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Digestive function and nitrogen balance in *Trypanosoma vivax* infected Scottish Blackface sheep given different levels of roughage fibre

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Appendix V

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EEC DG XII Project: The interaction between nutrition and genetic resistance to trypanosomiasis in trypanotolerant livestock.
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Introduction

Trypanosome infected N'dama heifers and their controls The Gambia were fed restricted levels of groundnut cake and groundnut hay in the morning and *ad lib* andropogon hay in the afternoon. It was found that the trypanosome infected heifers reduced their intake of andropogon hay but kept on eating all their groundnut cake and groundnut hay (Romney *et al.*, submitted). At the same time, results in sheep fed either lucerne hay or barley straw *ad libitum* indicated that although the intake of the straw was much lower, the amount of fibre consumed, as measured by neutral detergent and acid detergent fibre, was approximately the same (Wassink *et al.*, submitted). However, the digestibility of the diet was about 10 points higher in the lucerne hay than in the barley straw fed animals and was affected by a trypanosome infection in both groups. Changes were also found in the rate of passage after the trypanosome infection (Wassink *et al.*, submitted). These results indicate an influence of the amount and quality of the fibre consumed on the pathology of the disease.

Research in The Netherlands has shown that the nitrogen balance is altered after a *T.vivax* infection in West African dwarf goats, indicating a change in utilisation of the nutrients (Akinbamijo *et al.*, 1992). However, no differences were found in body weight changes between *T.congolense* infection in N'dama heifers and their pair-fed controls and that the body weight change patterns followed changes in feed intake (Romney *et al.*, unpublished), indicating no or insignificant changes in the efficiency of nutrient utilisation. Similar results were found in *T.congolense* infected Scottish Blackface sheep (Wassink *et al.*, unpublished).

The relative importance of the altered digestive function and the nitrogen balance was investigated during a *T.vivax* infection in Scottish Blackface sheep fed different levels of roughage fibre.

Materials and methods

Animals

Sixteen, six month old, healthy, castrated Scottish Blackface lambs were selected and divided into two groups of 8 Infected (I) and 8 Pair-fed Control (PC) animals. Each PC animal was offered the amount of ration eaten by its infected partner on the previous day. Each pair of I and PC are brothers. Four weeks before the experiment started the animals were introduced to the experimental feeds and the animals were put in the metabolic stalls two weeks prior to the start of the experiment.

Experimental diet

One group of 4 trypanosome infected Scottish Blackface sheep and their pair-fed controls received 150 g DM grass hay and 319 g DM crushed barley grain (plus mineral mix) in the morning and barley straw in the afternoon (Diet A). The other group of 4 trypanosome infected animals and their pair-fed controls were fed 300 g DM grass hay and 236 g DM crushed barley grain (plus mineral mix) in the morning and barley straw in the afternoon (Diet B). The roughage had a fibre length of approximately 5 cm. The barley straw was offered ad lib (20% more than previous days' intake) to the infected sheep but was restricted in the pair fed controls to the amount eaten by their infected partner the day before. The grass hay and barley grain was also given on a pair-feeding basis to the controls.

The expected ME intake was approximately 7.5 MJ and the CP intake was approximately 62 g (DCP $_c$32 g) by the animals on both diets.

Infection

Two weeks after the experiment starts the group I lambs will be infected with *T.vivax* Leeflang (Leeflang *et al.*, 1976). The trypanosomes will be obtained from irradiated mice during the first rising parasitaemia. Each animal will be inoculated intravenously with 5×10^5 trypanosomes in 3 to 4 ml phosphate buffered saline (PBS) (containing 1.5% glucose).

Measurements

General Measurements

Roughage, concentrate and water intake were measured daily by collecting refusals between 8.00 and 9.00 h. Clinical observations were made daily for any abnormal behaviour. The animals were weighed once a week on Wednesday.

Collection of feed, faecal and urine samples

Feed digestibilities and nitrogen balance of the I and PC groups were measured during two balance periods of 1 week each. During the 7 day balance periods composite samples of roughage and concentrate offered were taken. If available, leftovers for each animal were collected before the morning feed and stored. After the 7 day period a composite sample was taken. Total daily faecal output was collected using plastic bags connected to harnesses. The bags were emptied at 8 a.m. and 5 p.m. and the weight recorded. During each period cumulative daily faeces from each animal were stored in special bins. At the end of each period 2 composite samples of the fresh faeces were taken. One of the samples was slurred using approximately 5 ml of toluene and 20 ml of water to prevent nitrogen losses due to bacterial fermentation. The other sample was dried.

Urine was collected over the 7 day balance period by aspiration into 100 ml 5 mol/l hydrochloric acid. The weight of the daily urine production was recorded and 10 % of the daily urine production sampled for each animal.

Analysis of feed, faecal and urine samples

The slurred samples were subsequently analysed for nitrogen (N) and dry matter (DM).

The composite samples of the roughage and concentrates offered, the dried composite faecal samples and composite samples of leftovers were analysed for DM, ash, N, neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and gross energy (GE).

DM was determined by heating known quantities in a hot air oven at 80^{o}C for 48 - 72 h until constant weight. Ash content was measured after heating a feed sample at 500^{o}C in a muffle furnace overnight.

N content in the feed, faeces and urine were determined using an automated Kjeldahl method. NDF and ADF were analysed using methods described by MAFF *et al.*. (1981).

GE content of dried feed samples is measured in an automatic adiabatic bomb calorimeter.

Organic matter (OM) were calculated as the DM - Ash. The digestibilities of OM, N, NDF, ADF and GE are calculated from the difference in these values between intake and faeces.

Creatinine and urea concentrations of the urine samples were measured at the end of each balance period.

Rate of passage measurements

The rate of passage of the roughage through the digestive tract was measured using chromium as a marker. The chromium was mordanted to the roughage fibre using the method described by Uden *et al.* (1980, 1982). After feeding approximately 30 g of the chromium mordanted hay to the animals faecal samples were taken at 8, 11, 17, 23, 30, 38, 48, 72, 96 and 120 h. The concentration Cr in the faeces was determined using Atomic Absorption Spectroscopy after wet digestion according to the method by Christian and Coup (1954).

Haematological measurements

Collection of blood samples

On Mondays, Wednesdays and Fridays 5 ml of blood was collected into tubes containing ethylene tetra acetic acid (EDTA) for the measurement of parasitaemia, packed cell volume (PCV) and a range of haematological indices (red blood cell count (RBC), white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelet count (PLT), mean corpuscular volumes (MCV), and haemoglobin concentration (Hb)). The samples were collected from the jugular vein using 19 gauge needles between 7.00 and 9.00 a.m., just before the morning feed.

Two microhaematocrit tubes were filled with blood from each sample and the mean PCV determined by spinning the tubes in a microhaematocrit centrifuge for 5 minutes.

Parasitaemia estimation

The buffy coat of one of the haematocrit tubes per sample was examined using the dark ground/buffy coat method (Murray *et al.*., 1977; Paris *et al.*., 1982). The intensities of parasitaemia was graded 1 to 6 (Table 2).

Biochemical measurements

Collection of blood samples

On Mondays, Wednesdays and Fridays 10 ml of blood was collected into tubes containing lithium heparin for plasma cholesterol, free fatty acids, albumin and urea measurements. The samples were collected from the jugular vein using evacuated tubes and 19 gauge needles, between 8.00 and 9.00 a.m., just before the morning feed.

Statistical analysis

All parameters, except intensity of parasitaemia, were subjected to statistical analysis using a randomised block design with each block consisting of a pair of lambs (one I, one PC). Mean effect over time was calculated and subjected to split plot analysis of variance with consideration of variation between treatments, between animals within treatments and interaction between treatments. Intensities of parasitaemia were evaluated by the non-parametric Mann-Whitney test. Growth rates were determined using linear regression analysis. Differences in organic matter intake in the I group before and after infection with *T.vivax* were tested using the paired student's *t*-test.

The model of Dhanoa *et al.* (1985) was used to analyse the chromium excretion data, which contains an exponential term and a double exponential term derived by considering digesta flow as a multi-component exponential process.

The formula is:

$$y = Ae^{-k_1 t} e^{-Be^{-(k_2 - k_1)t}}$$

Where y is Cr concentration, A and B are constants, k_1 and k_2 are rate constants and t is the time of sampling. The rate constants k_1 and k_2 are outflow rate constants for the two largest compartments in the digestive tract likely to be the rumen and possibly the caecum. Mean retention time (MRT) is the mean retention time between the time of chromium administration and the appearance of chromium in the faeces. The transit time (TT) or lag time is the time between chromium administration and the first appearance of chromium in the faeces (Dhanoa *et al.*,1985).

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