# Effect of Helminthiasis on Zinc Metabolism

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**ABSTRACT** : The effect of helminthiasis on zinc metabolism was monitored using endogenous $^{65}$Zn after intraperitoneal injection of 1 g of $^{65}$Zn as zinc chloride. In the first experiment zinc turnover was investigated in 18 male weanling rats, which were randomly divided into 3 groups. One group was infected with 73 third stage larvae of *Nippostrongylus brasiliensis* per gram body weight ; the other groups were the pair-fed and *ad lib*-fed controls. The route of loss of zinc was investigated in the second experiment with the same design using 18 animals with a lower dose of infection (33 larvae per gram body weight). The biological half life of endogenous $^{65}$Zn was lower ($p<0.05$) in the infected group as compared to the controls. In the later phase of infection (9th to 16th day) there was reduced retention of $^{65}$Zn and increased loss ($p<0.05$) of $^{65}$Zn from the body though urine and faeces. It was concluded that infection of *N. brasiliensis* was accompanied by increased loss of endogenous Zn through faeces and urine. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 276-279*)

**Key Words** : Helminthiasis, *Nippostrongylus brasiliensis*, Rats, Zinc, Feed Intake

## INTRODUCTION

The body has poor zinc (Zn) turnover because most of it is found in the bone, hair, skin and muscle, which cannot be broken down specifically to release Zn during deficiency. However at times of low dietary Zn supply, deficiency can be prevented by increased tissue breakdown as may occur in protein-calorie malnutrition (Gordon et al., 1982). Following tissue breakdown, the adventitious release of the element into the vascular compartment leads to increased Zn loss in urine (Fell et al., 1973). Zinc deficiency on the other hand may arise in disease conditions, which cause malabsorption or increased body loss of nutrients, especially plasma proteins, in protein loosing enteropathy (Aggett and Harries, 1979). Malnutrition leading to mobilisation of body reserves have been reported in cases of worm infection (Crompton, 1984; Caroline et al., 1985) which is likely to increase the plasma pool of Zn followed by adventitious loss of Zn in urine. This experiment was designed to investigate the effect of worm infection on Zn metabolism in the body.

## MATERIALS AND METHODS

### Zinc turn over

Eighteen male Rowett black hooded Lister strain rats were used. The weanling rats were randomly assigned to 3 groups, whereby those in group 1 and 3 were fed *ad libidum* and those in group 2 were pair-fed to those in group 1. They were housed individually in plastic metabolic cages. Feed offered and residues were determined every day. The animals were weighed every other day before offering fresh feed. The diet used was a semi-synthetic diet with spray dried egg albumin prepared as in the method described by Williams and Mills (1970). Zinc was added to give a marginal level of 12 ppm.

The radioactive zinc used was $^{65}$Zn as zinc chloride (Radiochemical Centre, Amersham, Bucks, England). A solution with a specific activity of 3.6 $\mu$ci/ml and 6.8 $\mu$g Zn/ml was prepared, by dilution using Hank's balanced salt solution. The rats were injected intraperitoneally with 150 $\mu$ml of the solution containing an activity of about 0.5 $\mu$ci and 1 g of Zn per animal. The amount of $^{65}$Zn retained in the body was monitored in a small whole body gamma counter (Nuclear Enterprise, Sighthill, Scotland). Five counts, each of ten seconds, were recorded for each rat and the mean, corrected for the background, was taken as $^{65}$Zn retained in the body. Changes in counting efficiency and decay of the radioisotope were adjusted by daily counting 150 $\mu$ml of the solution retained as a standard.

Third stage larvae (L3) were isolated with the Baermann technique from faecal charcoal mixture containing L3 of *Nippostrongylus brasiliensis* obtained from the Veterinary School, University of Glasgow. Five days after administering $^{65}$Zn, the rats were infected by a subcuteneous injection of 73 L3 per gram body weight. Those in treatments 2 and 3 were sham infected by injecting with Hank's balanced salt solution.

### Route of zinc loss

Eighteen rats were used in this study with the same design as in the first experiment. However, the animals were housed in metabolic cages where faeces and urine were collected separately. The animals were

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also infected with a lower dose of larvae (33 L3 per gram body weight) because of the severe infection noted in the first experiment. Faeces and urine were collected over different intervals (0 to 2nd ; 3rd to 5th ; 6th to 8th and 9th to 12th days of infection) for gamma counting. The mean daily faecal and urine counts were calculated for every period of collection and were expressed as a percentage of the original count of $^{65}Zn$ administered in the rat.

**Statistical analysis**

The means were compared between infected rats and the pair-fed and the *ad lib*-fed controls using the students t-test as described by Steel and Torrie (1980).

## RESULTS

Two distinct phases of reduced feed intake were observed in the course of infection (figure 1). The first drop (5.3 vs 11.5 g $day^{-1}$) ($p<0.01$) was recorded on the 1st day of infection. A second drop was observed from the 6th to 8th day of infection, with the lowest intake (6.6 vs 12.3 g) ($p<0.01$) on the 6th day of infection. Changes in body weight corresponded to the daily feed intake (figure 2). However, despite the reduced feed intake on the 6th day of infection the infected rats continued gaining weight up to the 10th day of infection when a drop in body weight gain became evident (figure 2). In the later phase of infection (10th to 14th day) the infected rats were not gaining weight unlike the pair-fed controls, despite the improved appetite.

The semi-logarithmic retention curves of $^{65}Zn$ for each treatment are given in figure 3. The slopes representing the biological loss and decay rates of $^{65}Zn$ were similar in all the treatments before infection. Increased loss of $^{65}Zn$ was apparent in all animals from the 5th day of infection. However, a sharper slope was noted for the infected animals as from the

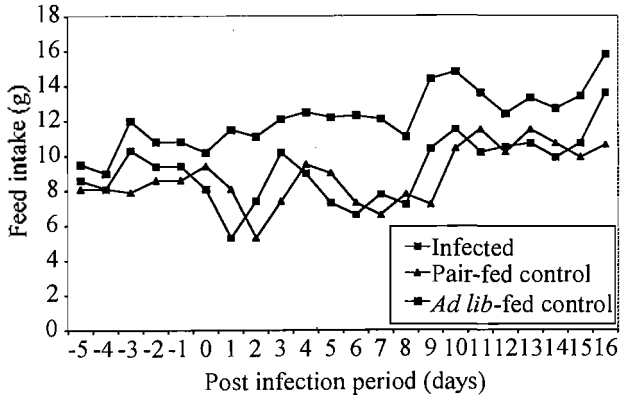


**Figure 1.** Daily feed intake of rats infected with *Nippostrongylus brassiliensis* and the pair-fed and *ad lib*-fed controls

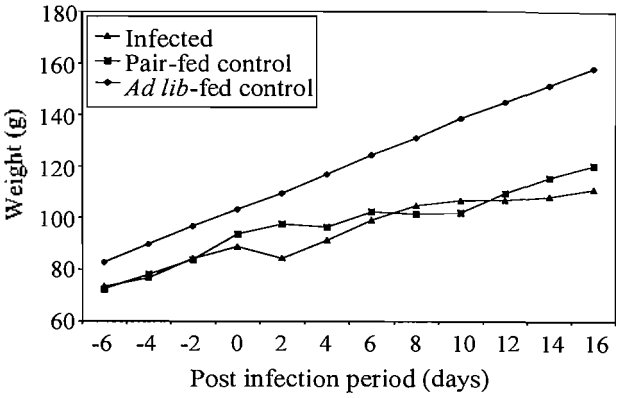


**Figure 2.** Growth of rats infected with *Nippostrongylus brassiliensis* and the pair-fed and *ad lib*-fed controls

6th day of infection. The biological half-live of $^{65}Zn$, calculated from regression lines of semi-logarithmic retention curves over different intervals, were lower ($p<0.05$) in the infected group as compared to the pair-fed controls from the 6th to 16th day of infection (table 1). The infected animals also lost more $^{65}Zn$ in faeces in the later phase of infection (9th to 12th day) as compared to both the pair-fed ($p<0.05$) and *ad lib*-fed ($p<0.01$) controls (figure 4). Similarly the loss of $^{65}Zn$ in urine was higher over this period than both the pair-fed ($p<0.05$) and *ad lib*-fed ($p>0.05$) controls (figure 5).

## DISCUSSION

The initial drop in feed intake and weight coincides with the systemic migratory phase of worms, and it may be due to parasitemia and verminous pneumonia induced by the presence of larvae in the blood and lungs. Earlier work has shown that larvae appear in the blood stream 10 hours after skin

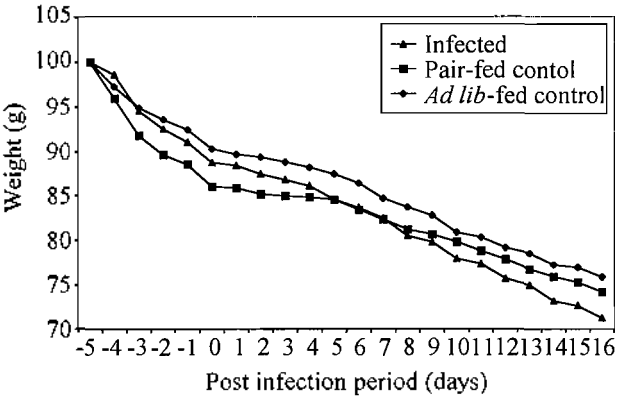


**Figure 3.** Percentage of $^{65}Zn$ retained in the body after an intraperitoneal does in rats infected with *Nippostrongylus brassiliensis* and the pair-fed and *ad lib*-fed controls

**Table 1.** Mean biological half-lives (days) of $^{65}Zn$ in infected (IR), uninfected pair-fed (PFC) and unin fected *ad lib*-fed rats (AFC) at various stages of infection with *Nippostrongylus brasiliensis*

| Days post infection | IR | PFC | AFC |
|---|---|---|---|
| -4 to 0 | 44.8±6.7 | 45.5±5.9 | 52.7±4.2 |
| 0 to 6 | 48.9±3.0[a] | 75.5±3.1[b] | 61.1±2.4[bc] |
| 6 to 16 | 43.1±3.5[a] | 60.1±4.8[b] | 51.0±1.7[ab] |

[a,b,c] Values on the same row with different superscripts differ significantly ($p<0.05$).
Values are expressed as means with standard error.

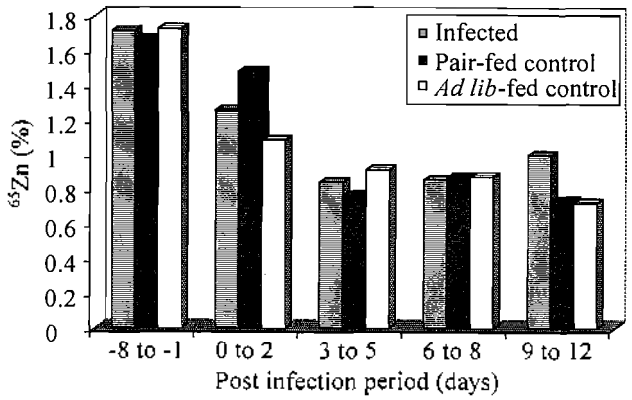


**Figure 4.** Percentage $^{65}Zn$ of the original dose lost in faeces after an intravenous injection in rats infected with *Nippostrongylus brassiliensis* and the pair-fed and *ad lib*-fed controls

penetration (Ogilvie & Jones, 1971) and by the 3rd day of infection, they migrate to the small intestine (El Hag, 1983). The 2nd period of reduced feed intake appears to coincide with accumulation of worms in the small intestines with the resultant enteropathy (Symons, 1957). However, it is intriguing that during the second period of low feed intake (6th to 10th day of infection), the infected rats continued gaining weight. This could be due to accumulation of fluid in the body or in inflamed intestine during the early phase of infection (Symons, 1957). The other reason that could explain this increase in weight is lethargy, which results in reduced nutrient requirements. The low feed intake recorded in infected animals could be enough to meet their low nutrient requirement unlike the active pair-fed controls that resort to tissue catabolism accompanied by loss in body weight. Changes in body weight observed in the later phase of infection are in agreement with the findings of Crompton et al. (1981) who showed that the fall in feed intake accounted for only half of the change in growth while the rest was attributed to inefficient feed utilisation. The low feed conversion efficiency from the 10th day of infection agrees with earlier work of Crompton (1984) and Caroline et al. (1985) who associated this with malabsorption, maldigestion and loss of endogenous nutrients from the body in protein loosing enteropathy.

The higher loss of $^{65}Zn$ which was observed in all the groups from the 5th day of infection coincides with the introduction of the second batch of diet with Zn levels of 16.23 ppm as compared to a concentration of 11.1 ppm in the first batch. This difference may have arisen from an error in weighing during the preparation of diets. There was increased retention of $^{65}Zn$ by pair-fed controls from the 1st day of infection (table 1) which is consistent with the observation that starvation induces increased synthesis of metallothionein (Bremner and Davis, 1975; Sas & Bremner, 1979) followed by a net influx of Zn into tissues. On the other hand, the infected rats showed increased loss of endogenous $^{65}Zn$ despite the low feed intake during this period. The infected group continued loosing more $^{65}Zn$ in the later phase of infection (figure 1 & table 1). This was due to higher losses in faeces and urine (figures 1 and 2). The possible routes of loss of radiolabelled endogenous pool of $^{65}Zn$ have been outlined earlier, but will be recapitulated here. In humans, Fell et al. (1973) associated the increased urinary loss of Zn following major surgery with evidence, such as increased urinary nitrogen loss, of net catabolism of body tissues. This may be the case with these experimental animals, thus it is possible that infected animals still had a net catabolic state which contributed to the loss of endogenous Zn in faeces and urine. However the cause of their continued tissue breakdown, especially since there is no evidence of it in pair-fed controls, is probably not that of reduced feed intake. In fact, in the later phase of infection (9th to 12th day) the daily feed intake of infected animals was increasing (figure 1). The clue to

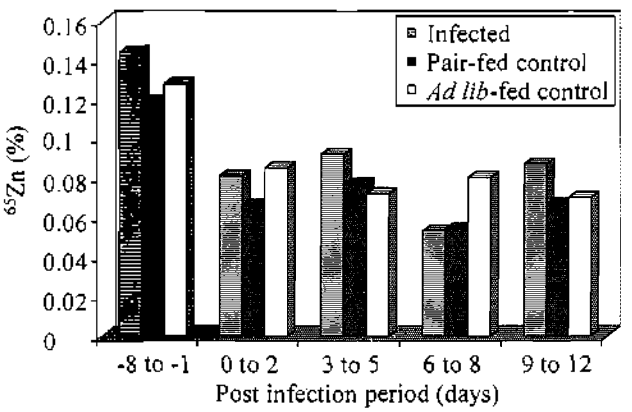


**Figure 5.** Percentage $^{65}Zn$ of the original dose lost in urine after an intravenous injection in rats infected with *Nippostrongylus brassiliensis* and the pair-fed and *ad lib*-fed controls

the probable cause of increased turnover of body tissues lies in the loss of $^{65}Zn$ in urine and faeces, which is possibly related to protein loosing enteropathy (Symons, 1957). The protein loss during protein loosing enteropathy include plasma albumin to which 80% of plasma pool of Zn is bound, additionally such enteropathies themselves cause catabolism of body tissues (Caroline, et al., 1985). In this experiment, faecal $^{65}Zn$ increased with time of infection with maximal loss being noticed between the 9th and 12th day of infection. This coincided with the time when gross intestinal damage reaches the peak (Symons, 1957). The high levels of $^{65}Zn$ in urine over this period (figure 5) in the infected group as compared to the pair-fed controls should be indicative of high plasma levels of $^{65}Zn$ most likely due to tissue catabolism. The evidence for the presence of tissue catabolism in infected rats could only be explained by the presence of protein loosing enteropathy or inefficient utilisation of feed. Inefficient utilisation of feed was evident in the infected group from the 10th day of infection when there was a drop in weight gain (figure 2) despite the improved appetite (figure 1), in contrast to the pair-fed controls. It was concluded that infection of *N. brasiliensis* was accompanied by increased loss of endogenous Zn through faeces and urine.

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