptation, not their eccentricity (3).

It is often claimed that nematodes occupy more biotypes than any other invertebrate group and yet show a relative constancy of form and life stages. One untested method of comparing them is to measure their calorie values. This method, rather than measuring a single parameter, i.e., a glycolytic enzyme or the grams of triglyceride per unit weight, is a holistic approach. It sums the relative energy investment of an organism in all its tissues, including food reserves at the moment of its capture. Such a measure is a profile of many interacting selective forces. So few studies have been performed on the energetics of helminths that we do not know whether the parasitic role has caused any fundamental change from that of free-living forms in the disposition of energy. It may be argued that the relative abundance of food, the homeostasis provided by many hosts, and the apparent increased fecundity claimed for parasites caused some deviation from free-living organisms.

Slobodkin and Richman (9) measured the calorie value of a wide range of invertebrates (and a small fish). They obtained values ranging from 4.397 to 6.962

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Kcal/g d. wt. (18.3 to 29.13 KJ/g d. wt.). They found that the distribution of calorie values was skewed with the modal value toward the lowest limit, and argued that this was consistent with selection for maximum progeny but not for high energy content per unit weight. No nematodes were included in their samples. A more detailed analysis of 21 species of free-living and parasitic flatworms (1) found a mean value of 5.841 Kcal/g, close to that of 5.821 Kcal/g, reported for a wide range of phyla (4). Calow and Jennings (1) interpreted their results in relation to parasitism, fecundity, and energetics. No similar studies have been reported on nematodes, although they show a wide biological spectrum of habitats and ecological niches similar to that of the Platyhelminthes. This paper describes a study of 16 species of nematodes and three additional parasitic invertebrates, one acanthocephalan, one crustacean and one digenean. A comparison is presented between different species and biotypes of nematodes and between nematodes and other invertebrates.

MATERIALS AND METHODS

The nematodes and other parasites used are listed below with approximate length and biological type. All were collected fresh from their natural habitats and were in good condition.

Nematodes: Anisakis simplex, (300 mm) larval stages (mainly L4), removed from the body musculature of the cod (*Gadus callarius*); commonly called "codworm." *Aphelenchus avenae,* (0.9 mm) plant-feeding form, grown in the laboratory on *Botrytis cinerea* (5); all stages were used. *Aplectana* spp./*Cosmocerca* spp., (6 mm) adults removed from the hind gut of toads (*Bufo bufo*) were used; both were very similar species of oxyuroid parasites and collections were pooled. *Ascaris lumbricoides* (= *suum*), (2500 mm) adult parasites removed from the intestine of pigs. *Aspiculuris tetraptera,* (2.5 mm) adult oxyuroid parasites of the caecum of laboratory mice were used; over 90% were females. *Deladenus siricidicola,* (20 mm) an entomophilic parasite of siricid wood wasps; the stages used were from the mixed eggs, larvae and adults from the mycetophagus phase in the life cycle, cultured on the fungus *Amylosterium* sp. *Ditylenchus dipsaci,* (1.0 mm) the stem and bulb eelworm of *Narcissus;* all stages of the life cycle occur in the plant, but a long-lived resistant "wool" develops as the over-wintering and most infective stage; samples contained over 97% L4; the "wool" was used. *Ditylenchus myceliophagus,* (1.0 mm) fungal-feeding tylenchid cultured on *Botrytis cinerea;* all stages were used. *Heterodera avenae,* (0.5 mm) infective second-stage larvae of tylenchid hatched from cysts containing eggs were used. *Heterodera trifolii,* (1.5 mm) tylenchid cyst nematodes, isolated from clover; females were used. *Litomosoides carinii,* (80 mm) filarial worms, isolated from the pleural cavities of the cotton rat, *Sigmodon hispidus;* adults, mostly females, were used. *Meloidogyne incognita,* (1.0 mm) plant-feeding tylenchid from roots of tomato; gelatinous egg sacs containing eggs extruded by the female were used. *Panagrellus redivivus,* (1.2 mm) bacteriophagus forms cultured in bacteria on cereal broth; all stages were used. *Proleptus obtusus,* (10 mm) a widespread stomach parasite of dogfish *Scylliorhinus cariculus.*

Platyhelminthes: Haplometra cylindracea, (10 mm) digeneans isolated from the lungs of the frog *Rana temporaria.*

Acanthocephala: Acanthocephalus ranae, (3 mm) spiny-headed worms isolated from the intestine of the toad *Bufo bufo.*

Crustacea: Chondracanthus gibbosus, (3 mm) adults removed from the gill chamber of the angler fish (*Lophius piscatorius*).

Calorie values were determined with a Phillipson microbomb calorimeter by standard calorimetric techniques (8). A Pye scale lamp galvanometer was used in determining the energy content of the material. All samples of dried material (minimum 3.0 mg) were stored in crucibles coated with silicone, which facilitated removal after vacuum or oven-drying. Pellets of 5 to 50 mg were prepared by pressing into a tablet maker.

The calorimeter was calibrated with benzoic acid standard of 6.319 Kcal/g. The number of samples varied with the availability of material (Table 1). The ash weight was less than 8-9% of the dry weight, and no correction factor was applied for possible endothermy (7).

Table 1. Comparative calorie values of nematodes and other invertebrates.

Species	No. samples	Mean Kcal/g
Proleptus obtusus, males, dogfish stomach	3	3.86
Heterodera avenae, L2	1	4.11
A. lumbricoides, males	3	4.15
Proleptus obtusus, females	5	4.27
Anisakis simplex, L4	5	4.50
A. lumbricoides, females	4	4.53
Contracaecum sp., adults	2	4.76
Ditylenchus myceliophagus, mixed	4	4.94
Meloidogyne incognita, egg sacs	1	5.05
Aspiculuris tetraptera, adults	1	5.17
Panagrellus redivivus, mixed	5	5.19
Deladenus siricidicola, mixed	2	5.21
Litomosoides carinii, adults	1	5.36
Aplectana/Cosmocerca, adults	1	5.36
Aphelenchus avenae, mixed	3	5.45
Heterodera trifolii, females	2	6.17
Ditylenchus dipsaci, adults, L4	4	6.78
D. dipsaci, L4	3	6.85
Acanthocephalus ranae, adults	2	5.71
Chondracanthus gibbosus, adult females	3	5.08
Haplometra cylindracea	2	5.22

Some *H. trifolii* females were collected from clover roots in October, November, and December. They were placed in soil, and by December they had tanned.

D. dipsaci "wool" was collected from the bulbs of *Narcissus* sp. and stored for 40 days at 10, 15, and 26 C. Calorie values were determined in comparison with fresh "wool" by bomb calorimetry.

Because of the size and availability of *A. lumbricoides* it was possible to measure the calorie values of various tissues of fresh worms. Eggs were obtained by dissecting the distal 1-cm portion of the uterus and dissolving the uterine wall in 10% KOH for 30 min. This procedure produced clean ova but also removed the albuminous outer layer. This was necessary for effective drying, a requirement for bomb calorimetry. Pellets of dried males and females were made by first powdering the dry nematodes and the pelleting portions of the homogenate so that all parts of the worm were proportionally represented.

RESULTS

Comparative calorie values (Table 1) varied from 3.86 to 6.85 Kcal/g. The lowest were males of *P. obtusus* and the highest were from "eelworm wool" of *D. dipsaci*. The calorie value of mature females of *H. trifolii* was also high, probably because of the eggs/larvae they contain. Other than the two extreme values, the calorie content of free-living plant-parasitic and animal-parasitic forms, in their various stages, did not differ greatly.

The calorie value of *H. trifolii* cysts was as follows: Oct. = 6.17 Kcal/g; Nov. = 5.51; Dec. = 5.52. This indicates a consumption of total calories during tanning.

The calorie value was higher for fresh *D. dipsaci* "wool" (Table 2) than for stored "wool", but there was no significant difference in energy content between larvae from the three storage temperatures.

Eggs and the intestine had the highest calorie values of the *A. lumbricoides* tissues (Table 3) and the higher value of the female homogenate was probably related to the contents of its ovaries and uteri. Fairbairn (6) found that 17.5% of the wet weight of fertilized eggs of *A. lumbricoides* was lipid.

Fig. 1 gives a frequency distribution plot of the calorie values of all the nematodes and of the three other parasites examined.

DISCUSSION

The calorie values for a wide selection of nematodes varied between 3.86 and 6.85 Kcal/g d. wt. While the bigger species had the lower values and the smaller free-living and parasitic forms had higher values, notable exceptions were both L2 of *H.*

Table 2. Calorie value (Kcal/g) of fourth-stage larvae of *Ditylenchus dipsaci*, freshly collected or after 40 days at different temperatures.

Sample	Fresh	Stored 8°	Stored 15°	Stored 26°
1	7.1271	6.1337	6.3589	6.4781
2	6.5218	6.1327	6.1601	6.1155
3	6.8903	6.3246	—	6.4116
Mean Kcal[a]	6.8464a	6.1970b	6.2595b	6.3351b

[a]Means followed by the same letter do not differ ($P = 0.01$).

Table 3. Calorie value of tissues of *A. lumbricoides.*

Composition of sample	Sample no.	Calorie value Kcal/g	Mean calorie value Kcal/g
Cuticle	1	4.41	
(males and females)	2	4.49	4.45
Body wall muscles	1	4.42	
(females)	2	4.49	4.41
	3	4.34	
Intestine	1	5.28	
(males and females)	2	4.83	5.06
Eggs	1	5.27	
(without albuminous layer)	2	4.99	5.13
Males	1	4.54	
(homogenates)	2	4.24	4.15
	3	3.68	
Females	1	4.44	
(homogenates)	2	4.78	4.53
	3	4.22	
	4	4.66	

avenae and adults of *L. carinii.* Calorie values for *D. dipsaci* L4 were very high; that is consistent with the view that resistant and long-lived stages have high values (9). The calorie values were higher for fresh "wool" of *D. dipsaci* than for stored "wool", demonstrating utilization of reserves even in this relatively anhydrobiotic state. The similarity in calorie loss from *D. dipsaci* "wool" stored at 8, 15, and 26 C may result from a thermal metabolic acclimatization (2). Values for cysts of *H. trifolii* varied with the period spent in the soil; this is consistent with a consumption of calories during tanning.

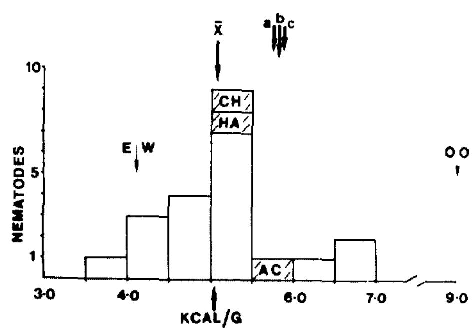

Fig. 1. A frequency distribution plot of the calorie values obtained in Table 1. CH, *Chondracanthus;* HA, *Haplometra;* AC, *Acanthocephalus.* All open space represents the nematodes. EW, egg white; OO, olive oil; x̄, mean for nematodes in this study. a, (5.821) mean of Cummins and Wuychek, (1971); b, (5.825) mean of Slobodkin and Richman, (1961); c, (5.841) mean of Calow and Jennings, (1974).

The mean value for all nematodes bombed was 5.095 Kcal/g d. wt., appreciably lower than in previous invertebrates studies (1,4,9). Fig. 1 plots these values and the mean value of other studies, all of which are very similar. The difference is rather striking and may be a feature of nematodes. The frequency distribution plot (Fig. 1) is skewed toward the lower calorie values, as was the study on flatworms (1).

Parasitism is not differentiated in this study. Indeed the only truly free-living organism, *P. redivivus,* falls very near the mean value. While the nematodes seem to have an energy content lower than other invertebrates, the adoption of parasitism in homeothermic and poikilothermic hosts or on fungi or higher plants shows no clear trend to have more or less calories per unit mass.

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