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The influence of plane of nutrition on the effects of infection with *Trypanosoma congolense* in trypanotolerant cattle

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SUMMARY

Thirty-two N'Dama heifers were offered *ad libitum* Andropogon hay plus 10·2 g/kg liveweight (LW) groundnut hay (GNH) (L) or 10·2 g/kg LW GNH and 3·9 g/kg LW groundnut cake (GNC) (H). After 4 weeks on the diets, half of each group were infected intradermally with *Trypanosoma congolense* clone (ITC 50) (LI and HI). Peak parasitaemia occurred 6–8 days after infection and started to decrease *c*. 56 days later. No differences in parasitaemia were observed between LI and HI animals. Packed cell volume (PCV) fell in all treatments (by 5·4, 13·8, 3·7 and 9·4 units after 49–63 days post-infection (p.i.) for the L, LI, H and HI groups respectively) and significant effects of infection and diet were observed. GNH and GNC intakes were maintained during the trial; however, infected animals had a decreased intake of Andropogon hay. LI animals lost significantly more weight during the experimental period than the non-infected controls (−71·4 *v*. −13·7 g/day). Meanwhile, HI animals gained less weight compared with the H group (52·2 *v*. 167·6 g/day). Weight losses appeared to be due to decreased food intake. In the period 54–68 days p.i., plasma concentrations of albumin were lower and plasma protein was higher in infected animals. Plasma cholesterol concentrations were also lower in infected animals 54–68 days p.i. Plasma urea concentrations were higher in supplemented animals but were not affected by infection. The results showed that animals on a higher plane of nutrition showed less severe clinical signs of infection. However, for all the parameters considered, the magnitude of the difference between groups on different diets was similar for both infected and control animals, suggesting that mechanisms of resistance were not affected by the planes of nutrition considered.

INTRODUCTION

The N'Dama breed of cattle found widely in West Africa is known to show a degree of tolerance to infection with trypanosomiasis. Male N'Dama cattle inoculated with *Trypanosoma congolense* had lower parasitaemias and a less severe degree of anaemia than Zebu animals consuming a similar diet (Dwinger *et al*. 1992) and appeared to recover from the disease spontaneously. The degree of trypanotolerance has been shown to be affected by a number of factors of which nutritional status of the host was considered to be the most important (Murray 1987). However, studies to examine the influence of nutrition on trypanotolerance have been limited.

A study with sheep suggested that, as plane of nutrition declines, the degree of trypanotolerance may also decline (Reynolds & Ekwuruke 1988) and in cases of extreme nutritional stress N'Dama cattle have been shown to be unable to control the anaemia caused by the disease (J. J. Bennison, unpublished). Previous studies carried out in the Gambia have shown that the severity of infection in grazing cattle under natural challenge can be reduced by supplementing the diet with small amounts of concentrate feed (Agyemang *et al*. 1990; Little *et al*. 1991). Agyemang *et al*. (1990) found that supplemented cattle recovered more rapidly from anaemia, while

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Table 1. *Content of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) as a percentage of DM for the feeds offered to N'Dama heifers*

		Feed composition			
		OM	CP	ADF	NDF
Feed	DM	(%DM)			
Andropogon hay	95·8	95·2	2·3	50·1	78·5
Groundnut hay	94·6	94·0	7·8	45·4	50·9
Groundnut cake	94·3	94·9	46·4	18·0	12·7

earlier work by Little *et al.* (1990) showed that packed cell volume (PCV) concentrations in animals on a lower plane of nutrition declined more rapidly than those on a higher plane.

Since detailed work in this area with cattle have been limited, the aim of the present study was to examine the effect of plane of nutrition on the resistance to trypanosomiasis in artificially infected N'Dama cattle. Blood parameters and changes in intake and liveweight were used as indicators of the severity of the disease.

MATERIALS AND METHODS

Thirty-two N'Dama heifers, aged 1–2 years (initial mean liveweight 113 ± 17·0 kg) and testing negative for the presence of anti-trypanosomal antibodies using the immunofluorescent antibody test (Katende *et al.* 1987) were used. Animals were allocated to four treatments, two groups (L and LI) receiving *ad libitum* Andropogon hay and groundnut hay (GNH), while the remaining groups (H and HI) received an additional supplement of groundnut cake (GNC). Diets were introduced to the animals over a 2-week period followed by a 4-week adaptation period. At the end of the sixth week, animals in groups LI and HI were inoculated intradermally with a *T. congolense* clone, ITC 50 (Dwinger *et al.* 1992), a bulk stabilate of the reference clone known as *T. congolense* IL1180, isolated in East Africa (Nantulya *et al.* 1984). A standard pour-on, 1%ww Fleumethrin (Bayer), was applied on a monthly basis to eliminate ticks and to

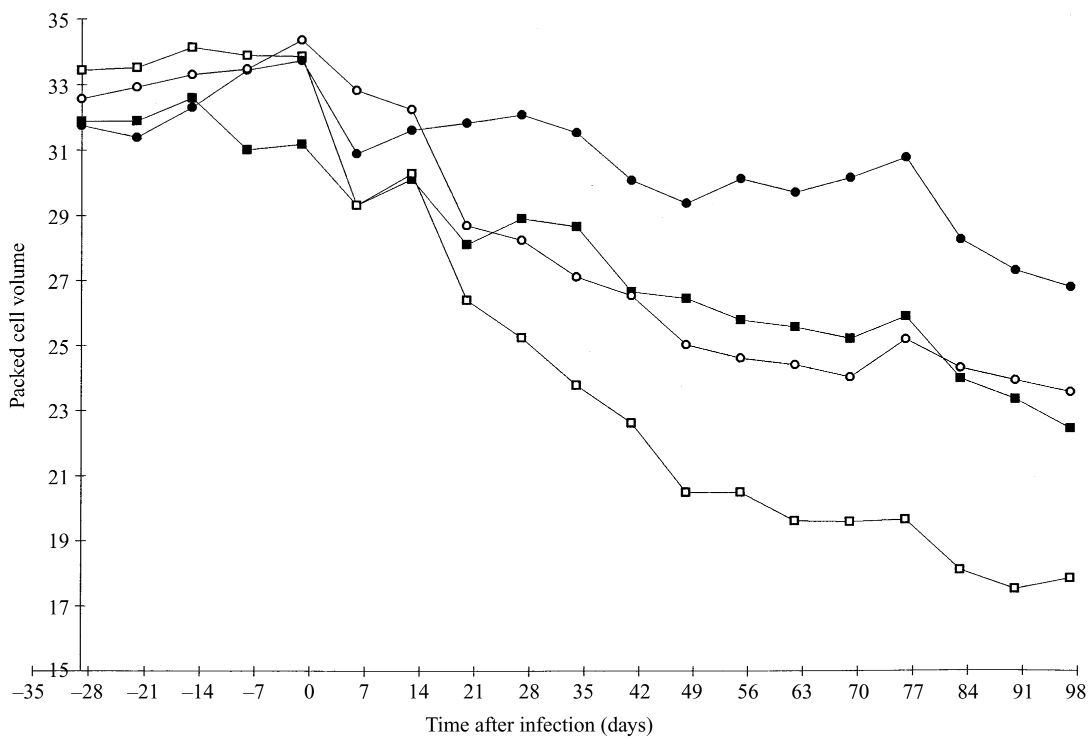

Fig. 1. The effects of a low (L) compared to a high (H) plane of nutrition and infection (I) on mean packed cell volume (PCV), before (−14–0 days, S.E. = 0·61) and after (49–63 days S.E. = 0·65) infection with *T. congolense* ($n = 16$, D.F. = 28). L (■), LI (□), H (●) and HI (○).

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minimize the possibility of cross-infection from external parasites. Measurements were continued until 16 weeks post infection (p.i.), when infected animals were treated with the trypanocidal drug, diminazine aceturate (Berenil), at 7 mg/kg LW.

Feed composition is presented in Table 1. Andropogon hay was hand chopped to a length of *c.* 200 mm and offered *ad libitum* at 130% of the previous day's intake. Animals were divided into two equal groups on the basis of weight (i.e. 89–110 kg and 116–146 kg), equal numbers from each group being allocated to each treatment. GNH was offered at 10·2 g/kg LW and GNC at 3·9 g/kg liveweight on a fresh weight basis, according to the mean liveweight of the animals in each weight group.

Animals were individually tethered 3 m apart in a fenced area, randomly distributing animals from the same treatment group. GNH and GNC were offered at 09.00 h and Andropogon hay at 10.00 h. Water was offered at 12.00 h and 15.00 h daily. Each animal had access to a mineral block *ad libitum*. Intakes of feed and water were measured daily and weekly subsamples of feeds and refusals taken for analysis of crude protein (CP) and ash. Animals were weighed once weekly before feeding. Blood samples were taken before feeding three times weekly for the determination of PCV using the standard micro-haematocrit method, and parasitaemia using the dark ground buffy coat method (Murray *et al.* 1977).

Once weekly, at the time of feeding, additional blood samples were taken using heparinized syringes for the determination of plasma metabolite concentrations. Commercial kits were used to measure the concentrations of plasma total protein, cholesterol (Biomerieux UK Ltd), albumin and urea (Roche Products Ltd), using a Cobas Mira autoanalyser (Roche Products Ltd).

All feeds were analysed for dry matter (DM), ash, nitrogen (N), acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to standard procedures.

Statistical analysis

Statistical tests comparing the effect of diet and infection on PCV, intake, and plasma metabolite concentrations, were carried out on mean values calculated over two 14-day periods, 14 days immediately pre-infection (−14–0 days) and 49–63 days p.i. (54–68 for plasma metabolite concentrations). The p.i. period was selected to coincide with the last 2 weeks before parasitaemia started to decline. For each period, an analysis of variance was conducted, separating out effects due to diet and infection as well as any interactions. Relative changes, p.i. values as a percentage of pre-infection values, were also compared for PCV and Andropogon intake. Liveweight gains in the p.i. period were examined in the same way. Mean parasitaemia scores in each week were compared using a *t*-test. Where data are presented graphically, relevant S.E.S of means and appropriate D.F. are given in the figure titles.

RESULTS

Peak parasitaemia scores of 3–5 occurred 9–13 days after inoculation and no significant differences ($P > 0·05$) were observed between dietary treatments in any week during the p.i. period. Animals in both groups were able to tolerate the disease, parasite numbers starting to fall around 56 days p.i. PCV (Fig. 1) concentrations fell in all treatments (by 5·4, 13·8, 3·7 and 9·4 units after 49–63 days p.i. for L, LI, H and HI groups respectively) and significant effects of both infection and diet were observed ($P < 0·001$) at 49–63 days p.i. The most severe drop in PCV was observed in the LI group, though there was no significant interaction between diet and infection ($P > 0·05$).

Animals in all treatments consumed all the GNH and GNC offered throughout the trial. Intakes of the Andropogon hay before infection were not significantly different ($P > 0·05$) between groups, but were lower ($P < 0·05$) in infected animals during the p.i. period (Table 2). Mean reduction in intake after 49–63 days was greater for the HI compared to the LI group (36 *v.* 19% of pre-infection intakes), although the apparent interaction was not significant. Furthermore, HI animals began to recover intakes after 10 weeks p.i., whereas depressed intakes were observed in LI animals until 3 weeks later. Estimated ME intakes were also lower ($P < 0·001$), while CP intakes were not significantly different ($P < 0·05$) for infected animals in the p.i. period examined (Table 2).

Animals on the L diet lost weight while those receiving the additional supplement of GNC gained weight. LI animals lost significantly ($P < 0·001$) more weight following infection than the non-infected controls (−71·4 *v.* −13·7 g/day). Meanwhile, HI animals gained less weight ($P < 0·001$) than H group controls (52·2 *v.* 167·6 g/day). Actual liveweights were not significantly affected by infection or diet by 46–63 days p.i. (Table 2).

There was a significant effect 54–68 days after infection of both diet ($P < 0·05$) and infection ($P < 0·01$) on plasma albumin concentrations (Fig. 2*a*), which were lower in animals consuming the non-supplemented diet and in infected animals. Plasma albumin concentrations were significantly higher pre-infection ($P < 0·05$) for animals receiving the H diet. Total protein concentrations in the plasma (Fig. 2*b*) were not affected by diet, with no significant differences being observed either before or after infection. However, in the p.i. period examined, infected animals had significantly higher concentrations of plasma protein ($P < 0·01$) than the non-infected controls, although this was mainly due to falling values in the control animals. Plasma urea con-

Table 2. *Effect of plane of nutrition and infection on liveweights and daily intakes of total dry matter (DM), Andropogon DM, total crude protein (CP) and metabolizable energy (ME), before (−14–0 days) and after infection (49–63 days) as well as liveweight change during the p.i. period (n = 16, D.F. = 28). (L = low; H = high; NI = non-infected; I = infected)*

	Plane of nutrition		Infection		S.E.
	L	H	NI	I	(*n* = 16)
Pre-infection (−14–0 days)					
Mean LW (kg)	112	116	115	114	4·7
Andropogon intake (g DM/kg $LW^{0.75}$)	30·8	31·8	32·0	30·6	1·92
Total DM intake (g/kg $LW^{0.75}$)	61·8	73·8	68·4	67·2	1·97
CP intake (g/kg $LW^{0.75}$)	3·2	8·5	5·9	5·9	0·11
ME intake (MJ/kg $LW^{0.75}$)*	0·53	0·69	0·62	0·60	0·014
Post-infection (49–63 days)					
Mean LW (kg)	109	120	116	113	5·0
Andropogon intake (g DM/kg $LW^{0.75}$)	29·4	28·7	33·0	25·1	2·13
Total DM intake (g/kg $LW^{0.75}$)	61·2	66·5	68·1	59·6	2·55
CP content of diet (g/kg $LW^{0.75}$)	3·1	8·6	6·0	5·7	0·12
ME intake (MJ/kg $LW^{0.75}$)*	0·51	0·66	0·63	0·55	0·021
Liveweight change in p.i. period (g/day)	−42·6	109·9	76·9	−9·6	12·51

* ME estimated from book values.

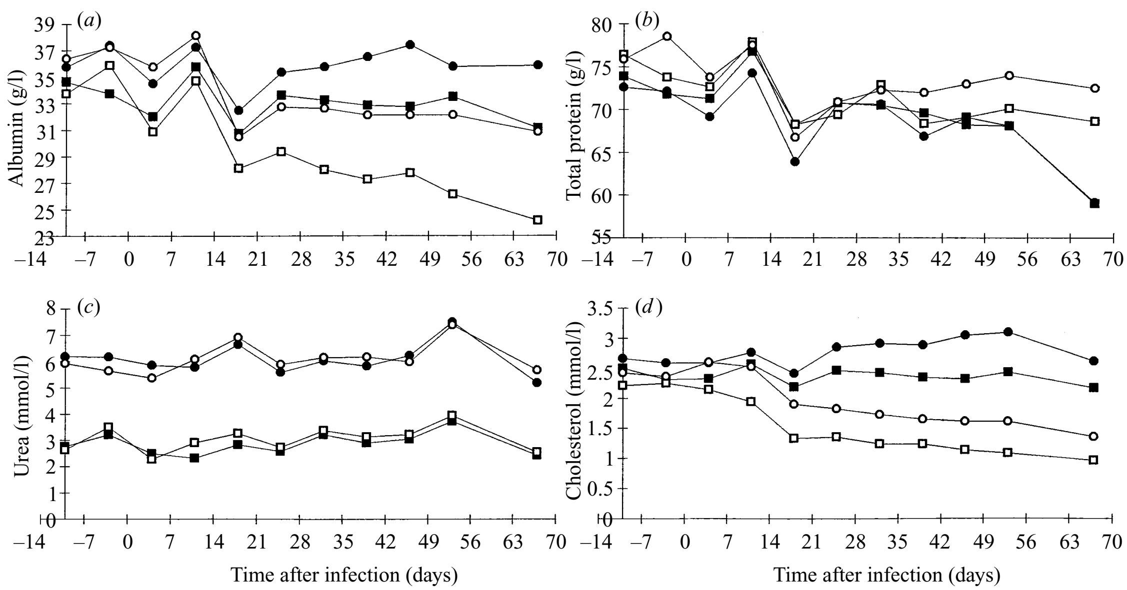

Fig. 2. The effects of a low (L) compared to a high (H) plane of nutrition and infection (I) on mean concentrations of (*a*) albumin, (*b*) total protein, (*c*) urea and (*d*) cholesterol, before (−14–0 days, S.E. = 0·69, 1·32, 0·20 and 0·12 respectively) and after (54–68 days, S.E. = 0·65, 1·72, 0·29 and 0·10 respectively) infection with *T. congolense* (*n* = 16, D.F. = 28). L (■), LI (□), H (●) and HI (○).

centrations (Fig. 2*c*) were significantly higher ($P < 0·001$) in animals in the H and HI groups, both before and after infection, reflecting a higher N intake. There was no significant difference due to infection. Plasma cholesterol concentration (Fig. 2*d*) started to fall in both infected groups in the second week after infection and were significantly ($P < 0·001$) lower in infected animals by 54–68 days after infection. Concentrations

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continued to fall throughout the period of observation. In both infected and non-infected animals, cholesterol was lower for animals consuming the L diet ($P < 0{\cdot}001$).

DISCUSSION

In this study with N'Dama cattle, infection with trypanosomes resulted in parasitaemia in all infected animals, with no significant difference observed due to diet. Although Katunguka-Rwakinshaya *et al.* (1993) observed significantly higher parasitaemias in sheep fed higher rates of protein, the current observations are in agreement with findings by other authors in infected sheep (Reynolds & Ekwuruke 1988; Katunguka-Rwakinshaya 1992), pigs (Fagbemi *et al.* 1990) and cattle (Little *et al.* 1990) compared at two planes of nutrition.

Infection of cattle in both nutrition groups resulted in anaemia with a more severe decrease in PCV being observed in animals receiving only GNH in addition to the Andropogon. Similar observations were made by Little *et al.* (1991) in a study comparing infected N'Dama cattle under village conditions. Studies in sheep (Katunguka-Rwakinshaya 1992; Katunguka-Rwakinshaya *et al.* 1993) and pigs (Fagbemi *et al.* 1990) using isoenergetic and isonitrogenous diets suggested that the PCV decline in infected animals responded to alterations in energy but not in protein intakes. However, GNC provides both extra protein and energy and further work would be required to clarify whether such an effect also occurs in cattle.

The reason for the PCV decline in both non-infected control groups is not clear. This may have occurred as a result of the frequent blood sampling (*c.* 40 ml/week) as Reynolds & Ekwuruke (1988) observed in sheep. However, a diet effect was also observed. Akinbamijo *et al.* (1994*a*) also showed a more severe decrease in PCV in sheep on a medium, compared to a high, plane of nutrition and Liu *et al.* (1994) showed that PCV of sheep nourished by intragastric infusion declined in response to decreasing protein supply below maintenance. The presence of some additional factor causing depressed PCV may explain why spontaneous recovery of PCV values, as observed by Dwinger *et al.* (1992), did not occur.

Work with trypanotolerant West African Dwarf goats (Zwart *et al.* 1991; Wassink *et al.* 1993) and sheep (Reynolds & Ekwuruke 1988; Akinbamijo *et al.* 1994*a*, *b*) infected with *T. vivax* showed total DM intake to decrease in response to infection in some animals. In the present trial, cattle in both infected groups decreased intake of the Andropogon hay as parasitaemia developed and it may be that changes in digestive function due to infection caused a decrease in the total quantity of feed that could be consumed. Veenendaal *et al.* (1976) observed a slight inhibition of extrinsic ruminal contractions in goats during the acute phase of infection with *T. vivax*, while sheep infected with *T. congolense* had increased mean retention times compared to control animals (G. J. Wassink, unpublished). Such changes might be expected to increase gut-fill, thus decreasing intake (Forbes 1986). The selective refusal of the Andropogon hay may have resulted from cattle selecting for higher nutritive value or against the more fibrous feed, which might be expected to have the greatest effect on gut-fill. Altering the fibre content or energy concentration of the diet may allow cattle to avoid anorexic effects of infection and maintain productivity; however, further work would be required to verify this hypothesis.

In studies with both goats (Verstegen *et al.* 1991) and lactating ewes (Akinbamijo *et al.* 1994*b*) increased requirements of energy for maintenance were observed in response to infection with *T. vivax*. However, in the present trial, changes in liveweight appeared to follow alterations in Andropogon intake and there was no evidence that energy requirement was altered in these animals. Supporting evidence was obtained in a subsequent trial with pair-fed N'Dama and Zebu cattle (O. O. Akinbamijo, unpublished) in which liveweight gains for control and *T. congolense*-infected N'Dama cattle were very similar. It may be that trypanotolerant cattle exhibit different mechanisms of disease control than small ruminants.

Higher total protein concentration in the plasma in infected animals probably occurred as a result of immunoglobulin production, while decreased albumin concentrations may have been due to catabolism in the liver, with the resulting amino acids being used to synthesise these immunoglobulins. However, the data were insufficient to confirm this and alternative causes would include a decreased synthesis of albumin or a switch between intra- and extra-vascular pools. Similar decreases in plasma albumin have also been observed in sheep (Katunguka-Rwakinshaya 1992; Katunguka-Rwakinshaya *et al.* 1993), although in one trial plasma total protein also decreased and it was suggested that the change in albumin concentration was, in part, due to haemodilution. Verstegen *et al.* (1991) observed increased serum urea concentrations in response to infection. These workers suggested that the increase occurred due to an increase in body protein catabolism in response to an increased demand for N, linked to an increased energy demand as a result of infection. Despite increased weight loss in LI animals, no differences in plasma urea concentration were observed.

Cholesterol concentration decreased following infection for animals on both diets. Similar decreases have been observed in sheep (Katunguka-Rwakinshaya 1992; Katunguka-Rwakinshaya *et al.* 1993) and a study in cattle suggested that *T. congolense* can take up serum cholesterol directly (Traore-Leroux *et al.* 1987). The difference in concentrations between infected and control groups was similar between dietary groups, which may reflect the similar parasite numbers observed for the two diets.

The present trial clearly showed that with the higher plane of nutrition clinical signs of infection were less severe, despite there being no difference in the degree of parasitaemia. Further study is required to clarify whether efficiency of utilization of energy in N'Dama cattle is altered in response to infection and to explore further the mechanisms which control intake in infected animals.

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