

## THE INFLUENCE OF THE REGIONAL BASIC DIET FROM NORTHEAST BRAZIL ON HEALTH AND NUTRITIONAL CONDITIONS OF MICE INFECTED WITH *SCHISTOSOMA* *MANSONI*.

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*Protein nutritional status indicators were studied in weanling albino Swiss mice infected with S. mansoni and fed the Regional Basic Diet (RBD) from Northeast Brazil, a multideficient diet of low-protein content. Each mouse was infected percutaneously with 80 cercariae. The experiment lasted 63 days. The growth curve, food consumption, protein intake, weight gain, Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) were the parameters investigated. RBD-fed mice showed a marked weight loss, a lower food and protein intake, a slower body weight gain and lower rates of food protein utilization when compared to casein-fed animals. Differences between infected and non-infected mice were not consistent. The present results suggest that the effects of RBD-induced malnutrition on health and nutritional conditions of the mice are more severe than those of Manson's schistosomiasis, in the initial phase of the disease.*

*Key-words:* Schistosoma mansoni. Mice. Malnutrition. Protein.

Malnutrition areas and major *Schistosoma mansoni* endemic foci overlap considerably in Northeast Brazil<sup>9</sup>. Multiple deficiencies, mainly of the calorie-protein type, have been detected in the population living in these areas<sup>5 8 9 10 12 14 23</sup>. Based on food habits of this population, a definition was possible for the Regional Basic Diet (RBD), a food blend of low protein content (kidney beans-*Phaseolus vulgaris*, manioc flour-*Manihot esculenta*, salted and dried meat-"charque" and sweet potatoes-*Iponoea batatas*), which reproduces dietary deficiencies prevalent in the region<sup>9 11 32</sup>. To manioc flour and sweet potatoes have been ascribed the amino acid imbalance and further decrease in the protein content of this multideficient diet.

A type of malnutrition rather similar to the marasmatic form of calorie-protein malnutrition seen in humans, which is quite common in Northeast Brazil, has been induced in weanling mice<sup>9 11</sup> and

rats<sup>21 32</sup> fed RBD. In addition to growth disturbances<sup>9 11 21 32</sup>, RBD intake results in a deficient glycemic regulation<sup>33</sup>, a reduction in the sciatic nerve conduction velocity<sup>28</sup> and impaired propagation of spreading depression in the cerebral cortex of Sprague-Dawley rats<sup>19</sup>.

For further information on the effects of RBD on the initial phase of experimental mansonic schistosomiasis in albino mice, particularly on the protein nutritional status of the host, biological assays (Protein Efficiency ratio-PER and Net protein Ratio-NPR) were performed to determine the utilization of dietary protein. In addition, some other indicators of protein metabolism<sup>25</sup>, such as the weight gain, growth curve, food consumption and protein intake were investigated.

The proposed experimental model reproduces the relationship between undernourished host and parasite infection as it is found among Brazilian populations living in schistosomiasis endemic areas. The experiment was designed: 1) to study the effects on the host, of malnutrition induced by RBD intake; 2) to detect, by using some indicators of protein nutritional status, the influence of this diet on protein utilization in the body of mice infected with *S. mansoni* as compared to non-infected controls.

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Recebido para publicação em 19/08/91.

## MATERIALS AND METHODS

### Diets

The Regional Basic Diet (RBD) is based on human staple foods usually ingested in Northeast Brazil (kidney beans-*Phaseolus vulgaris*, manioc flour-*Manihot esculenta*, salted and dried meat and sweet potato-*Iponaea batatas*). It has the following composition (g/100 g): kidney beans (proteins-3.96, carbohydrates-10.66, fats-0.24, minerals-0.57, fibers-1.09); manioc flour (proteins-0.82, carbohydrates-48.59, fats-0.12, minerals-0.43), fibers-5.64); "charque" (proteins-2.74, carbohydrates-0.43, fats-0.21, minerals-0.06); fat from "charque"(fat-0.35); sweet potato (proteins-0.30, carbohydrates-9.99, fats-0.03, minerals-0.20, fibers-0.48). This food blend contains, per 100 g, 7.82% proteins, 69.67% carbohydrates, 0.95% fats and 1.26% fibers. Both control diets contained casein and had different total protein levels: 7.82% (control diet I) and 22.60% (control diet II).

The control diet I had the following composition (g%): casein (protein-7.06), soybean oil (fat 8.00), corn starch (protein-7.76, carbohydrate-74.37), cellulose (fibers-3.50).

The control diet II had the following composition (g%): casein (protein-22.09), soybean oil (fat-13.00), corn starch (protein-0.51, carbohydrate-50.19), cellulose (fibers-4.00).

Both control diets were supplemented with mineral salts (2.50 g%) and vitamin mixtures (1 g% water-soluble and 1 g% fat-soluble vitamins), according to Tagle & Donoso<sup>31</sup>. All these diets were given in pellet form.

The non-protein diet, used in the Net Protein Ratio biological assay (NPR), was composed as follows (g%): soybean oil (fat-8.00), mineral salts (4.00), fat-soluble vitamin mixture (1.00), water-soluble vitamin mixture (1.00), corn starch (protein-0.82, carbohydrates-72.03, fat-0.16, mineral salts-0.12), cellulose (fibers-4.00). It was given during 10 days to a group of 10 animals used as controls for that assay.

### Experimental animals

A total of 70 albino Swiss mice were used. Twenty male and female animals were fed each diet and divided into infected and non-infected subgroups

of 10 mice each. An additional group of 10 non-infected mice was given a non-protein diet and was used as control for the Net Protein Ratio biological assay.

### Infection

A *S. mansoni* strain isolated from São Lourenço da Mata (Pernambuco State, Brazil) and maintained regularly in our laboratory was used. Each animal was infected percutaneously with 80 cercariae shed from *Biomphalaria glabrata* reared and infected in the laboratory (infections up to 100 to 150 cercariae per mouse are well tolerated<sup>24 34</sup>).

### Experimental protocol

Twenty-one-day old weanling mice weighing 9 to 12 g were kept in individual wire bottom cages. Water and diets were provided "ad libitum". The body weight was determined weekly and the food intake was recorded daily. The experiment lasted 63 days.

Subgroups of 5 mice per control diet were submitted to a pair-feeding trial along the first 28 days of the experiment. So, the daily food intake of control mice (diets I and II) was restricted to the "ad libitum" intake of RBD-fed animals.

The post-weanling rapid growth period of the mouse was observed during the first 28 days of the investigation (Phase I). Henceforth, the *S. mansoni* oviposition and maturation of hepatic and intestinal lesions were investigated (Phase II).

### Indicators of the protein nutritional status

The weight curve, total food consumption, protein intake, weight gain, Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) values were the parameters studied. PER is a biological assay devised to determine the grams gain per gram protein consumed, while NPR measures the weight loss of a negative control group fed a non-protein diet, added to the weight gain of the experimental group (RBD-fed mice) and divided by the protein consumed by the latter<sup>22</sup>.

### Statistical analysis

The analysis of variance and Student's "t" test

were used, proportions being compared by normal approximation. The probability level considered significant was  $p < 0.05$ .

## RESULTS

### Weight curves

In general, the weight curve values for infected and non-infected animals fed casein diets were higher than those fed RBD (Figure 1). As expected, the highest values were achieved by mice fed the 22.60% casein diet. The weight of the infected RBD animals decreased in Phase II of the experiment, but this reduction was less apparent in casein-fed infected animals. Also, the mean body weight of RBD non-infected mice was lower than that of animals fed casein diets.

### Food intake

The *S. mansoni* infection resulted in a significant reduction in RBD intake. Regarding the casein-fed groups, however, data were non-significant (Table 1).

### Protein intake

Regarding protein intake, both infected and non-infected mice fed RBD showed a significant

difference ( $p < 0.001$ ) when compared to the other groups, the lowest values for the infected ones. A comparison among the groups showed that: a) protein intake was always higher for the animals fed the 22.60% casein diet; b) during the overall experimental period significant differences were detected between the low-protein fed infected and non-infected animals and those fed the 22.60% casein diet (Figure 2).

### Weight gain

The mean body weight gain was significantly and consistently higher for the non-infected RBD-fed group, when compared to infected mice under the same diet. The infection, however, did not seem to affect in the same way the weight gain of the casein-fed groups. A comparison among the different groups showed that the highest values for body weight gains were found in casein-fed mice, particularly in the 22.60% casein group, irrespective of the presence of schistosome infection.

Body weight gains of infected mice in relation to the non-infected ones taken as controls (i.e., ascribing to these a 100% body weight gain level line), under the three diets, are seen in Figure 3. At the end of the experiment, body weight gains of the infected mice were 6.39%, 62.52% and 87.25% (RBD, 7.82% casein and 22.60% casein groups, respectively). Again, a comparison among the groups

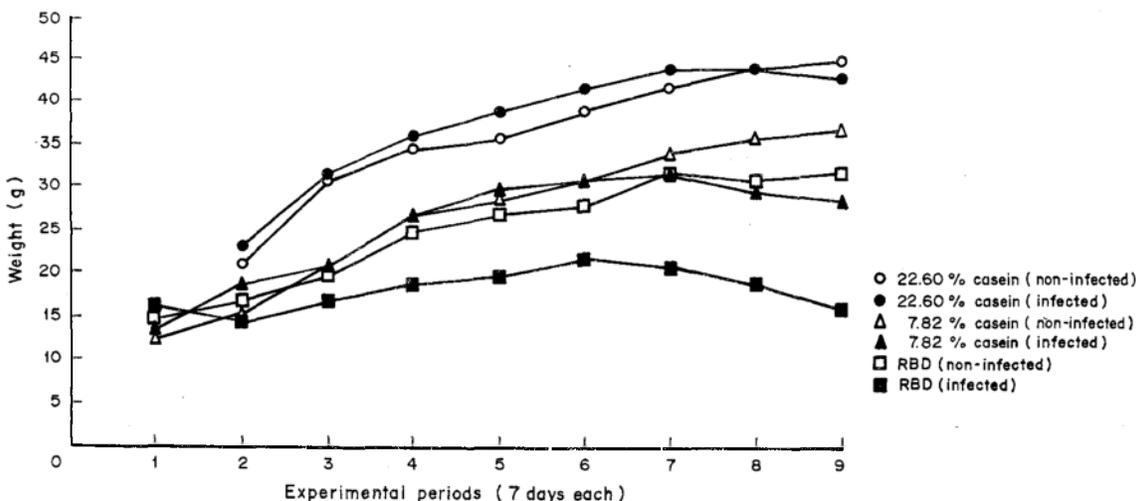


Figure 1 - Weight curves of infected and non-infected mice fed RBD and Casein diets.

Table 1 - Mean food intake(g) of infected and non-infected mice fed RBD and Casein diets.

Diets and Animal subgroups	Phase I ( $\bar{X} \pm \text{SEM}$ )	Phase II ( $\bar{X} \pm \text{SEM}$ )	Overall experimental period ( $\bar{X} \pm \text{SEM}$ )
RBD (infected)	89.15±9.31	88.94±7.97	191.99±21.54
RBD (non-infected)	114.60±4.29	141.00±8.16	257.01±10.68
	Significant (p < 0.05)	Significant (p < 0.0001)	Significant (p < 0.02)
7.82% casein (infected)	107.05±6.87	135.35±11.53	242.40±11.95
7.82% casein (non-infected)	94.72±7.26	172.91±11.62	270.23±14.57
	Non-significant	Significant (p < 0.05)	Non-significant
22.60% casein (infected)	121.68±7.73	125.55±7.29	243.83±13.61
22.60% casein (non-infected)	108.46±7.36	138.20±3.75	240.84±12.77
	Non-significant	Non-significant	Non-significant

$\bar{X}$  = arithmetic mean

SEM = standard error of the mean

RBD = Regional Basic Diet

Phase I = The first 28 days after weaning.

Phase II = From the 29th day to the end of the trial

Table 2 - Proteins efficiency ratio of infected and non-infected mice fed the Regional Basic Diet and Casein diets (controls)\*.

Diets and Animal subgroups	PER ( $\bar{X} \pm \text{SEM}$ )	Student's "t" test
RBD (infected)	0.59±0.08	Significant (p < 0.001)
x 7.82% casein (infected)	1.64±0.08	
RBD (infected)	0.59±0.08	Significant (p < 0.001)
x 7.82% casein (non-infected)	1.93±0.08	
RBD (non-infected)	1.04±0.08	Significant (p < 0.005)
x 7.82% casein (non-infected)	1.93±0.23	

$\bar{X}$  = arithmetic mean

SEM = standard error of the mean

RBD = Regional Basic Diet

PER = protein efficiency ratio biological assay

(\*) This assay was not performed for the subgroup fed 22.60% casein diet because a protein content lower than 10% is required.

showed that the infected RBD-fed mice had the lowest values for the body weight gain. Pair-fed animals also showed significantly (p < 0.01) lower body weight gains in RBD-fed mice.

#### Indicators of food protein utilization

PER was determined for the two low-protein diets (RBD and control diet I), both with a protein

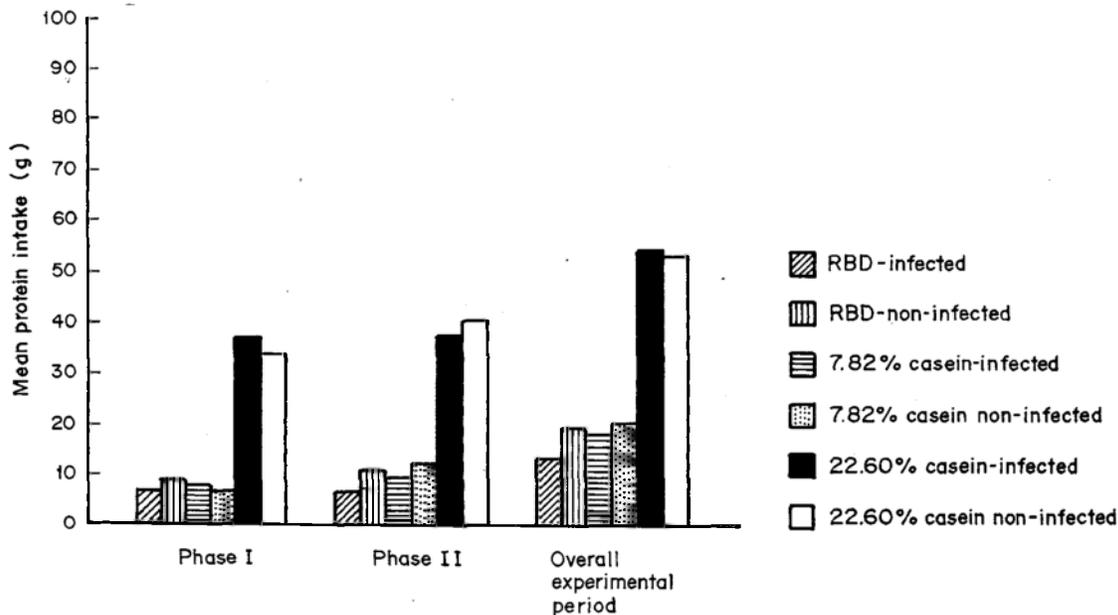


Figure 2 - Mean protein intake of infected and non-infected mice fed RBD and Casein diets.

Table 3 - Net Protein Ratio of infected and non-infected mice fed the Regional Basic Diet and Casein diets\*.

Diets and Animal subgroups	NPR (X ± SEM)	Student's "t" test
RBD (infected)	1.68 ± 0.23	
RBD (non-infected)	2.08 ± 0.17	Non-significant (p > 0.05)
7.82% casein (infected)	3.99 ± 0.52	
7.82% casein (non-infected)	4.90 ± 0.50	Non-significant (p > 0.05)

X = arithmetic mean  
SEM = standard error of the mean

RBD = Regional Basic Diet  
NPR = net protein ratio biological assay

(\*). This assay was not performed for the subgroup fed 22.60% casein diet because a protein content lower than 10% is required.

level below 10% as required for this biological assay. Only RBD groups showed a significant difference regarding PER values ( $p < 0.002$ ) between infected and non-infected mice. When the groups were compared to each other, again the lowest values were detected in the RBD-fed mice, particularly when *S. mansoni* infection was associated (Table 2). NPR values were apparently

higher for the non-infected mice. However, results were not statistically significant (Table 3).

## DISCUSSION

Although over the past 35 years significant relationships between schistosomiasis and nutritional

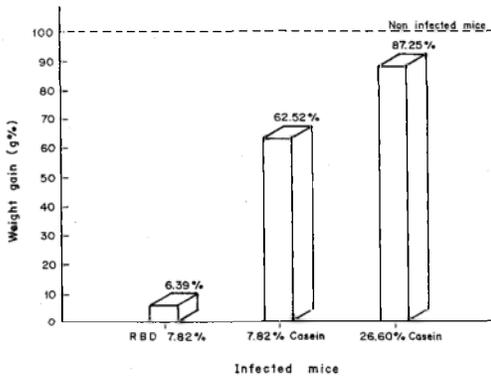


Figure 3 - Weight gain of infected and non-infected mice fed RBD and Casein diets.

status have been reported, the public health importance of these findings in schistosomiasis-infected communities is still unclear<sup>30</sup>. On the other hand, these investigations are important in estimating to what extent a community may benefit from the control of schistosomiasis *per se* even if the nutritional situation remains unchanged<sup>6</sup>.

The lack of proteins is the principal nutritional problem in our days, being a constant finding among the populations of developing areas.

The nutritive value of several diets used by human populations has been assessed in experimental animals<sup>18 26 29</sup>. Although human and murine growth rates and nutritional needs can not be assumed to be at all closely related, the present experimental model seemed to us more adequate. Results obtained from experimental studies using purified or semi-synthetic diets fed to laboratory animals can hardly be extrapolated to man, because they do not reproduce the natural conditions to which human populations are exposed. Our diet (RBD), based on northeastern staple foodstuffs, is a multideficient diet with a low-protein content and is responsible for different nutritional deficiencies occurring in this region of the country. The essential and non-essential amino acid contents of RBD ranges from 16 to 44% (methionine and arginine, respectively) and from 10 to 81% (proline and cystine, respectively).

Clear cut alterations of protein metabolism have been described in man<sup>6 7 13 17</sup> and experimental animals<sup>3 15</sup> infected with *S. mansoni*, particularly in the hepato/splenic stage of the disease.

A decreased protein and lipid utilization was reported in infected mice and was ascribed to the altered hepatic function or to the acute intestinal inflammation seen in this experimental host<sup>16</sup>.

PER and NPR assays have been extensively used in evaluating the nutritive value of food proteins<sup>22</sup>. In the present investigation, these indicators were consistently lower in RBD-fed mice; infected animals presented the lowest values.

Clinical, epidemiological and experimental studies have suggested that deficiency states induced by malnutrition depress host defense mechanisms, aggravating the course of infectious and parasitic diseases, including schistosomiasis<sup>27</sup>. However, this has not been supported by some investigations in humans<sup>9 30</sup>. Some experiments suggest that the appearance of the clinical symptoms of schistosomiasis depends on the host's nutritional status<sup>1 2 3 4 20</sup>. According to previous findings, however, schistosome infection only contributes to aggravate malnutrition<sup>9 10 11</sup>.

In conclusion, the data presented in this paper indicate that, at least in the initial stage of the disease and under our experimental conditions, the harmful effects of RBD-induced malnutrition are more severe than those of Manson's schistosomiasis.

## RESUMO

Indicadores do estado nutricional de proteínas foram estudados em camundongos albinos suíços recém-desmamados e infectados com *S. mansoni*, aos quais foi administrada a Dieta Básica Regional (DBR) do Nordeste do Brasil. Cada camundongo foi infectado com 80 cercárias, por via percutânea. O experimento teve a duração de 63 dias. Os parâmetros investigados foram: evolução ponderal, consumo alimentar, ingestão protéica, ganho ponderal, Coeficiente de Eficiência Protéica (PER) e Coeficiente de Eficiência Protéica Líquida (NPR). Os camundongos alimentados com a DBR revelaram acentuada perda de peso, menor consumo alimentar e protéico, maior lentidão no ganho em peso relacionado com a fase de crescimento e taxas menos elevadas de utilização da proteína dietética, quando comparados com os controles (alimentados com dieta balanceada, à base de 22,60% de caseína). As diferenças encontradas entre camundongos infectados e não infectados, não foram consistentes. Os resultados sugerem que os efeitos provocados pela má-nutrição induzida pelo consumo da DBR são muito mais perniciosos para a saúde e estado

nutricional do hospedeiro, do que aqueles resultantes da infecção pelo *S. mansoni*, na fase inicial da doença.

Palavras-chaves: *Schistosoma mansoni*. Camundongos. Má-nutrição. Proteínas.

## ACKNOWLEDGEMENTS

We are indebted to Miss Lúgia Pedrosa for her critical review and Miss Ivanilda V. Nascimento for typing the manuscript.

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