THE RELATIONSHIP BETWEEN PROTEIN-CALORIE MALNUTRITION AND TRICHINOSIS: 1. STUDIES ON THE NUMBER OF INTESTINAL WORMS AND MUSCULAR LARVAE IN RATS FED LOW AND HIGH PROTEIN DIETS

SASTRI SAOWAKONTHA

Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok 4, Thailand.

INTRODUCTION

The relevance of interactions of nutrition and infection have been well documented (WHO, 1965; Scrimshaw *et al.*, 1968). Helminthic diseases been reported to interfere with the nutritional status of the host, e.g. food intake and food absorption (Venkatachalam and Patwardhan, 1953; Maegraith, 1967). On the other hand, alterations in the nutritional status of the host can affect the course of infestations (Platt and Heard, 1965). Foster and Cort (1932) and Giron Mendez (1963) experimentally showed that the numbers of *Ancylostoma caninum* and *Toxocara canis* in dogs were higher and more eggs were excreted by animals fed on diets with deficient protein or protein of low quality than by animals fed on diets with high protein or protein of good quality, respectively. There has been a report on the relationship between protein-calorie malnutrition and numbers of intestinal worms of *Trichinella spiralis* in mice (Kwan *et al.*, 1965). The present experiments were performed to study more extensively the effects of different levels of protein in diets on the numbers of intestinal worms and muscular larvae of *T. spiralis* in rats of different weights and ages, and the effect on the different intensity of infection.

MATERIALS AND METHODS

Weanling male black and white hooded Lister rats, weighing 40-60 gm were randomly divided into two groups. The rats in each group were fed *ad lib* on experimental low-and high-protein diets (Platt and Stewart, 1968) for 3 to 4 weeks before being infected with the infective stage of *T. spiralis* larvae. The preparation of the parasites and the method of infection were slightly modified from Nelson *et al.*, (1965) in which the incubating temperature was at 37°C for 5 hours. The studies were categorised into 3 experiments as follows:-

In Experiment I, rats of the same age were given 6,000 larvae per rat. The rats were serially killed after different periods of infection, 3 and 10 days after infection. No rats in the low protein infected group (LPI) survived after 10 days of infection, therefore only the intestinal worms were examined in both dietary groups.

In Experiment II, rats of the same weight were given 3,000 larvae per rat. In this experiment a lower dose was used and to prevent the low protein rats (LP) from receiving a relative overdose, the experiment was designed so that low and high protein rats would have the same body weight at the time of infection. Their ages, however, were necessarily different.

In Experiment III, rats of the same age were given 25 larvae per gram body weight. This experiment was attempted to standardise the dose rate and avoided using rats of different ages. Each rat received an infective dose based on its own body weight (25 larvae/gm body weight).

In the last two experiment (experiment II and III) both the intestines and three muscles (masseter, tongue and diaphram), were collected for counting the worms and larvae respectively at 4, 11, 21 and 32 days after infection. In trichinosis, the highest recovery of larvae is made from these muscles (Stryker, 1947; Forrester *et al.*, 1961 and Olsen *et al.*, 1964).

The worms and larvae in all experiments were expressed as total number per rat or number of worms per infective dose of 3,000 larvae, and total number of larvae per three muscles or total number or concentration of larvae present per infective dose of 1,000 larvae. The differences in the numbers of worms and larvae were tested by a non-parametric method, Mann-Whitney test (Snedecor and Cochran, 1967).

RESULTS

Experiment I : The LP rats gained significantly less weight than the high protein group (HP). From the beginning of the experiment until day 23, the day of infection, the LP rats had increased their weights by 109% and the HP rats by 218%. After infection the weight of the infected rats dropped sharply by about 34% and 24% of the LP and HP respectively by 10 days of infection (Fig. 1).

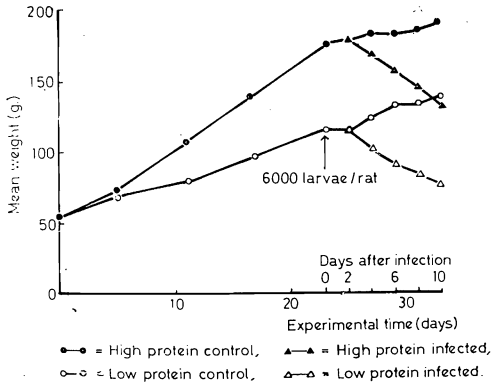

Fig. 1—The mean body weight of rats fed low and high protein diets before and after infection with 6,000 *Trichinella* larvae per rat.

The mortality rate of infected rats on both diets is shown in Table 1. It was found that the mortality rate of the LPI group was higher than the high protein infected group (HPI), especially after 10 days of infection and no rat in the LPI survived after 10 days of infection. Thus, this dose of larvae proved to be too heavy, particularly for the LP rats (Fig. 1). Consequently, this experiment represent only the intestinal phase.

Total number of worms was significantly higher in LPI than in HPI at day 10 after infection ($P < 0.01$) but not at day 3 after infection as shown in Table 2.

Experiment II : In 10 days in HP rats attained the weight which had taken the

Table 1

Mortality rate of rats infected with 6,000 larvae per rat (number that died from each infected group of 12 rats).

Protein diet	Days after infection			
	3	10	21	32
Low	1 (8.3)	2 (16.6)	12 (100)	-
High	0 (0)	1 (8.3)	1 (8.3)	3 (25.0)

Number in parentheses are percentages.

Table 2

Mean number of adult worms (± S.E.M.) in the intestines of rats fed low and high protein diets, expressed as number per rat (Exp. I and II.) and per infective dose of 3,000 larvae (Exp. III).

Experiment	Infective dose	days after infection	No. of rat	Low protein	No. of rat	High protein	P
I	6,000 larvae per rat	3	11	2,178±181	12	2,015±215	>0.05
		10	9	3,617±133	11	2,833±216	<0.01
II	3,000 larvae per rat	4	6	1,873±128	6	1,605±132	>0.05
		11	6	1,070±152	6	999±206	>0.05
		21	6	151± 70	6	5± 4	<0.01
		32	7	22± 18	6	0	0.05
III	25 larvae/gm body weight	4	6	2,239±124	6	2,215±143	>0.05
		11	6	1,899±123	6	1,785±178	>0.05
		21	6	207± 63	6	1	<0.01
		32	5	52± 43	5	0	<0.01

LP rats 30 days to achieve. The LPI group reach their maximum weight loss 12 days after infection (16.6% loss) compared with 6 days (2% loss) in the HP group. At day 30 after infection, the weight of the LPI rats differed from the low protein control (LPC) by 70 gm compared with a 30 gm difference between HPI and the high protein control (HPC). The difference between the weight of the LPC and HPC rats was only 10 gm. (Fig. 2).

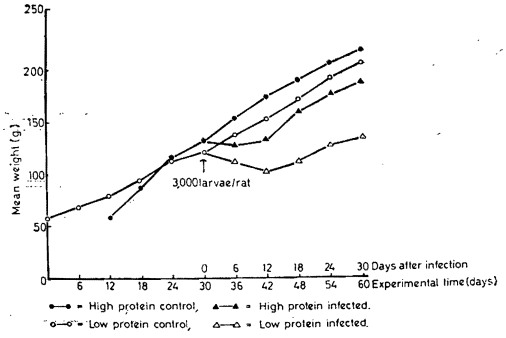

Fig. 2—Mean body weight of rats fed low and high protein diets before and after infection with 3,000 *Trichinella* larvae per rat, at the time when the rats in each dietary group were of approximately the same weight (120–130 gm).

At the infective dose (3,000 larvae per rat), the rats in both dietary groups survived throughout the experiment, except for one HP rat which died of an unknown cause before being infected.

Total number of intestinal worms were not different between LPI and HPI ($P > 0.1$) at day 4 and 11 but significantly higher in LPI than in HPI groups at day 21 and 32 after infection ($P < 0.01$ and $P = 0.05$) as shown in Table 2.

The total number of larvae were not significantly different between the diets at day 21 but day 32 the numbers were significantly higher in LPI than HPI groups ($P < 0.05$) as shown in Table 3.

Experiment III : The LP rats gained significantly less weight than the HP, during the first 23 days of the experiment; thereafter the growth rate of uninfected LP and HP rats was similar. After infection, the HPI animals did not lose weight but gained more slowly than the HPC group, whereas the LPI rats showed a slight loss of weight, the lowest being at day 12 after infection (6.4% loss) (Fig. 3). No rats died in this experiment.

Table 3

Mean number of muscular larvae (±S.E.M.) in three muscles (Tongue, masseter and diaphragm) of rats fed low and high protein diets, expressed as number per rat (Exp. II) and per infective dose of 1,000 larvae (Exp. III).

Experiment	Infective dose	Days after infection	No. of rat	Low protein	No. of rat	High protein	P
II	3,000 larvae per rat	21	6	31,074±5,096	5	29,601±3,280	>0.05
		32	6	77,422±6,232	6	56,762±5,749	<0.05
III	25 larvae/gm. body weight	21	6	13,694±584	6	10,772±1,292	>0.05
		32	5	41,867±5,345	5	33,431±3,171	>0.05

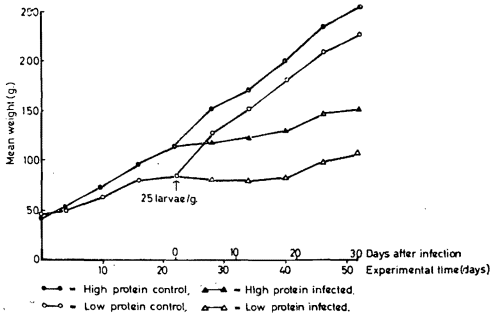

Fig. 3— Mean body weight of rats fed low protein and high protein diets before and after infection with 25 larvae /gm body weight.

Total number of worms per infective dose of 3,000 larvae were significantly higher in LPI than in HPI at day 21 but were not different at day 4, 11 and 32 after infection as shown in Table 2.

The number of muscular larvae per infective dose of 1,000 larvae were not found to differ between the diets on either day 21 or 32 after infection, although the mean values were higher in the LPI than the HPI groups as shown in Table 3.

DISCUSSION

Rats fed on low protein grew more slowly than those fed on high protein diets, but the differences between the two groups varied somewhat from one experiment to another, despite the fact that the starting weights and other conditions were kept the same in all experiments. There was a considerable difference in growth rates in the two diets in Experiment I (Fig. 1), whereas in Experiment II and III (Fig. 2 and Fig. 3), after an initial setback in growth in the low protein groups, subsequent growth rates were not significantly impaired. A low protein diet was therefore suboptimal rather than severely deficient with respect to protein for the weanling rats. With an *ad lib* feeding regime, slight differences in food consumption could make a considerable difference in growth performance.

The effects of infection on the growth rate of rats also varied. The weights of the infected rats on both low and high protein diets in Experiment I dropped equally steadily after infection. The infective dose of 6,000 larvae per rat proved to be too heavy. This dose amounted to about 52 larvae per gram body weight for the LPI rats, and 35 larvae per gram body weight for the HPI rats which would be regarded as severe to moderate doses (McCoy, 1931, 1932). The LPI rats did not survive more than 2 weeks after infection although the rate of weight loss was

similar in both dietary groups, the HP rats could stand this loss rather better than deficient rats which had a higher rate of mortality (Table 1). In Experiments II and III when either body weight or dose was adjusted to avoid overdosing the LP rats, the infection impaired the growth rate of the LPI groups slightly more than the HPI groups. However, in Experiment II, all rats in both dietary groups were of the same weight but different ages and all the rats survived up to 5 weeks after infection. The disadvantage of this procedure was that two different batches of rats were needed and the animals were of different ages which were not comparable due to differences in growth rate and biochemical maturation of the body. Therefore, Experiment III seemed appropriate where all rats were started on diets at the same time and age, and the dose was then adjusted to the body weight of the rats.

Castro and Olson, (1967) showed that the severity of weight loss after infection was proportional to the dose in that the greater the dose, the more severe the weight loss. The authors reported that infected guinea pigs looked miserable, with a staring coat, and were apathetic and diarrhoeic. Their food and water intake was also reduced. Rogers (1942), Platt and Heard (1965), and Orraca-Tetteh (1964) reported that the digestibility and utilization of protein diets were reduced in animals infected with *T. spiralis*, *T. canis* and *Nippostrongylus muris*. The impairment of protein utilization was more marked in animals fed on low protein that those fed on high protein diets (Platt and Heard, 1965; Orraca-Tetteh, 1964). These factors could account for the loss of weight of infected rats in the present experiments.

The three experiments showed higher numbers of intestinal worms in the LPI rats than in the HPI animals, particularly during the later stages of infection. In Experiment I the LPI rats showed higher total numbers of worms at day 10. These finding could have been due to low resistance of the LPI rats to the infection; the fact that the LPI rats received very much higher infective doses than the HPI animals; or the possibility that the low protein diet might have changed the intestinal flora, and hence made the biochemical environment more favourable to the worms (Haenel, 1970; Hentges, 1970). Increased worm numbers due to low protein diets (Kwan *et al.*, 1965) or to increased infective doses (Kozar and Kozar, 1965; Castro and Olson, 1967) have both been reported previously. The present work offers no evidence on changes in intestinal flora or environment, but in Experiments II and III attempts were made, by two different methods to standardise the dose, and thus test the contribution of the lower protein diet itself to increased worm numbers.

The effect of differences in doses of infection is worth mentioning here. If the same relatively heavy dose of infection is given to small and large animals (animals fed on low and high protein diets for a certain period), remarkable differences in numbers of worms may be expected between the two dietary groups as shown by Kwan *et al.*, (1965) and by Experiment I in the present work.

Although, the results were less dramatic with lower doses, than with high doses, the LPI rats still showed higher worm numbers particularly during late infection. The main effect of the low protein diet was to delay the passage of worms down the gut and therefore to delay expulsion. This would inevitably have resulted in more worms being found in the LPI rats than in the HPI rats during the later stages of infection.

Mean number of muscular larvae were higher in the LPI than in HPI rats (Experiments II, III) and the differences were statistically significant in Experiment II. The increased numbers of larvae found in the

three muscles of the LPI rats is in parallel with longer persistence of worms in the gut of these animals. No difference in the numbers of muscular larvae were found at day 21 after infection indicating that the penetration of the larvae into the muscle was not complete until about 32 days after infection.

SUMMARY

Infected rats on a low protein diet developed lower resistance to infection than rats on a high protein diet. For whatever reason, the intestines of the rats fed on a low protein diet provided a more favourable environment for *Trichinella spiralis* than those of animals fed a diet of high protein value, thus allowing the worms to live longer in the low protein animals, as demonstrated by increased numbers of intestinal worms and, ultimately of muscular larvae.

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