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Effect of infection with lungworms (*Dictyocaulus viviparus*) on energy and nitrogen metabolism in growing calves

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1. Ten Friesian male calves of about 100 kg and 3 months old were reared similarly and were worm-free. From 13 weeks of age five calves received a dose of 640 infective larvae (L_3) of lungworms (*Dictyocaulus viviparus*) twice weekly for 8 weeks to simulate continuous infection. Animals not infected were fed to the same level as the infected animals (about 1·2–1·3 kg concentrates and 1·4–1·5 kg good-quality hay/d).

2. Heat production was measured twice weekly during 48 h (days 2 and 3, and days 5 and 6) in each group of experimental animals.

3. Infection caused considerable damage to the lungs, increased respiration frequency and clearly produced antibody titres against *D. viviparus*.

4. Animals infected with lungworms had on average a lower rate of weight gain, reduced by 70 g/d per animal. Digestibility was not affected. Nitrogen retention was much lower in infected animals (12·0 *v.* 14·6 g/d per animal in controls).

5. Metabolizability of energy was slightly reduced in infected animals. Heat production as found in infected animals may be associated with an increased maintenance energy requirement of 30 kJ/kg live weight$^{0.75}$ per d or reduced partial efficiency of feed conversion above maintenance in animals infected with lungworms (58·5 *v.* 64·1% in the control animals).

6. It was concluded that the depression in rate of gain was related to reduced intake of feed and to decreased N retention.

Reduced growth rate in animals infected with parasites is often associated with reduced appetite (Steel, 1974; Coop, 1982; Symons, 1982; Boon *et al.* 1984). Moreover, performance may be impaired when conversion of nutrients after absorption from the gastrointestinal tract is less efficient (Coop, 1982; Symons, 1982). Steel (1974), Poppi *et al.* (1981), Coop (1982) and Symons (1982) suggested that loss of endogenous nitrogen into the intestine is the major reason for a reduced N gain. This loss implies, however, that the apparent digestibility of N is also reduced. This phenomenon is observed sometimes in calves infected with parasites (Coop, 1982). A higher maintenance requirement of metabolizable energy in sheep with gastrointestinal worms compared with treated animals has been shown to be associated with the presence of parasites (Van Adrichem *et al.* 1981).

In addition to information on feed intake and pathology, more information on energy balances is required. Therefore, we decided to use the balance technique for the determination of the use of dietary energy and N in calves infected with lungworms (*Dictyocaulus viviparus*). This infection causes an obstructive bronchitis–bronchiolitis (Jarett *et al.* 1957; Lekeux *et al.* 1985) resulting in reduced elasticity and damaged epithelial tissue of the lungs and a high activity of macrophages and eosinophilic granulocytes. In heavy infections the epithelium of the intestines is sometimes also changed, due to a massive penetration of larvae (Jarett *et al.* 1960). The clinical symptoms that are most frequently observed are an increased respiration rate and coughing. Additional N may thus be required for repairing the damage to lung tissues and for production of macrophages and granulocytes. It is to be expected that the increased respiratory frequency and the reduced elasticity of the lung

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tissue are associated with more work in respiration and thus with an increased metabolic rate.

The aim of the present experiment was to study the effect of a continuous experimental infection with *D. viviparus* on metabolizability of energy, N retention and energy metabolism during a period of 6 weeks (weeks 3–8) after initial infection (PII). This period was chosen because Boon *et al.* (1984) found it to be the most detrimental period for the animals in terms of clinical signs and rate of weight gain during continuous *D. viviparus* infections.

MATERIAL AND METHODS

Animals

Ten Dutch Friesian male calves, born within a period of 1 week and weighing about 42 kg, were reared worm-free and housed individually at the Department of Animal Husbandry. During the first 12 weeks of rearing, the calves received liquid milk-substitute. From 4 weeks onwards they also received, *ad lib.*, good-quality hay and water. From 4 weeks of age onwards, concentrates were supplied in small quantities, increasing to about 1 kg/d at 12 weeks of age. All animals were weighed once weekly on Monday at 15.00 hours. At the end of the experiment the calves were slaughtered and the respiratory tracts were examined for pathology. For histological studies, tissue samples were taken from the diaphragmatic lobes.

Infection

When the calves were 3 months old (and weighed about 100 kg live weight), they were randomly allocated to either a control (C) or an experimental (I) group of five animals per group. Twice weekly the calves of group I received orally a gelatine capsule, containing 640 infective larvae (L_3) of *D. viviparus* (see Boon *et al.* 1984). This was done throughout the 8-week experimental period. At 2 weeks after the first infection (PII) the animals were placed in a calorimeter, each in a balance cage. Five animals were housed per calorimeter and stayed there throughout the experimental period.

Feeding

During the first 3 weeks PII the animals received hay *ad lib.* and 1 kg concentrates/d. During the following 5 weeks (weeks 4–8) each animal received 1·5 kg concentrates and 1·3 kg hay/d (Table 1). It was expected that the infected animals would eat less (Steel, 1974; Coop, 1982; Symons, 1982; Boon *et al.* 1984). Therefore, it was decided to adjust the daily feed intake of the control animals to the mean *ad lib.* intake of the infected animals.

Concentrates were sampled at each weighing of feed and the composite sample of each week was analysed for crude protein (N × 6·25; CP; Kjehldal) and energy (bomb calorimeter). The concentrates contained (/kg): 888 g dry matter (DM) and, on a DM basis, 160 g CP and 18·8 MJ gross energy (GE). The hay was sampled and analysed in the same way. The corresponding values for hay were 800 g DM, 110 g CP and 20·7 MJ GE.

Analytical procedures

Clinical, parasitological and serological indices. To determine the severity of the infection in the individual calf the live weight, respiration frequency, serum antibody titre and the faecal larvae output were measured for each animal every week. The respiration frequency (respirations/min) was determined twice weekly on Monday and Thursday at about 14.00 hours. Blood samples were taken from the jugular vein from each calf on Thursday. The sera were analysed using IgG antibodies against *D. viviparus* according to the Elisa

technique (Boon *et al.* 1982). The faecal larvae output was counted each week from a rectal sample of 5 g faeces (Boon, 1979).

Digestibility of DM, N and energy. The amounts of feed ingested and faeces excreted by each animal were recorded and collected daily. Feed and faeces were sampled and the composite samples of 1 week were analysed for DM, N content (Kjeldahl) and energy content (bomb calorimeter). Digestibility of DM, N and energy of feed were thus measured on a per animal per week basis. The amount of urine voided by each animal was collected under acid (hydrochloric acid) and a portion sampled daily. The samples for 1 week for each animal were mixed and analysed for N and energy. The energy contents of faeces and urine were determined in a bomb calorimeter after freeze-drying the samples.

Metabolizability. Metabolizable energy (ME) intakes for individual animals were calculated for each week from energy in the feed, feed residues and energy in faeces, urine and methane. The volume of methane was measured for each group of animals. Correction of ME for methane was done by assuming that each animal in a treatment group had a similar percentage of energy ingested produced as methane.

N balances. N balances were calculated over weekly periods for each animal from N in feed, feed residues, faeces and urine. Moreover, they were adjusted for N escaping as ammonia in the air of the calorimeter by analysing condensation for N and sampling the air for ammonia. The air sample was obtained by leading chamber air at a constant rate through a bottle containing sulphuric acid. It was assumed that the contribution of ammonia to the air was proportional to the amount of N ingested by each animal in a treatment group.

Energy balances. Energy gain was calculated from ME intake and heat exchange. The heat exchange was measured for 48 h on days 2+3 and days 5+6 in each week. Weekly energy gains for each animal were then calculated from individual ME intakes by subtracting heat production (H) from the ME value. H was calculated for each animal by assuming, on a per unit metabolic weight and a per unit ME intake basis, that each animal in the group had the same H. Fat gain was calculated by subtracting energy deposited in protein estimated from N balance from the total energy gain.

Statistics

The differences between treatment groups within each week were subjected to analysis of variance according to the model:

$$y_{ij} = \mu + A_i + e_{ij},$$

where y_{ij} is the index, μ is the mean, A_i is the treatment ($i = 1, 2$) and e_{ij} is the error term.

The overall means of treatment results were tested by Student's t test. All analyses were done using the SPSS statistical Package (Nie *et al.* 1975).

RESULTS

Performance of the animals

Animals infected with *D. viviparus* showed a reduced rate of weight gain. The weight of the animals, the mean daily weight gain and values for the average daily feed consumption per treatment are presented in Table 1.

Moving animals to balance cages in week 2 PII caused a reduction in live weight in weeks 2 and 3. The reduction of 2·5 kg was similar for all animals. In weeks 4–8 PII, the daily weight gain was 359 g for the infected animals and 429 g for the controls. Variation in rate of weight gain in the infected animals was higher than that in the controls (see Table 1).

Table 1. *Weight, average daily weight gain and average daily feed consumption of calves*
(Mean values with their pooled standard errors for five animals per group)

Group...	Control	Experimental	Pooled SE
Initial wt at infection (kg)	102·2	102·2	1·8
Wt (kg) after infection			
2 weeks (in calorimeter)	113·9	112·4	2·2
3 weeks	111·4	109·8	2·0
8 weeks (final)	125·4	122·4	2·1
Average wt gain (g/d) (weeks 3–8)	429	359	50
Feed consumption (kg/animal per d)			
Concentrates	1·51	1·47	0·016
Hay	1·30	1·23	0·022

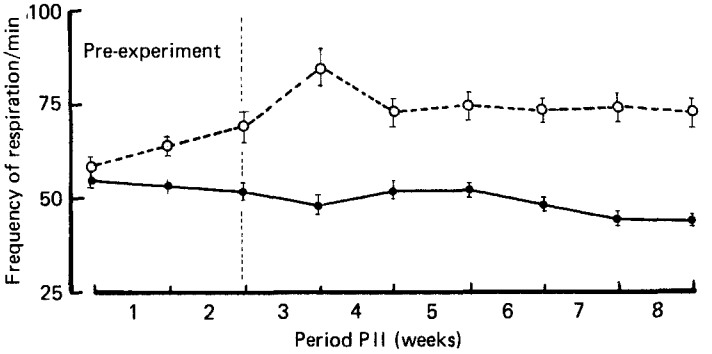

Fig. 1. Respiration frequency for control animals (●) and animals infected with *Dictyocaulus viviparus* (○) in various weeks after infection (PII). Points are mean values with their standard errors represented by vertical bars.

Clinical, parasitological and serological indices

Fig. 1 shows that animals infected with *D. viviparus* had a markedly increased respiration frequency compared with that of the controls. The average respiration frequency for animals in the experimental group was 74·6 /min and for the control animals 48·0 /min. In Fig. 2 the faecal larvae output and the serum antibody titre expressed as Elisa titre counts are given for the experimental group. The control animals were worm-free during the experiment and they did not show any positive Elisa titre count or larvae in the faeces.

Pathology findings

Only in the infected calves were affected lungs found. There was a variation in the total affected area of the lungs between the calves; however, in all the lungs local emphysema and oedema as well as lobular bronchopneumonia were observed, especially in the diaphragmatic lobes. In the bronchial tree of two calves some adult lungworms were found. Microscopically, mainly thickening of the bronchial epithelium, obstruction of some bronchioles with exudate and epithelialization of the alveoli with an increase of the interstitial connective tissue compared with the lungs of the control calves were observed. The lungs of the control calves showed no pathological lesions.

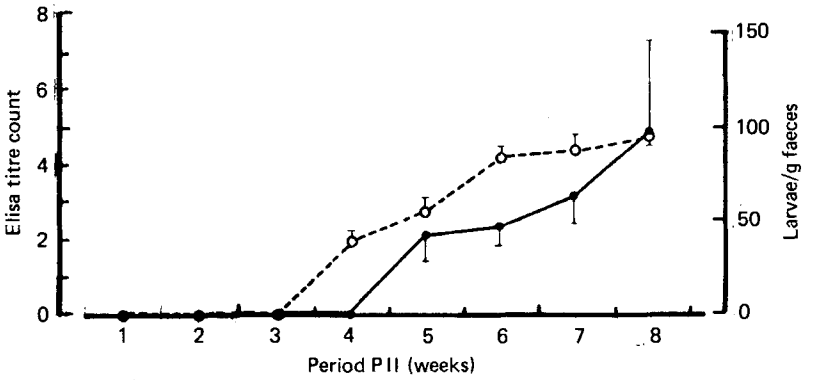

Fig. 2. The serum antibody titre against *Dictyocaulus viviparus* (Elisa titre count, ○) and faecal larvae output (larvae/g faeces, ●) for the experimental group in various weeks after infection (PII). Points are mean values with their standard errors represented by vertical bars.

DM intake, digestibility of DM and ME

In Table 2, values for the intake of DM and apparent digestibility of DM are given. Digestibility of DM in the groups of animals was similar. During the 5-week period, average digestibility was 0·700 in the infected animals and 0·708 in the control animals. The control animals consumed more feed than the infected animals, on average about 100 g DM/d more which was about 4·5% of the average daily intake (Table 2). In addition to the small difference in digestibility (−0·8%, see Table 2) there was some additional difference between treatment and control animals in metabolizability of GE. Control animals had a slightly higher ME:GE. Differences, however, were not significant.

N balance

Results presented in Table 3 show that N intake tended to be reduced in infected animals ($P < 0{\cdot}10$) compared with controls. Digestibility of N was not affected by infection with *D. viviparus*. The results also show that N retention, expressed as a percentage of N ingested, was 23·2 in control animals and 19·7 in infected animals ($P < 0{\cdot}10$). This difference was associated with the 4·2% extra N ingested found in the urine of infected animals.

In Fig. 3 the values for urinary N excretion are expressed as a percentage of the apparently-digested N. More N was found in the urine of infected animals than in the controls. The increase in the percentage of the digested N excreted in the urine started in week 4 after infection PII. The difference between infected and control calves remained fairly constant until the 8th week when the difference decreased.

Energy balance

Fig. 4 shows that GE intake, ME intake and energy gain were somewhat higher in control animals than in infected calves. H was slightly higher for the infected animals (not significant).

Methane production as a percentage of GE intake in both groups was similar (3·1% of GE). In Table 4 the energy balance indices are presented as averages of weeks 4–8 PII.

Results presented in Table 4 show that animals infected with *D. viviparus* had a somewhat lower ME intake ($P < 0{\cdot}10$) and a clearly-reduced energy balance ($P < 0{\cdot}05$). H in infected animals was about 1·2% higher than that in the control animals despite the reduced ME intake (not significant).

Table 2. *Intake of dry matter (DM), apparent DM digestibility and calculated metabolizability of gross energy (ME/GE; %) in various weeks after infection (PII) in control animals (C) and animals infected with* Dictyocaulus viviparus *(I)*

Period after PII (weeks)...	4		5		6		7		8	
Group...	C	I	C	I	C	I	C	I	C	I
DM intake (g/d)	2210	2075	2105	2020	2510	2380	2560	2510	2530	2460
Apparent DM digestibility	0·72	0·70	0·70	0·71	0·72	0·70	0·70	0·70	0·70	0·69
Difference (%)	−1·3		+1·0		−1·4		−0·3		−0·6	
ME/GE (%):										
Mean	65·8	63·5	63·7	64·0	66·8	64·8	65·7	64·9	64·5	63·5
Pooled SE	1·4		0·7		0·8		0·7		0·5	
Difference (%)	−2·3		+0·3		−2·0		−0·8		−1·0	

ME, metabolizable energy: GE, gross energy.

Table 3. *Nitrogen intake, digestible N, digestibility of N, urinary excretion of N, N retention and N retention as a percentage of protein ingested in control animals (C) and animals infected with* Dictyocaulus viviparus *(I)*

(Mean values over 4–8 weeks after infection with their pooled standard errors for five animals per group)

Group...	C	I	Pooled SE
N intake (g/d)	63·0	60·8	1·6
Digestible N (g/d)	38·1	36·3	1·4
Digestiblity of N (%)	60·6	59·9	0·9
Urinary excretion of N (g/d)	23·0	23·8	1·6
N retention (g/d)	14·6	12·0	0·7
N retention as a percentage of N intake	23·2	19·7	3·0

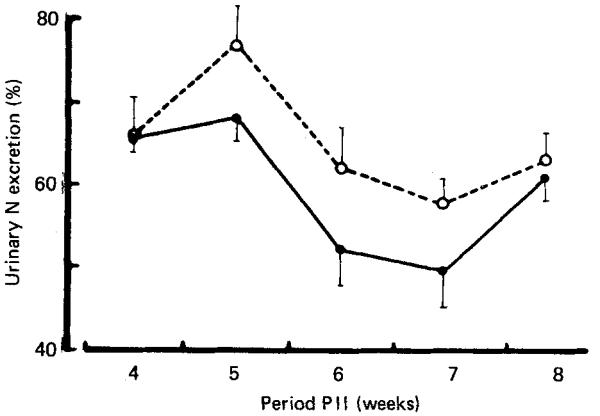

Fig. 3. The urinary nitrogen excretion for control animals (●) and animals infected with *Dictyocaulus viviparus* (○) (% of the apparently digested N) in various weeks after infection (PII). Points are mean values with their standard errors represented by vertical bars.

Table 4. *Gross energy (GE) intake, metabolizable energy (ME) intake, heat production (H) and energy gain (E) expressed per unit metabolic weight (kJ/kg live weight$^{0·75}$ per d), calculated maintenance requirement (efficiency assumed 64%) and efficiency (maintenance assumed 460 kJ/kg live weight$^{0·75}$) over weeks 4–8 in control animals (C) and animals infected with* Dictyocaulus viviparus *(I)*

(Mean values with their pooled standard errors)

	Treatment		Pooled	Statistical significance of
	C	I	SE	difference
GE intake	1308	1277	19·8	NS
ME intake	855	819	11·7	$P < 0·10$
H	604	611	4·2	NS
Estimated E	253	210	7·4	$P < 0·05$
ME_m	460	491		
E/ME_p (%)	64·1	58·5		

NS, not significant; ME_m, ME at maintenance; $ME_p = E/k_g$, where k_g is partial efficiency; $ME_m = ME - ME_p$; if for experimental animals a similar k_g for energy gain was assumed then the ME_m for the infected animals would be increased by 31 kJ ME/kg live weight$^{0·75}$.

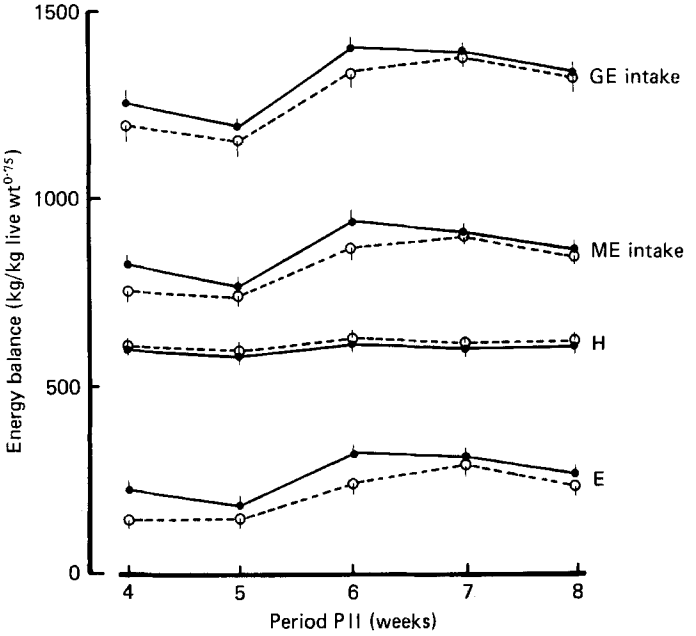

Fig. 4. Gross energy (GE) intake, metabolizable energy (ME) intake, heat production (H) and energy gain (E) expressed per unit metabolic weight (kg live weight$^{0.75}$) determined per week after infection (PII) for control animals (●) and animals infected with *Dictyocaulus viviparus* (○). Points are mean values with their standard errors represented by vertical bars.

DISCUSSION

Numerous studies have shown that various aspects of feed intake and metabolism may be responsible for a reduced weight gain in animals infected with parasites other than *D. viviparus* (Steel, 1974; Sykes & Coop, 1976; Symons, 1976; Poppi *et al.* 1981; Coop, 1982; Symons, 1982). Jarett *et al.* (1957) reported that an infection with *D. viviparus* causes an obstructive bronchitis–bronchiolitis. The respiration frequency is raised by infection due to less-efficient respiration (Jarett *et al.* 1957; Lekeux *et al.* 1985). In the present study the respiration frequency of the infected animals was 55% higher than that in the control animals.

It has been reported previously that calves infected with endoparasites show a reduction in their rate of weight gain and in feed intake (Sykes & Coop, 1976; Boon *et al.* 1984). A reduced rate of weight gain was found in the *D. viviparus*-infected animals and the intake of DM was reduced by 4% in the infected animals. It should be noted also that due to this reduced intake, rates of weight gain were rather low. This suggests a growth-reducing effect of *D. viviparus*. This is in accordance with earlier findings in ruminants infected with gastrointestinal parasites (Steel, 1974; Coop, 1982). The daily weight gain of the control animals was higher by 70 g/d per animal 4–8 weeks after the first infection. This reduction was about 16% of the rate of weight gain of the control animals.

In experiments with gastrointestinal parasites in calves and sheep the higher relative amount of N in the faeces of infected animals was not casued by malabsorption (Steel, 1974; Symons, 1976; Coop, 1982; Symons, 1982). These authors explained the lower apparent digestibility of N by higher endogenous losses of N into the intestine. This effect may be present in calves infected with gastrointestinal worms (Symons, 1982), but not in calves infected with lungworms. An effect on apparent digestibility can hardly be expected since

D. viviparus mainly affects the lungs. The damage to the epithelial cells of the intestinal tract caused by penetrating-lungworm larvae by this level of infection is considered to be very small (Jarett *et al.* 1957).

Values presented in Table 3 show that the infected animals absorbed 36·3 g N/d and the control animals 38·1 g N/d. This was mainly due to a reduced intake of 2·2 g N/d and is in accordance with the literature (Sykes & Coop, 1976). Expected N retention in infected animals at the efficiency of the control animals (23·2%, see Table 3) would be expected to yield 14·1 g N/d but we found a retention of 12·0 g N/d. The difference was partly excreted in the urine (Table 3). The decrease of urinary N excretion during week 7 was remarkable. This could be related to the development of immunity which starts after 2 weeks PII and is well developed at week 10 PII (Jarett *et al.* 1957). After that period the animals would be expected to cope with the parasites so they could control re-infections. Thus it can be concluded that the reduced N gain in addition to reduced intake for a major part is associated with the urinary output. This suggests a reduced efficiency of N utilization and is in agreement with the findings of Steel (1974) and Symons (1976).

Production of sputum in the lungs is also increased (Boon *et al.* 1984). The sputum protein that is swallowed passes through the gastrointestinal tract and it is likely that this protein will be reabsorbed since damage of the small intestine is not likely to be found with this moderate level of infection (Jarett *et al.* 1960). This reabsorption is consistent with the same digestibility of N found for the two groups of animals (Table 3).

In the present experiment the infected animals showed a reduced total energy gain. H was not reduced in the infected animals despite the reduced intake. Van Adrichem *et al.* (1981) noted that H in mature sheep given a maintenance ration and not treated with anthelminthica was higher than that in treated animals. They associated the higher H with an increased maintenance energy requirement in animals with gastrointestinal parasites. In the present experiment, growing animals were used and therefore it cannot be distinguished if maintenance was increased or partial efficiency decreased. If a similar maintenance (ME_m) of 460 kJ/kg live weight$^{0.75}$ (Van Es & Nijkamp, 1967) is assumed, the partial efficiency (k_g) of the conversion of ME above maintenance (ME_p) for energy gain (E) can be calculated for both groups. The average partial efficiency, as presented in Table 4, shows that the infected and control animals had a k_g of 58·5% and 64·1% respectively. The partial efficiency of 64% in control animals is similar to that found in the literature (Blaxter, 1962). If, on the other hand, a similar k_g for E (0·64 for the controls) is assumed, then maintenance for the infected animals would be increased by 31 kJ ME/kg live weight$^{0.75}$ (Table 4). Both decreased k_g and increased maintenance can be associated with a relatively increased H in infected animals. In normal uninfected calves, about 2% of total H may be associated with performance of breathing. The observed alveolar epithelialization and increase of interstitial connective tissue in the affected lungs could be related to a lowered compliance of the lungs which resulted in a higher energy requirement for breathing. This is in agreement with results of Lekeux (1984) and comparable to the results of Guyton (1981) who found that in human lungs with lowered elasticity the energy requirement for breathing may be a multiple of the normal level.

In conclusion, the reduced performance of calves infected twice weekly with infective larvae of *D. viviparus* was associated with reduced intake of food and an increased urinary N output.

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