## ORIGINAL ARTICLE





# Condition factor and organosomatic indices of parasitized *Rattus rattus* as indicators of host health

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**Abstract** This study describes the influence of parasitism (Trypanosoma lewisi and Cysticercus fasciolaris) on the condition factor, hepato and splenosomatic indices of the common house rat, Rattus rattus Linnaeus, 1758 from Rohilkhand, Uttar Pradesh, India. Examination of R. rattus (n = 389) revealed T. lewisi (prevalence 12.40 %; intensity14 parasites/1000 RBC) from the blood and C. fasciolaris (larval Taenia taeniaeformis) (prevalence 46.70 %; intensity 2-4 par/host) from the liver. Condition factor (K) and organosomatic indices [hepatosomatic index, spleenosomatic index (SSI)] were evaluated in two groups (Group I non pregnant, Group II pregnant) of female rats which were further subdivided into four subgroups each (a Uninfected, b T. lewisi infected, c C. fasciolaris infected, d T. lewisi and C. fasciolaris infected) belonging to three weight groups (A 50-100 g; B 100-150 g; C 150-200 g). The results indicated that reduction in K-factor was more apparent in young rats, Group Ic (weight category A) showing the maximum depletion (21.62 %), hepatomegaly and splenomegaly were frequent outcomes of parasitic

infection and maximum change (50 %) was recorded in dual-infected pregnant rats (Group IId) expressed as SSI of the infected rat. The abnormal condition factor and organosomatic indices indicate perturbations in the biological systems at the organismal level. Thus, the information generated through this piece of work is a warning of an incipient or impending problem.

**Keywords** Rattus rattus · Trypanosoma lewisi · Cysticercus fasciolaris · Condition factor · Organosomatic indices

## Introduction

Mice and rats are the most common laboratory animals used in research and testing. Experimental results from research performed with living laboratory animals may be affected by environmental conditions provided by breeding and maintaining of these animals and by infectious agents (Pakes et al. 1984; Homberger and Thomann 1994). Application of indicators that use relationship between organs like the liver, kidney and spleen measures of length and weight and the study of the relative condition factor, in relation to parasitism levels, have proved to be important tools in the study of ecological relationships between parasites and their hosts (Lizama et al. 2006). The pathogenic activity of parasites necessarily affects the host condition in a negative way (Bauer 1970). Condition factor relates to weight and length and is an organism level response with factors such as nutritional status, pathogen effects and toxic chemical exposure causing greater than normal or lessthan-normal weights. It is regarded as a quantitative indicator of fitness. Organosomatic indices indicate the proportional sizes of organs and reflect the status of organ

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systems which may change in size more rapidly than organism weights and lengths increase or decrease. Condition factor and organosomatic indices have been used extensively as indicators of the well being of individual organisms and in host health and population assessments (Hoque et al. 1998). As it integrates many levels of suborganismal processes (molecular, cellular, organ system), an index such as Fulton's condition factor (Carlander 1969) may signify the overall condition and nutritional status of individual host (Adams et al. 1992). The size or weight of the liver and spleen relative to host weight may assist in signifying the overall health of the host. This study addresses the relationships between the parasites infra populations (Trypanosoma lewisi and Cysticercus fasciolaris) of Rattus rattus with the condition factor and hepato, splenosomatic indices.

## Materials and methods

## Animal sampling and processing

Wild rats, *R. rattus* (n = 389) used for the study were trapped from different localities of Rohilkhand region and maintained in the Animal House of the Department of Animal Science, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh, India (28.35°N; 79.42°E). They were kept in cages (3–4 rats per cage) and fed with bread, vegetables and water. The experiments were conducted according to the prevailing guidelines for animal care. The parasites were collected from the blood (*T. lewisi*) and liver (*C. fasciolaris*) of the animals.

# **Experimental groups**

Female rats were divided into two major groups (Group I non pregnant, Group II pregnant) which were further subdivided into four subgroups each as follows:

Group I	Non pregnant
Ia	Uninfected
Ib	T. lewisi infected
Ic	C. fasciolaris infected
Id	$T.\ lewisi + C.\ fasciolaris$ infected
Group II	Pregnant
IIa	Uninfected
IIb	T. lewisi infected
IIc	C. fasciolaris infected
IId	T. lewisi + C. fasciolaris infected
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#### Weight groups

The results were tabulated in the following weight groups:

Weight group A	50-100 g
Weight group B	100–150 g
Weight group C	150–200 g

## Collection of blood and slide preparation

Blood was collected from lateral tail vein, jugular vein, lateral saphenous vein, orbital plexus or by cardiac puncture using a 16 gauge, 5 cm long, hypodermic syringe, covered with a cover slip and examined microscopically to observe live parasites. Blood was also centrifuged in microhematocrit (7000 rpm for 7 min) to confirm the presence of blood parasites. However, their presence was also confirmed by preparing stained blood smears. A fresh drop of blood was placed on grease-free clean slides at a distance of about one inch from the right end and a smear prepared by holding another slide at an angle of 45 degrees and pushed gently to the left, exhausting the blood and forming a 'tail'. The film was allowed to dry rapidly, fixed with pure methyl alcohol (CH<sub>3</sub>OH) for 3-5 min and again allowed to dry. Giemsa, azur- eosin- methylene blue was diluted with buffer solution (pH 6.8) in the ratio of 1:7, poured over the film and kept for 45–60 min. The slide was flushed in gentle flow of tap water till the stain stopped running, dried in upright position in a slide rack and examined at 40X and 100X power objective using oil immersion under LEICA-DMLB photo automat or OLYMPUS BX-21 microscope to confirm the presence of parasites.

## Post mortem examination

Subsequent to blood examination, rats were sacrificed for observing helminth infection. Rats were anaesthetized in mild chloroform by holding them in glass jars till they were unconscious and failed to respond to touch stimuli. They were then sacrificed and their organs examined for parasites.

## Weighing/measuring rats and organs

The length (cm) of rats was recorded by a linear scale; rats were weighed (g) on Docbel Braun common balance (capacity 1 kg). After dissection, the fresh liver and spleen were weighed (g) in Adair Dutt electronic single pan balance (sensitivity 0.001 g).



#### Helminth examination

C. fasciolaris were observed as white creamish cysts on rat liver. The infected liver was removed and the parasite carefully extracted with the help of needle and brush and collected in the normal saline. The cyst was opened with a brush and the parasites collected under a stereoscopic microscope. They were fixed in 10 % formalin, pressed and stained in Borax Carmine as per routine techniques.

## Data analysis

The prevalence and intensity were calculated (Bush et al. 1997). The condition factor, hepatosomatic indices and spleensomatic indices were calculated according to the following formulae:

Prevalence 
$$\% = \frac{\text{Total no. of infected rats} \times 100}{\text{No. of rats examined}}$$

Mean Intensity = T. lewisi: Total no. of parasites/1000 RBC C. fasciolaris: No. of parasites/ host

$$Condition \ factor \ (K) \ = \frac{Weight \, (gm)}{Length^3 (cm)}$$

$$HSI = \frac{Fresh \text{ weight of liver } (gm) \times 100}{Fresh \text{ body weight } (gm)}$$

SSI = 
$$\frac{\text{Fresh weight of spleen (gm)} \times 100}{\text{Fresh body weight (gm)}}$$

# Statistical analysis

The group mean  $\pm$  SE (standard error) was calculated for each analyte and value of significance (P values) were calculated by SPSS software. Data obtained was analyzed using analysis of variance (ANOVA) to determine value of significances. Values of P < 0.05 were considered to be statistically significant.

## Results and discussion

The blood parasite discovered from *R. rattus* was identified as *T. lewisi* Kent, 1880 (Hoare 1972) and the cestode as *C. fasciolaris* (larval *Taenia taeniformis*) (Yamaguti 1959).

# Parasite profile

Type host R. rattus Linnaeus, 1758

Type locality Rohilkhand region, Bareilly (U.P.), India

(28.35°N; 79.42°E)

Prevalence 12.40 % (*T. lewisi*), 46.70 % (*C. fasciolaris*) Intensity 14 parasites/1000 RBC (*T. lewisi*), 2–4 parasites/host (*C. fasciolaris*)

Biometrical data and analyses of condition factor and organosomatic indices are given in Table 1.

## **Condition factor**

 $T.\ lewisi$ -infected (Group Ib) rats showed insignificant (P>0.05) change in the values of condition factor as compared to control rats (Group Ia). Values of  $T.\ lewisi$  infected rats fell by 5.40 % in weight category A. Similarly, rats of weight category B showed a 3.03 % fall in Group Ib whereas, rats of weight category C (Group Ib) were infection- free. Rats belonging to weight category A of Group Ic ( $C.\ fasciolaris$ -infected rats) showed a significant (P>0.05) change in the value of condition factor: showing the greatest fall (21.62 %), weight categories B and C of  $C.\ fasciolaris$ -infected rats being 6.06 and 11.42 % respectively. Rats of weight category B and C of Group Id showed a similar trend [12.12 and 14.28 % fall respectively (significant P<0.05)] whereas, weight category A (Group Id) was infection-free.

T. lewisi infected pregnant rats (Group IIb) showed decreased value of condition factor as compared to infection-free pregnant rats (Group IIa). Weight categories B and C (Group IIb) showed 2.70 and 2.94 % fall respectively whereas, weight category A (Group IIb) was free from infection. In Group IIc, only rats belonging to weight category B were infected showing 16.21 % fall. In Group IId rat of weight category C showed 11.76 % fall (values shown in Table I; percent change Fig. 1).

Parasites presented significant negative correlation between their abundance and the relative condition factor of the host. Group Ib hosts, presented K values that were lower than those of the nonparasitized rats in weight categories A and B. In *C. fasciolaris* infestation (Group Ic), K factor values lowered in infected rats as compared to uninfected rats of all 3 weight categories. Similarly, in Group Id, decreased values of K factor were noticed in weight categories B and C.

K-factor is a quantitative indicator of fish fitness, reflecting recent feeding conditions and is obtained using the weight-length relation of the individual (Le Cren 1951). As the relative K-factor considers expected weight and observed weight, events involving reproduction or construction of the gonads are minimized if the relation is equal to one (1) under normal conditions. Any alteration (influenced by environmental change, lack of food or even parasitism) to this relation causes variations in this calculation. Accordingly, the factor is totally dependent on the



Table 1 K-factor, HSI and SSI under normal and parasitized conditions

	Weight categories#	Group I*	Group I*				Group II*			
		Ia	Ib	Ic	Id	IIa	IIb	IIc	IId	
K factor	A	$0.037 \pm 0.004$	$0.035 \pm 0.0001$	$0.029 \pm 0.001$	_	_	_	_	_	
	В	$0.033 \pm 0.001$	$0.032 \pm 0.001$	$0.031 \pm 0.0006$	$0.029 \pm 0.005$	$0.037 \pm 0.007$	0.036	$0.031 \pm 0.007$	_	
	C	$0.035 \pm 0.001$	_	$0.031 \pm 0.0008$	0.030	$0.034\pm0.0005$	0.033	_	0.030	
HSI	A	$4.580 \pm 0.160$	$4.610 \pm 0.260$	$4.670 \pm 0.260$	_	_	-	_	_	
	В	$4.020 \pm 0.060$	$4.120 \pm 0.200$	$4.290 \pm 0.070$	$4.730 \pm 0.550$	$3.520 \pm 0.530$	3.670	$3.810 \pm 0.610$	_	
	C	$3.570 \pm 0.070$	_	$3.760 \pm 0.070$	3.820	$3.530 \pm 0.170$	3.770	_	3.880	
SSI	A	$0.510 \pm 0.030$	$0.540 \pm 0.004$	$0.550 \pm 0.020$	_	_	-	_	-	
	В	$0.480 \pm 0.010$	$0.500 \pm 0.020$	$0.530 \pm 0.020$	$0.570 \pm 0.010$	$0.530 \pm 0.010$	0.580	$0.600 \pm 0.040$	_	
	C	$0.470 \pm 0.010$	_	$0.480 \pm 0.020$	0.630	$0.440 \pm 0.030$	0.490	_	0.660	

<sup>\*</sup> Group Ia Uninfected rats, Group IbT. lewisi infected rats, Group Ic Cestode (C. fasciolaris) infected rats, Group Id T. lewisi and cestode (C. fasciolaris) infected rats, Group IIa Uninfected pregnant rats, Group IIb T. lewisi infected pregnant rats, Group IIc Cestode (C. fasciolaris) infected pregnant rats, Group IId T. lewisi and cestode (C. fasciolaris) infected pregnant rats

<sup>&</sup>lt;sup>#</sup> A 50–100 g, B 100–150 g, C 150–200 g

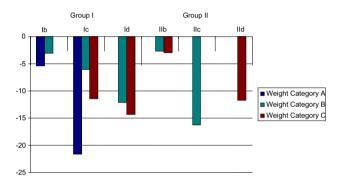


Fig. 1 Percentage fall in K-factor of parasitized rats. *Group I* non pregnant *Group II* Pregnant a Uninfected, b T. lewisi infected, c C. fasciolaris infected, d T. lewisi + C. fasciolaris infected (groups are same in all figures)

length and weight of analyzed animals (Verani et al.1997). Due to this, the relative K-factor defined by Le Cren (Le Cren 1951) and applied by Eckmann (Eckmann 1984) was chosen to evaluate the influence of parasitism in the general health state of the specimens studied. Measurements and standard deviation of K give a better foundation for statistical comparisons than tests comparing a and b values of weight/length relationship.

There is controversy over the host-parasite relationship. According to Bauer 1970, the pathogenic activity of parasites necessarily affects host condition in a negative way. A negative correlation between parasitism intensity and the K-factor in juveniles of this host was established in bluegill (Lemly 1980). Data using the same age and length classes indicated that the condition factor and weight can be used to quantify the effects of parasites in small fish. A negative correlation has been seen between disease and host condition (Moller 1985). Similarly, in the present findings, rats of all the weight categories of Group I and II, showed

negative correlation with the K-factor of nonparasitized rats. The parasitized fish presented a relative K-factor significantly higher than the non parasitized fish (Lizama et al. 2006). This was confirmed when the species were analyzed separately, where Rhinonastes pseudocapsaloideum, Saccocoelioides magnorchis and Gamispatulus sp. presented significant positive correlation between K-factor and abundance of parasitism. Considering that parasites are pathogenic, a negative correlation was expected. However, parasitic abundance was relatively low in *Prochilodus lineatus* in relation to other species. Thus, the largest individuals with the highest K-factor tolerate higher levels of parasitism and this was further supported (Cone 1995). A reduction in the relative K-factor occurred in fish infected with Ichthyophthirius multifiliis (Kurovskaya and Osadchaya 1993) whereas endoparasites of Prochilodus scrofa (P. lineatus) from the Upper Parana river floodplain, showed no significant differences between parasitized and non parasitized fish (Takemoto et al. 1998).

With regards to K-factor, it has been shown that total weight was close to that expected for each standard length in fish implicating that fish parasitized by different groups of parasites do not show any condition change (Ranzani-Paiva et al. 2000) which were contrary in *Plagioscion squamosissimus* infected by metacercariae of *Diplostomum* (Austrodiplostomum) compactum (Silva-Souza 1998). K-factor formulas used in assessing the state of fish health were also developed (Morton and Routledge 2006).

# **Hepatosomatic index (HSI)**

All the weight categories of Group Ib and Ic showed an insignificant (P > 0.05) rise in the HSI values as compared to control rats (Group Ia); weight categories A and B of



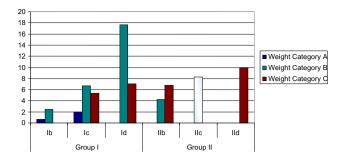


Fig. 2 Percentage rise in HSI of parasitized rats

Group Ib showed 0.65 and 2.48 % rise respectively whereas weight category C was infection-free. The rise in rats of Ic was 1.96 % (Group A), 6.71 % (Group B) and 5.32 % (Group C). Rats of weight category B of Group Id showed a significant (P < 0.05) rise (17.66 %) and that of category C, showed 7.00 % rise.

Pregnant *T. lewisi* parasitized rats (Group IIb) showed an increase (4.26 % Group B; 6.79 % Group C). In Group IIc, rats of category A and C were infection free, those of category B, showed 8.23 % rise, rat (weight category C) showed 9.91 % rise (values shown in Table 1, percent change, Fig. 2).

The liver is formed of hematopoietic tissues (Matushima 1995) plays a major role in metabolism and performs the functions of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification (Lagler et al.1977). It produces bile which emulsifies lipids during digestion. The highly specialized tissues of liver regulate a variety of high volume biochemical reactions many of which are necessary for normal vital functions of the body. Therefore, infection of the organ by the parasites may play a crucial role in host metabolism. HSI and SSI are regarded to establish ponderal relationships between the organs and the entire body. In some parasitic infections, an increase in liver volume can occur, with low levels of glycogen (Quentel and Obach 1992) and possible alterations to erythropoietic and leucopoietic tissues (Lizama et al. 2006).

Hepatosomatic index (HSI) is the weight of the liver expressed as a percentage of total body weight (also known as liversomatic index). One of the earliest finding dealing with hepatosomatic relation was reported in young perch (*Perca fluviatilis*) parasitized by cestodes (Kuperman 1973). Significant differences were observed only between the dimensions and host weight in massive infections, where parasite weight was between 20 and 60 % of liver weight. Similarly, in the present study, comparatively higher values of hepatosomatic index were recorded in *C. fasciolaris* infected rats (Group Ic) belonging to weight categories B and C and in weight categories B and C rats having dual infection (Group Id). Alterations to the

hepatosomatic indices was also recorded in infestations by *Cryptobia salmosistica* (Lowe-Jinde 1980).

Fishes infected by *Ichthyophthirius multifiliis* showed reduction in their liver weight (Kurovskaya and Osadchaya 1993). The hepatosomatic and splenosomatic relations in intensely cultivated fish were analyzed showing that correlation existed between liver and spleen weight and body weight, and liver weight and healthy host length (Tavares-Dias et al. 2000a).

Insignificant correlation was observed between the hepatosomatic index of uninfected rats and weight category A and B of T. lewisi -infected (Group Ib) rats and weight category A of rats infected with C. fasciolaris (Group Ic). Findings were reported where significant differences in the HSI for Oreochromis niloticus, Leporinus macrocephalus and the hybrid "tambacu" in parasitized and non parasitized hosts were not recorded (Tavares-Dias et al. 2000b). Only *Piaractus mesopotamicus* presented lower HSI values in parasitized hosts. For *P. lineatus*, significant correlations between the HSI and parasitic abundance were not observed either. In the present findings, rats of Group Id (weight category B) showed the maximum values of HSI suggestive of hepatomegaly perhaps due to the coupling effect of C. fasciolaris present in the liver itself and T. lewisi present in the blood. This may be due to the reduction in glycogen content and probable alterations to erythropoietic and leucopoietic tissues as proposed elsewhere (Quentel and Obach 1992).

# **Spleensomatic index (SSI)**

The findings of SSI of Groups Ib and Ic were similar to those of HSI, both showing an insignificant rise (P > 0.05). The SSI of Group Ib rats of weight categories A and B showed 5.88 and 4.16 % rise respectively. Weight categories A, B and C of Group Ic indicated 7.84, 10.41 and 2.12 % rise respectively. A significant (P < 0.05) rise in SSI was recorded in rats belonging to weight categories B and C (18.75 and 34.04 % respectively) of groups Id and weight category C of Group IId (50 % rise), the latter being the peak change observed in all values during the present experimentation. Weight categories B and C of Group IIb showed 9.43 and 11.36 % rise. Weight category B rat of Group IIc showed 13.20 % rise in SSI value (values shown in Table 1, percent change, Fig. 3).

The spleen primarily acts as a blood filter. It plays important roles in regard to red blood cells and the immune system. It eliminates RBC's and holds a reserve of blood in case of hemorrhagic shock and recycles iron. As part of the mononuclear phagocyte system (MPS), it metabolizes haemoglobin from senescent erythrocytes. Antibodies are synthesized in the white pulp and remove antibody coated



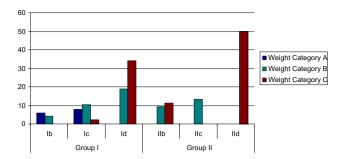


Fig. 3 Percentage rise in SSI of parasitized rats

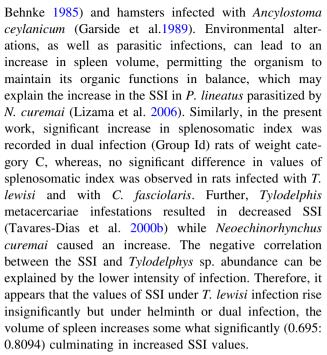
pathogens along with antibody coated blood cells by way of blood and lymph node circulation. It is one of the centers of activity of the RE system and is regarded analogous to a large lymph node.

Increase in SSI was recorded in rats infected with *T. lewisi* (Group Ib) in weight categories A and B, *C. fasciolaris* infected (Group Ic) rats of weight categories A, B and C, as compared to uninfected ones. Rats having dual infection (Group Id) showed comparatively higher values of SSI in weight categories B and C.

SplenoSomatic index is the weight of the spleen expressed as a percentage of total body weight. Alterations in this index could indicate an abnormal condition in the spleen such as necrosis or swelling due to infection (Goede and Barton 1990). Spleen size is considered to be a useful diagnostic factor as it is an important hematopoietic and leucopoietic organ (Anderson 1990) thus serving as an immune system organ. It plays an important role in production in lymphocytes that are involved in fighting infection (John 1994). Immune investment varies by sex (Klein 2004); this may be related to pregnancy (Roberts et al. 1996), a reason why we chose females only for both groups, I and II to make comparison more viable. The importance of the spleen to vertebrate immunity is reflected by more intense infections with various parasite taxa in both splenectomized (Hillgarth and Wingfield 1997) and splenomegalous individuals of various birds and mammals species in the lab (Kristan and Hammond 2000). The relationship between splenic mass and parasitism in wild population has not been studied too well (de Bellocq et al. 2007). It has also been tested whether splenic mass of masked shrews, Sorex cinereus is related to intensity of bladder nematode, Liniscus (= Capillaria) maseri infection (Cowan et al. 2009).

Alterations in related spleen size could signal a dysfunction and have effects at the whole organism level. SSI has not been as extensively studied as HSI but certain endogenous and exogenous factors are known to affect it. An increased SSI appears to be linked to a diseased state.

Splenomegaly is known to persist for several weeks in mice infected with *Nematospiroides dubias* (Ali and



When the results of above findings were summarized it was found that the small sized rats showed a greater fall in the K-factor as evident in T. lewisi infected rats (Group Ib). Moreover, under dual infection of T. lewisi and C. fasciolaris, the fall in K-factor was higher in weight category C (Groups Ic, Id, IIb) indicating that the growth of the large rats was being affected. On the other hand, the changes in HSI values in Group Ib were insignificant but those under cestode and dual infections were suggestive of hepatomegaly as depicted by their HSI values. Here, in Groups Ic and Id, rats of the growing age (weight category B) showed greater hepatomegaly as compared to weight category C. In pregnant rats, it was interesting to note that in weight categories B and C, under T. lewisi -infectivity, C. fasciolaris-infectivity and dual infectivity, there was a gradual increase in hepatomegaly. It was further observed that the SSI values also increased under infectivity with respect to the different age groups, the older rats were more susceptible to splenomegaly showing a maximum increase of 50 % in pregnant rats having dual infection (Weight Category C, Group IId). The relationship between splenic mass and parasitism in wild populations has not been much studied (de Bellocq et al. 2007). It has also been observed by other authors that splenomegaly is a manifestation of helminth infections (Ali and Behnke 1985, Garside et al. 1989). The present investigation also indicates splenomegaly in infected hosts. The cause for splenomegaly observed in the present case is presumed to develop in association with the parasitic infection and may result from an increase in the defense activities of the organ. The demand for increased antigen clearance from the blood may lead to increased numbers of reticuloendothelial (RE)



cells in the spleen and stimulate accelerated antibody production with resultant lymphoid hyperplasia. The literature on similar studies on protozoan infectivity are less documented and the present findings also do not show any conclusive evident on HSI and SSI values of T. lewisi infected rats, however the findings fall on similar lines as observed by earlier authors with respect to helminth infection. From the above studies, it is apparent that condition factor, HSI, and SSI can be regarded as useful indicators of parasitic infection with reference to the two parasites investigated, T. lewisi and C. fasciolaris. Hepatomegaly and splenomegaly have been correlated in congenic strain of BIO.LP male mice experimentally infected with Leishmania donovani 3S strain from the spleen of a hamster donor (Premvati 1979). It is recommended that the study protocol must be consistent and conservative in terms of sampling particularly since HSI and SSI can be altered within minutes by capture and holding stress. The decrease in condition factor can be considered to be a reflection of depletion in energy reserves (Goede and Barton 1990) as the index is positively related to total muscle and liver energy content (Lambert and Dutil 1997). The increase in HSI maybe linked to pathological damage (Hinton and Lauren 1990) caused by the parasites.

Investigations on K-factor, HSI and SSI of female parasitized (*T. lewisi*and *C. fasciolaris*) rats indicated that growth of young rats (Weight category A) was more affected, hepatomegaly and splenomegaly were frequent outcomes of parasitic infection, hosts with dual infection showing greater significant changes.

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