The interaction between nutrition and metabolism in West African Dwarf goats, infected with trypanosomes

Promotoren: dr. D. Zwart,
Emeritus hoogleraar in de tropische veehouderij

dr. ir. M.W.A. Verstegen,
Buitengewoon hoogleraar in de veevoeding, in het bijzonder de voeding der eenmagigen

Co-promotor: dr. T. Wensing,
Universitair hoofddocent van de vakgroep Inwendige Ziekten en Voeding der Grote Huisdieren, Universiteit Utrecht.

The interaction between nutrition and metabolism in West African Dwarf goats, infected with trypanosomes

J.T.P. van Dam

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
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dr. C.M. Karssen,
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In a series of experiments the interaction between nutrition and energy- and nitrogen metabolism of West African Dwarf goats, infected with trypanosomes was studied. Animals were injected with trypanosomes, and feed intake, energy and nitrogen balance and blood metabolites and hormones were measured for a period of six weeks post infection. *Trypanosoma vivax* infection caused fever and anaemia. The degree of anorexia, as indicated by the ratio [dry matter intake during infection / dry matter intake before infection] differed between experiments and between animals. Some indications were found for a relation between MHC genotype and dry matter intake ratio, but also environmental factors like stress and the age of the animals probably affected this ratio. Trypanosome infection caused a reduction of the energy retention, by decreasing gross energy intake and by increasing maintenance requirements with 28 %. This increased maintenance requirement was mainly related to the fever. Blood biochemical parameters reflected the undernourished state of infected animals. No indications were found for an interaction between the quality of the offered diet and the course of infection with respect to feed intake and nitrogen metabolism; instead, the effects of diet quality and of trypanosome infection were additive. Also no interaction occurred between nutritional history (as indicated by growth retardation which had developed prior to infection) and the course of subsequent trypanosome infection with respect to energy and nitrogen metabolism.

Ph.D. thesis, Wageningen Agricultural University, Department of Animal Husbandry, Section of Animal Production Systems, PO Box 338, 6700 AH Wageningen, The Netherlands.

Stellingen

1. Tijdens de eerste 6 weken van een trypanosomiasis infectie zijn de haematocrietwaarde en voederopname niet gecorreleerd.
dit proefschrift

2. Slechts in extreme gevallen is tijdens de eerste 6 weken van een trypanosomiasis infectie een interactie tussen de kwaliteit van de voeding en het verloop van infectie te verwachten.
dit proefschrift

3. De gevolgen van trypanosomiasis infectie voor de dierlijke produktie zijn vrijwel uitsluitend gelegen in een verlaagde voedselopname en een verhoogde onderhoudsbehoefte.
dit proefschrift

4. De bevinding van Kyriazakis *et al.* (1994) dat dieren kunnen compenseren voor de negatieve effecten van nematode infectie door middel van selectie van hoogwaardiger voer, is in het voordeel van 'browsers' (geiten, schapen) ten opzichte van 'grazers' (koeien).
Kyriazakis, Oldham, Coop and Jackson. 1994. Br. J. Nutr. 72: 665-677.

5. Een duurzame relatie tussen 'veeteelt' en 'milieu' vergt toewijding.

6. Het is te verwachten dat de opheffing van het Landbouwschap negatieve gevolgen zal hebben voor de gehele landbouwsector, vooral met betrekking tot het overleg tussen werkgevers en werknemers, en de belangenbehartiging richting de politiek.

7. Het is te betreuren dat in de maatschappelijke discussie over ethische kwesties uitzonderingsgevallen vaak fungeren als breekijzer om de bestaande wetgeving aan te vechten.

8. De term 'samenleving' verliest gaandeweg haar inhoud naarmate meer nadruk op individuele ontplooiing wordt gelegd.

9. Voor zowel liefhebbers als haters van de soap 'Goede Tijden, Slechte Tijden' (GTST) zou een verandering van deze naam in 'Goede Fijne Tijden' duidelijker de aard van de serie aangeven.

10. 'Onder'zoeken levert kennis op; echte wijsheid kunnen we alleen Boven vinden.

Stellingen behorend bij het proefschrift 'The interaction between nutrition and metabolism in West African Dwarf goats, infected with trypanosomes'.
J.T.P. van Dam, 25 juni 1996.

Voorwoord

En dan zit het werk erop... Het proefschrift ligt klaar voor de drukker. Het tot-stand-komen van dit proefschrift zou dit niet mogelijk zijn geweest zonder de hulp en inzet van vele mensen.

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Voor Marjo

Eerbiedig ontzag voor de Here is de basis van alle wijsheid,
en het kennen van God geeft meer inzicht.

Het Boek, Spreuken 9:10.

Chapter 1

General Introduction

General Introduction

The disease

Trypanosomiasis, one of the most important livestock diseases in sub-Saharan Africa, is caused by the protozoan parasite *Trypanosoma spp.*, and is transmitted by tsetse flies (*Glossina spp.*). The wide occurrence of the disease retards agricultural development (Stephen, 1986; ILRAD, 1994). Clinical signs include loss of appetite, anaemia, pyrexia and eventually death (Tizard, 1985; Stephen, 1986). The immune response to trypanosome infection is antibody mediated; however, the parasite is capable of frequently changing its antigenic structures (variable antigen types; VAT's), thus creating a chronic course of disease with successive parasite subpopulations emerging in the blood (Morrison *et al.*, 1985). During infection, activated phagocytic cells produce cytokines, like TNF-α and interleukin-1 (Sileghem *et al.*, 1993, Sileghem *et al.*, 1994); these are thought to induce many of the pathological signs, observed during infection, like anaemia (Sileghem *et al.*, 1994), anorexia (McCarthy *et al.*, 1986; Plata-Salaman *et al.*, 1988), fever (Van Miert *et al.*, 1992) and immunosuppression, leading to an increased susceptibility to opportunistic infections (Griffin *et al.*, 1980; Van Dam *et al.*, 1981; Mwangi *et al.*, 1990).

Trypanotolerance

Several local cattle, goat and sheep breeds like the West African Dwarf (WAD) goats and sheep, and the N'Dama and Muturu cattle are tolerant to the effects of infection (Griffin and Allonby, 1979; ILCA, 1979, 1986). The well recognized tolerance to trypanosomiasis of N'Dama cattle (Roberts and Gray, 1973; Starkey, 1984) consists of the ability to limit greatly the reduction in packed cell volume (PCV), and keep parasitaemia counts at a low level. Also animal production is maintained to a reasonable degree: in N'Dama's, the ability to limit the reduction in PCV was positively correlated with reproductive performance and cow productivity (Trail *et al.*, 1991; Trail *et al.*, 1992). Therefore, in tsetse infested areas the production potential of the N'Dama is superior to that of susceptible cattle breeds like the Zebu (Paling *et al.*, 1991; Dwinger *et al.*, 1992).

Because small ruminants (goats and sheep) are regarded as ideal for small scale farming systems (Luckins, 1992), having a high production potential (Armbruster and Peters, 1993), more attention has been given recently to the investigation of the nature of the trypanotolerance of the small ruminants in trypanosomiasis endemic regions.

Studies indicated that infection of WAD goats caused serious anaemia, and that the protein and energy retained in these animals was reduced (Verstegen *et al.*, 1991; Adah *et al.*, 1993; Akinbamijo, 1994; Osaer *et al.*, 1994). Osaer *et al.* (1994) suggested that the nature of trypanotolerance of small ruminants may be different from that of trypanotolerant cattle breeds. They suggested that the observed ability to maintain production, should be taken as the real index of resistance, rather than the ability to maintain PCV at a normal level.

Zwart *et al.* (1991) and Wassink *et al.* (1993) described a large variation in feed intake among trypanotolerant WAD goats during *T. vivax* infection and consequently large variation in body weight gain. This observation corresponds with studies of Roelants *et al.* (1983) and Clausen *et al.* (1993) in trypanotolerant Baoulé cattle. More knowledge is needed on the question what determines inter- and intra-breed variation in the degree of trypanotolerance with respect to feed intake and animal production, *i.e.*, has trypanotolerance a genetic basis, or do environmental factors (also) determine the degree of tolerance. Studies of Clausen *et al.* (1993) and Wassink *et al.* (1993) indicated a high repeatability of individual responses to successive infections of trypanotolerant animals, with respect to clinical paramters. This implies the possibility of a genetic basis for these traits.

However, trypanotolerance is also found to interact with environmental factors. Indications for an interaction with nutrition was detected by Reynolds and Ekwuruke (1988), and Agyemang *et al.* (1990). Agyemang *et al.* (1992) found indications for an interaction of tolerance with the physiological status of the animal, and Kaufmann *et al.* (1992) reported an interaction between the degree of tolerance and secundary infections. If substantial interaction between tolerance and nutrition exists, then strategic feed supplementation might increase animal production of trypanotolerant breeds to a great extent.

Energy and nitrogen metabolism

An important area of study is the energy and nitrogen metabolism of the infected host during infection. Animal production is mainly determined by the intake of nutrients, relative to the needs of the animal. The absorbed nutrients are firstly used for maintenance processes and the remainder for production, like body weight gain, milk, wool and traction (Blaxter, 1989). As mentioned before, nutrient intake was reduced by trypanosome infection (Zwart *et al.*, 1991; Wassink *et al.*, 1993). Also partitioning between protein and energy may be changed by infection. Beisel (1985) described increased N losses due to parasitic infection. This may be caused by protein losses via

urine or faeces, due to intestinal or renal lesions, or by an increased protein turnover. Verstegen *et al.* (1991) studied the energy and nitrogen metabolism of WAD goats during *T. vivax* infection. They found that maintenance requirements were increased by infection, leading to reduced productivity. In this study only group means were measured, and mean feed intake level differed between infected and control animals. More research was needed on the relation between metabolic rate and feed intake in infected and control animals, by measuring the variation in energy metabolism parameters between animals.

Aim of the thesis

This thesis describes a series of studies on the effect of experimental trypanosome infection on energy and nitrogen metabolism of trypanotolerant animals, and the possible interaction with nutrition. As a model the WAD goat was chosen. The animals were infected with the strain *T. vivax* Y486 (Leeflang *et al.*, 1976) by intravenous injection. Energy and nitrogen metabolism traits were measured during a period of 4 - 6 weeks after infection, to study how these were affected, and what were the metabolic costs in terms of energy and nitrogen. Special attention was given to the variation in feed intake among trypanotolerant animals and to possible mechanisms behind this variation. In addition, it was studied if the quality of the offered diet, and the nutritional history of an animal influenced the course of *T. vivax* infection. Because the degree of anaemia due to infection is thought to indicate the level of trypanotolerance (Trail *et al.*, 1991), this variable was studied too.

For the series of experiments, which are described in this thesis, three different research themes were defined. Firstly, the variation in feed intake reduction between animals due to infection was studied. Secondly, the effect of trypanosome infection on energy and nitrogen metabolism was studied, and thirdly, the interaction between nutrition and trypanosome infection with respect to energy and nitrogen metabolism was studied.

Outline of the thesis

In chapter 2 and 3, the studies on the variation in *ad libitum* dry matter intake during trypanosomiasis (research theme 1) are described. In chapter 2, a study on the dry matter intake (DMI) response to successive trypanosome infections of individual animals is reported. Analysis of the variation between successive responses per infected animal in

relation to the variation between animals was carried out, and from this indications on the relative importance of genetic sources of variation in the feed intake response to infection could be obtained.

Chapter 3 deals with the variation in DMI response to *T. congolense* infection between animals; it was studied if possible genetic mechanisms of feed intake regulation during infection exist. Therefore the phenotypic variation among animals was related to polymorphism in the Major Histocompatibility Complex (MHC) region of the genome.

The studies into the effect of *T. vivax* infection on energy and nitrogen metabolism, and energy partitioning, at different feed intake levels (research theme 2) are described in chapter 4 and 5. The effect of *T. vivax* infection on energy and nitrogen retention of individually housed WAD goats is presented in chapter 4. To make an isonutritional comparison between infected and control animals over a range of intake levels, a design with high and low responder animals and a restricted feeding regimen for part of the animals was chosen. The relation between energy retention (ER) and metabolizable energy (ME) intake, and the relation between nitrogen retention (NR) and ER were studied, the latter relationship would give insight in the question if trypanosome infection leads to increased N losses. Also blood biochemical parameters, which are informative about the energy and protein status of the animal, were measured.

Chapter 5 describes in more detail the heat production during infection, and the relation between body temperature and body posture. Therefore, these traits were measured on a continuous basis during infection, and the relation between short term variation in heat production and in body temperature was measured. Also the effect of body posture on heat production and body temperature was estimated.

In the chapters 6 - 8, the effect of nutrition on the course of *T. vivax* infection with respect to energy and nitrogen metabolism is described (research theme 3, 2). In chapters 6 and 7, an infection trial in which goats were fed either a good quality roughage (*i.e.*, lucerne), or a poor quality roughage (*i.e.*, grass straw) is described; in chapter 6, the organic matter intake, body weight change and nitrogen metabolism during infection is described, whereas in chapter 7 the metabolic profile and pathological (PCV, post mortem analysis) findings are given.

Chapter 8 reports on an experiment in which the effect of nutritional history of young growing goats on the course of *T.vivax* infection was studied. Before infection, therefore, restricted feeding at maintenance level was applied for half of the experimental goats, and the other goats had *ad libitum* access to feed. The effect of the induced retarded growth pattern on energy and nitrogen metabolism during infection was examined.

Finally, in the general discussion, the major findings from the previous chapters are discussed, and conclusions and directions for future research are formulated.

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Chapter 2

The relation between feed intake responses to successive trypanosome infections of trypanotolerant West African Dwarf goats

J.T.P. van Dam, W. van der Hel, P. Hofs and D. Zwart.

Dept. of Animal Husbandry, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands.

Submitted.

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By J.T.P. van Dam, W. van der Hel, P. Hofs and D. Zwart.

Dept. of Animal Husbandry, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands.

Abstract

Twelve adult West African Dwarf bucks were infected with successively *Trypanosoma congolense* and *T. vivax*, to study the individual response to infection with respect to the variables dry matter intake, body weight change and packed cell volume. Large variation between animals with respect to feed intake parameters was observed. The ratio [dry matter intake during infection / dry matter intake before infection] of individual animals was different for the two infection periods. This means that repeatability of dry matter intake ratio was low, indicating that genetic factors played a minor role. Probably housing in social isolation during the second infection period induced changes in the individual feed intake response to infection.

Introduction

A number of indigenous livestock breeds of West Africa are recognized as tolerant to the devastating effects of trypanosome infection, e.g. N'Dama and Muturu cattle and West African Dwarf (WAD) goats and sheep (Trail *et al.*, 1980). This tolerance exists in the capability to maintain Packed Cell Volume (PCV) level and productivity at an acceptable level. Trypanotolerance has a genetic basis (Murray, 1988). Roelants *et al.* (1983), however, found that within the trypanotolerant Baoulé cattle breed both sensitive and tolerant animals can be found. Also Zwart *et al.* (1991) observed large variation in feed intake and productivity due to *T. vivax* infection, among animals from the WAD goat breed. Wassink *et al.* (1993) infected WAD goats with successively *T. congolense* and *T. vivax*, with a recovery period between the two infections. They measured the dry matter intake (DMI) per animal before and during both infections separately, and calculated the ratio [DMI after infection / DMI before infection]. From this study a ranking correlation between individual DMI response to successive infections of 0.59 ($P < 0.05$) was calculated; thus indications were found that at least the DMI response to trypanosome infection, irrespective of the trypanosomal species or strain used, can be predicted from the response during previous infections. This may imply that individual

DMI response of WAD goats to trypanosomiasis may be genetically determined. However, the correlation between two repeated measurements comprises both variation which can be attributed to genotype and variation which can be attributed to constant environmental conditions during the two observations. In the study of Wassink *et al.* (1993), environmental conditions (type of feed, housing) were similar for both infection periods, which implies that the estimated correlation probably contained a relatively high proportion of variation attributable to environmental conditions.

In the present experiment, therefore, we infected WAD goats with successively *T. congolense* and *T. vivax* and changed the environmental conditions during the second infection period to study the variation in DMI response to infection between animals and between successive observations within animals. It was expected that this would give more insight in a possible genetic basis of feed intake response to trypanosomiasis.

Material and methods

The reported results were part of a larger experiment, in which the effect of feed intake level on the course of *Trypanosoma vivax* infection with respect to energy and nitrogen balance was studied (Van Dam *et al.*, in press).

Animals, feeding and housing

A group of 48 adult castrated West African Dwarf bucks with a mean body weight (BW) of 27.7 (± 1.0) kg and mean age of 21.0 (± 1.4) months, was used.

Before, during and after infection 1, all animals were housed in individual ground pens in which eye and ear contact with congeners was possible. During infection period 2, animals were housed individually in one of two identical respiration chambers, as described by Verstegen *et al.* (1987).

Throughout the experiment, animals had free access to pelleted lucerne. The lucerne contained 93 % dry matter, with on average 18 % crude protein in the dry matter. Water and salt lick were freely available.

Experimental design and time schedule

Five weeks before infection with *T. congolense* (Infection 1), 48 West African Dwarf goats were housed in individual pens. All animals were infected intravenously with *T. congolense subakia* stabilate, isolated in Nairobi, Kenya in 1961 at a dosage of approximately $1x10^6$ parasites per goat. Five weeks after infection the animals were treated with 7 mg diminazene aceturate per kg body weight (Berenil, Hoechst Veterinär,

München; double dosage), so that they could recover and regain pre-infection weight and PCV levels.

After infection 1, animals were allocated to one of three groups, depending on the observed DMI ratio (see under Measurements), *viz.* low ratio (LRat; Ratio $<$ 0.40), medium ratio (MRat; 0.40 $\leq$ Ratio $\leq$ 0.60) and high ratio (HRat; Ratio $>$ 0.60). For some animals, this ratio could not be calculated because daily DMI was not stable. From the animals with a known DMI ratio, 12 animals were selected for a second infection; 4 animals were selected from the HRat group, and 8 animals were selected from the LRat group. Thus only animals that had shown a large or a small reduction of dry matter intake during infection with *T. congolense* were selected, to minimize the use of animals. After a mean recovery period of 11 months, the selected animals were individually housed one after the other in one of two respiration chambers in complete social isolation. The selected goats had a mean age of 29 ($\pm$ 1.1) months at the moment of infection 2.

After 1 week in the respiration chamber, the goats were infected intravenously for the second time, at a dosage of approximately $1x10^6$ parasites per goat. For this infection a different trypanosomiasis species, *i.e.*, *T. vivax* Y486, isolated in Zaria, Nigeria by Leeflang *et al.* (1976), was used. This challenge with another species was done, because trypanotolerant animals are able to better control a secundary (or rechallenge) infection with the same trypanosomiasis strain than a primary infection (Paling *et al.*, 1991); it was assumed that by using a different species, the response to infection 2 would be comparable with that to infection 1.

After 6 weeks of infection the experiment was terminated and the animals were euthanized, according to Dutch welfare regulations.

Measurements

Feed intake was measured three times per week, from 3 weeks before infection until the end of the infection period. Therefore the amount of offered and refused feed was measured three times per week, and a weekly composite sample of both fractions was taken. Dry matter content of the samples of offered and refused feed was measured (ISO 5984). DMI per kg metabolic weight per day (g DM·$kg^{-0.75}$·d^{-1}) was calculated as the difference between offered DM and refused DM.

The mean DMI in a 2-weeks period prior to infection 1 was calculated, as well as the mean DMI during infection 1, (day 5 until 35 post infection; a prepatent period of 4 days was assumed). Moreover, the mean DMI in the week before infection 2, when the animals were already housed in the chamber, was calculated, and DMI during infection 2 (day 5 until 35 post infection). The ratio DMI [during infection / before infection] was calculated per animal per infection period.d

Body weight (BW) was measured weekly in the morning just after feeding, throughout the experiment.

Statistical analysis

Statistical analysis was done with General Linear Models procedure (GLM) of SAS Statistical Package (SAS, 1990) using 1-way analysis for analysis of DMI before and during infection, as well as DMI ratio for the second infection (with *T. vivax*; n = 12):

$$Y_{ij} = \mu + R_i + e_{ij} \quad [1]$$

where: Y_{ij} = dependent variable; μ = overall mean; R_i = effect of Ratio group (LRat or HRat; classification was based on results in infection period 1); e_{ij} = error term.

Moreover, the normal and the ranking (Spearman) correlations between subsequent measurements on DMI ratio of individual animals were calculated.

Results and discussion

In Table 1 BW at the start of infection, average DMI before and during infection and the DMI ratio are given per infection period per treatment. The BW at the start of infection was higher in period 2 compared with period 1 ($P < 0.01$). Both the infections with *T. congolense* and *T. vivax* caused a reduction of DMI and BW. This corresponds with studies of Zwart *et al.* (1991). Two animals died before the end of the second infection period on day 35 and 39 post infection, respectively.

The DMI before and during infection 1 and DMI ratio were higher, compared with the same parameters, measured during infection 2. During the first infection the DMI ratio was higher in HRat than in LRat; however, this was induced by the experimental design and therefore an artefact. In the second infection, however, only a small and non-significant difference between HRat and LRat was found ($P = 0.23$).

In Table 2, ranking (Pearson) correlations between clinical parameters, measured on the 12 experimental animals during both infections 1 and 2, are presented. The ranking correlation between DMI before infection 1 and DMI before infection 2 was positive ($P < 0.01$). Also the correlation between DMI before infection 1 and DMI during infection 1 was significant ($P < 0.05$), as well as the correlations between DMI during infection and DMI ratio, within each of the infection periods (at least $P < 0.01$). However, the correlation between DMI before infection 2 and DMI during infection 2, as well as the correlation between DMI ratio during infection 1 and infection 2 was very low and not different from zero.

Table 1. Least squares means of body weight (BW) at infection, dry matter intake (DMI) before and during successive infections with *Trypanosoma congolense* and *Trypanosoma vivax* and ratio DMI [during infection / before infection] of West African Dwarf goats, selected for either a high or a low DMI ratio.

Treatment[1]	LRat	sem	HRat	sem	P-Value
No. of observations	8		4		
		Infection 1			
BW at infection, kg	29.3[a]	1.1	24.4[b]	1.5	*
DMI, $g \cdot kg^{-0.75} \cdot d^{-1}$					
Pre-infection	66.1	4.0	79.0	5.6	ns
Infection	19.7[a]	2.3	58.0[b]	3.2	***
DMI ratio	0.30[a]	0.024	0.73[b]	0.034	***
		Infection 2			
BW at infection, kg	34.5	1.3	31.3	1.8	ns
DMI, $g \cdot kg^{-0.75} \cdot d^{-1}$					
Pre-infection	38.4	4.2	45.9	6.0	ns
Infection	10.3	2.1	16.8	3.0	ns
DMI ratio	0.28	0.056	0.40	0.079	ns

[1]: LRat = Low DMI ratio. HRat = High DMI ratio; selection based on DMI ratio in infection period 1;
[a,b]: means with different superscripts per infection period within a row are different ($P < 0.05$).

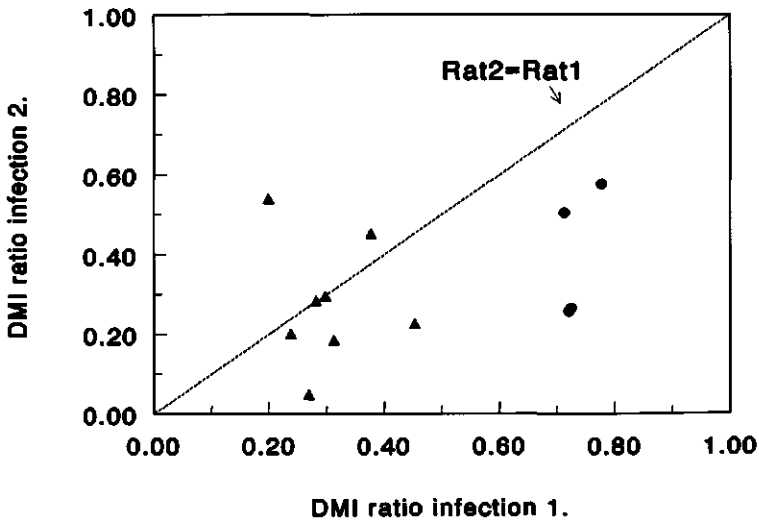
Table 2. Ranking (Spearman) correlation r between DMI before infection (DMI-1), DMI during infection (DMI-2); DMI ratio (DMI-R), of subsequent infections with *T. congolense* and *T. vivax*[1].

	T. congolense			*T. vivax*		
	DMI-1	DMI-2	DMI-R	DMI-1	DMI-2	DMI-R
T. congolense infection:						
DMI-1	-	0.64 (*)	0.37 (ns)	0.77 (**)	0.24 (ns)	-0.23 (ns)
DMI-2		-	0.94 (***)	0.40 (ns)	0.37 (ns)	0.20 (ns)
DMI-R			-	0.16 (ns)	0.36 (ns)	0.35 (ns)
T. vivax infection:						
DMI-1				-	0.35 (ns)	-0.34 (ns)
DMI-2					-	0.75 (**)
DMI-R						-

[1]: significancy between brackets: ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

The relation between DMI ratio after infection 1 and DMI ratio after infection 2 is also depicted in Figure 1; no relation (P = 0.27) was found. This agrees with findings of Dwinger *et al.* (1992), who observed a small and non-significant ranking correlation between body weight change of N'Dama cows during successive trypanosome infections, the body weight change being related to feed intake. Trail *et al.* (1991), however, estimated a heritability of 0.39 for body weight change of N'Dama cattle in trypanosomiasis-endemic areas. Also Wassink *et al.* (1993) found a high ranking correlation between individual DMI ratio's to successive trypanosome infections of 0.59. However, re-analysis of the data shows that the normal correlation in their experiment was low (r = 0.31; P = 0.29); this was due to the fact that one animal showed an extremely different response during the second infection. This observation could be qualified as an outlyer (P < 0.01), according to Cook's Distance test (Cook, 1979). Exclusion of this animal from the dataset brought correlation to 0.81 (P < 0.001; Wassink *et al.*, 1993).

Figure 1. The relation between DMI ratio during *T. vivax* infection (infection 2) with DMI ratio during *T. congolense* infection (infection 1) of individual animals. ▲ represents LRat animals, ● represents HRat animals.



The low correlation between subsequent DMI ratio's found in the present study, compared to Wassink *et al.* (1993), possibly can be attributed to different factors. Firstly, the social isolation of the animals in the respiration chambers may have affected DMI and DMI ratio. Carbonaro *et al.* (1992) observed an increase of norepinephrine in goats undergoing short periods of social isolation, which was mainly associated with exercise and physical stressors but probably also psychological stress. Van Adrichem and Vogt

(1993) found that metabolism of sheep was strongly affected by social isolation for periods of 1 week. Therefore possibly in the present study, feed intake and consequently DMI ratio was changed by the social isolation.

Secondly, the age of the animals may have played a role. At the start of infection 2 the animals were 8 months older than at the start of infection 1 (29 and 21 months, respectively). Ketelaars and Tolkamp (1991) observed a decrease of digestible organic matter intake per kg metabolic weight, with age, in a group of WAD goats aged between 22 and 32 months. Together with increasing age, also the body condition and amount of body fat increased (our unpublished observations) which may also have led to a reduced feed intake. Lee *et al.* (1995), described a negative relation between fat depth and feed intake of mature ewes. Nevertheless, a significantly positive correlation was found between DMI before infection 1 and DMI before infection 2, indicating that the ranking of animals had not changed largely in the intermediate period. Only during the second infection period, infected animals showed a different ranking with respect to DMI and DMI ratio.

It can be concluded, therefore, that little indication was found for a genetically determined DMI and DMI ratio during trypanosome infection. Environmental factors probably largely determined the DMI and DMI ratio and perhaps also interaction between genotype and environment has played a role.

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Chapter 3

A relation between CLA polymorphism and dry matter intake of West African Dwarf goats, infected with *Trypanosoma congolense*

J.T.P. van Dam[1], E.J. Hensen[2], B. Bissumbhar[2] and D. Zwart[1].

[1]: Dept. of Animal Husbandry, Wageningen Institute of Animal Science, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Infectious Diseases and Immunology, Utrecht University, PO Box 80.165, 3508 TD Utrecht, The Netherlands.

Submitted.

A relation between CLA polymorphism and dry matter intake of West African Dwarf goats, infected with *Trypanosoma congolense*

J.T.P. van Dam[1], E.J. Hensen[2], B. Bissumbhar[2] and D. Zwart[1].

[1]: Dept. of Animal Husbandry, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Infectious Diseases and Immunology, Utrecht University, PO Box 80.165, 3508 TD Utrecht, The Netherlands.

Abstract

A group of 25 mature West African Dwarf goats was experimentally infected with *Trypanosoma congolense* to study possible genetic mechanisms behind variation in clinical variables among animals. Therefore, before and during infection, dry matter intake (DMI), body weight change and packed cell volume (PCV) were measured. The ratio [DMI during infection / DMI before infection] was calculated, and the PCV ratio analogously. From all animals, the class I and class II caprine leucocyte antigens (CLA) were determined, by 1-dimensional iso-electric focussing. Indications were found for a correlation between on the one hand DMI during infection and DMI ratio, and on the other hand CLA genotype polymorphism. It was suggested that the class I and II CLA genes acted as a genetic marker for the TNF-α gene or its regulators, and that therefore differences in CLA genotype may have been related to different TNF-α production levels during infection. These results therefore offer some evidence for a genetic basis of the degree of trypanotolerance.

Introduction

Over 90 % of sheep and goats in sub-Saharan Africa are found in East and West Africa where they provide respectively 30 % of the meat and 15 % of milk consumed (ILCA, 1990). Many aid workers and international agricultural organizations consider these small ruminants ideal for increasing livestock productivity of low input systems (Luckins, 1992). A major constraint, however, for viable animal production systems in sub-Saharan Africa is the disease trypanosomiasis, caused by the protozoan *Trypanosoma spp.* and in Africa transmitted by the tsetse fly *Glossina spp.* The disease causes anorexia, anaemia and high mortality if left untreated (Stephen, 1986). Some local goat, sheep and cattle breeds, however, are tolerant to infection (ILCA, 1986). Trypanotolerance is described as the ability of individual animals to limit the anaemia and to maintain animal production (Murray and Morrison, 1981; Murray, 1988; Trail *et al.*, 1991). Griffin and Allonby

(1979) observed that tolerant breeds not only controlled the anaemia during infection, but also lost less weight in the first 4 months of infection. In studies on trypanotolerant West African Dwarf (WAD) goats, however, a reduction of feed intake was observed, which varied largely between animals (Zwart *et al.*, 1991). Roelants *et al.* (1983) also reported large differences in tolerance to infection among cattle from the trypanotolerant Baoulé breed.

Notoriously antigen specific immunity is activated during infection, but due to the antigenic variation of the parasite less effective. The innate immune system of the host has a central role with respect to tolerance to infection in many studies (Murray, 1988; Sileghem *et al.*, 1993). During infection, several hormone-like polypeptides are produced by activated mononuclear cells, such as tumour necrosis factor-α (TNF-α) and interleukin-1 (Sileghem *et al.*, 1993; Sileghem *et al.*, 1994). These cytokines regulate local inflammatory reactions by cell-to-cell communication, but may also gain access to the circulation and induce systemic effects such as anorexia and fever (Plata-Salaman *et al.*, 1988; Socher *et al.*, 1988; Van Miert, 1995). Karunaweera *et al.* (1992) demonstrated a close relation between short term variation in serum TNF-α levels and magnitude of fever.

It was found by Freund *et al.* (1992), that polymorphism in the gene, encoding for TNF-α production in mice correlated with levels of TNF-α mRNA in infected brain tissue and the level of resistance to the development of toxoplasmic encephalitis. Thus different alleles of the TNF-α gene may explain variation in TNF-α production between animals, and therefore provide a possible mechanism behind the observed variation in feed intake response to infection among animals.

The gene encoding for TNF-α is located in the central region of the major histocompatibility complex (MHC) in mammalian species including the goat, within the MHC class I and class II region (Andersson and Davies, 1993). In goats the MHC is called the caprine leucocyte antigen system (CLA). The class I and II regions of the CLA are also extremely polymorphic; the recombination rate between the class I and II region is low (less than 3 % in cattle) and therefore these genes usually cosegregate (Andersson and Davies, 1993). The CLA class I and II can be regarded as ideal marker genes for TNF-α gene polymorphism, based on the criteria for marker genes (Van der Beek, 1996).

Therefore, in the present study the individual response to artificial *T. congolense* infection of West African Dwarf goats with respect to the clinical parameters dry matter intake (DMI), body weight change and packed cell volume (PCV), was related to CLA class I and II polymorphism; these genes were expected to act as marker genes for the gene encoding for TNF-α. Results from this study could therefore give insight in the possible mechanisms behind the clinical signs due to trypanotolerance.

Material and methods

I. Biochemical genotyping of CLA genotypes

The method described below was based on the protocol for isoelectric focussing in goats (Joosten *et al.*, 1993).

preparation of the cells

From each goat, 10 mL of blood was taken by puncture from the *vena jugularis* using vacuumized tubes containing Lithium-heparin. Lymphocytes were isolated by centrifugation under Ficoll/NaMetrizoate (S.G. 1.078) for 45' at 850 g. Freshly isolated lymphocytes were washed 3 times in Hanks Balanced Salt Solution. Approximately 20×10^6 cells were kept in methionine free MEM (Gibco Ltd, Paisley, UK), with 10 % (v/v) foetal calf serum (FCS; Flow-Lab, Irvine, UK) before labelling, and were incubated at 37°C with 5 % CO_2, for 45'. Then cells were labelled by adding 100 μCi of ^{35}S-Methionine (Amersham Laboratories, UK) and incubated overnight.

Immunoprecipitation

After labelling, each cell suspension was transferred to two 1-mL eppendorf tubes (approximately 10×10^6 cells per tube) and was spinned and resuspended two times (centrifugation for 3' at 13,000 g). After spinning for the 3rd time, 1 mL of cold NP40 1 % lysis buffer was added to each eppendorf tube. Also fresh phenyl methyl sulphon fluoride (PMSF) was added (10 μL·mL^{-1} of a 10 mM solution) and the cells were resuspended. After 60' incubation on ice, the supernatant was transferred to a clean tube.

Preclearing of the supernatant was done twice; each preclearing consisted of the following steps. Normal rabbit serum (NRS; 3 μL) was added to the supernatant and was incubated on ice for 60', after which the tubes were spinned for 1' at 13,000 g. Then 75 μL of a 10 % Staph A solution was added, and after 30' incubation on ice, the tubes were spinned again for 3' at 13,000 g.

In the 2nd preclearing step, incubation with NRS was done overnight on ice. For standard precipitation of the Class I products, 3 μL of mAb B1.1G6 was added to one tube of each animal, and for precipitation of the Class II products, 3 μL of mAb human poly α-II was added to the other tube. Both tubes were incubated on ice for 90'. The pellet was washed 4 times with NNet buffer. Then it was incubated with 50 μL neuraminidase for 3 h, and, after spinning and washing, incubated overnight with 50 μL neuraminidase after which the samples were frozen at -80°C.

One-dimensional isoelectric focusing (1D-IEF)

1D-IEF was done in vertical polyacrylamide gels. The composition of the used electrode buffer, the overlay buffer and the sample buffer, was as reported by Joosten *et al.* (1993). The precipitates were thawed and resuspended in 40 μL IEF-sample buffer, incubated for 2 h at room temperature, and spinned for 2' at 13,000 g. 20 μL of supernatant was loaded in the wells, and 20 μL of overlay buffer was added carefully. Then the wells were filled with upper buffer and the electrophoresis buffer was added. After prerunning of the gels for 2 h at a V_m of 400 V, the gels were run for 18 h at a V_m of 800 V and a constant current of 15 mA.

After running, the gels were treated twice with DMSO for 30' and were fluorographed by treating the gels with DMSO-PPO for a minimum of 3 h. The gels were washed with water and were dried. Class I and II band patterns were autoradiographed on Kodak X-AR film for 1 week.

II. Infection experiment

Animals, feeding and housing

From a flock of WAD goats, established at the Agricultural University about 15 years ago (Montsma, 1986), a group of 25 mature castrated male goats with a minimal disease history were selected. All animals had previously been vaccinated against ecthyma and had received anthelminthic treatment with Ivomec (Ivermectin, MSD, AGVET, Hoddesdon, UK). The animals were 18 $\pm$ 0.1 months old and had a mean body weight of 27 $\pm$ 0.6 kg. They were the offspring from four unrelated sires; the selected subpopulation included four pairs of full sibs.

The animals had *ad libitum* access to pelleted lucerne, drinking water and salt lick. The lucerne consisted of 93 % dry matter, 16.8 $kJ \cdot g^{-1}$ gross energy and 18 % crude protein. Animals were housed individually in pens on a bedding of wood shavings. They were able to have visual contact with adjoining animals. The lights were on from 7.00 h till 19.00 h. Ambient temperature ranged between 18 and 20°C.

Experimental design

Before infection, all animals were housed in individual pens for 4 weeks. On day 0, the animals were infected intravenously with 1 x 10^6 trypanosomes. Therefore, a stabilate of *T. congolense subakia*, isolated in Nairobi, Kenya in 1961 was inoculated in mice, for multiplication of the parasite. This mouse blood was used for infection of the goats.

After 5 weeks of infection, the animals were treated intramuscularly with 7 mg Berenil/kg bodyweight after which recovery started. The time schedule of the experiment is given in Table 1.

Table 1. Time schedule of the experiment.

Day to infection	
-28	Housing of animals in individual pens; feeding of the experimental feed; start measurements on feed intake, body weight and PCV.
-10	Start measurement body temperature
0	Infection
35	Treatment with 7 mg Berenil/kg bodyweight; start recovery.

Measurements

The CLA class I and II alleles were determined by 1D-IEF. The definition of the class I alleles and the class II alleles was done by the authors, because no literature was available on CLA haplotypes of West African Dwarf goats. The nomenclature of the different genotypes resulted from the two haplotypes combined in each animal.

Before and during infection, individual feed intake was measured three times per week by offering *ad libitum* feed and by collecting feed residues afterwards. Composite samples of both offered feed and refused feed were analyzed for dry matter content (ISO 5984); from this, dry matter intake (DMI) per kg metabolic weight ($kg^{0.75}$) per day was calculated. The mean DMI in the two weeks preceding infection, as well as the mean DMI from day 5 until 35 of infection (leaving the prepatent period of 4 days out of the calculation) were calculated per animal. Also the ratio DMI [during infection / before infection] was calculated per animal.

The body weight (BW) was measured weekly, in the morning after feeding. For each animal, the body weight change during infection was calculated as the difference between BW at week 3 p.i. and BW at infection.

A blood sample was collected weekly from the jugular vene in heparinized tubes. The packed cell volume (PCV) in blood was measured by centrifugation of the blood in capillaries using a micro-haematocrit centrifuge. The ratio PCV [after 3 weeks of infection / before infection] was calculated for each animal.

Statistical analysis

Preliminary analysis showed no effect of sire on the studied parameters. The effect of CLA genotype on DMI before and during infection, on the DMI ratio, and on the body weight change and PCV ratio was tested using the following model:

$$Y_{ij} = \mu + G_i + e_{ij} \quad [1]$$

where: Y_{ij} = parameter of study; μ = overall mean; G_i = effect of Genotype (i = 1,..,6); e_{ij} = rest error term.

The model was tested using the General Linear Models Procedure of the SAS Statistical package (SAS, 1990). Only 6 CLA genotypes from 20 animals were included because of low incidence of the other genotypes. Differences between genotypes, calculated as least square means, were tested simultaneously. For all parameters involved a normal distribution of variation was found.

For all studied parameters, also a mean value per group of animals that shared a specific haplotype, was calculated. However, because goats are diploid, animals which were heterozygotic for CLA genotype, were included twice in this dataset; therefore, no statistical analysis could be done.

Table 2. Composition of CLA haplotypes and genotypes, and distribution frequencies in a flock of West African Dwarf goats.

Haplotypes	A	B	C	D	E	F
Number of animals	4	5	8	15	7	1
Class I allele[1]	1	3	4	5	6	7
Class II allele[1]	1	3	4	5	8	9

Genotypes in the goat population, observed as combinations of haplotypes[2]

AA (1)	AB (*)	AC (*)	AD (*)	BE (*)	FF (1)
AB (1)	BD (2)	CC (4)	BD (*)	DE (*)	
AC (1)	BE (2)	CD (3)	CD (*)		
AD (1)			DD (4)		
			DE (5)		

[1]: numbers refer to Figure 1;
[2]: number of animals between brackets;
*: already counted.

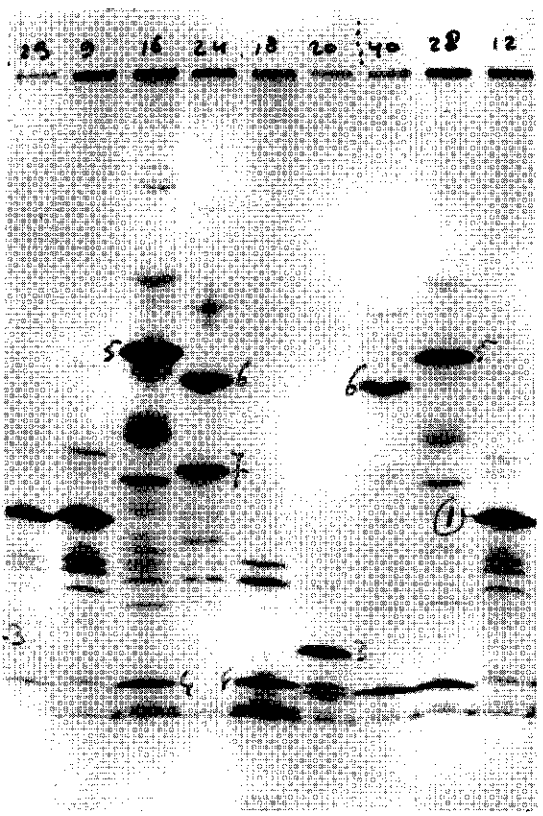
Results

CLA genotyping

In the studied population 6 different Class I and also 6 different Class II alleles were distinguished. In Figure 1 an autoradiograph of the 1D-IER analysis for class I is given; in Figure 2 an autoradiograph for class II analysis is given. An interpretative drawing of

the focussing bands of each allele is presented in Figure 1 and 2, besides the autoradiographs. No recombination between class I and class II was detected in the animals studied; therefore 6 different CLA haplotypes were defined by both class I and II polymorphism of a particular haplotype (A to F; Table 2). Because no CLA Class 1 and II typing in WAD goats has been reported before, the definition of the different haplotypes was arbitrarily chosen by the authors. The pairwise combination of haplotypes in each animals resulted in 11 different genotypes (Table 2).

Figure 1. One-dimensional isoelectric focusing (1D-IEF) autoradiographic analysis of CLA class I antigens, precipitated from cell lysates from non-stimulated peripheral blood mononuclear cells (PBMC), using mAb B1.1G6, from a flock of 25 West African Dwarf goats; included is an interpretative drawing of the focussing bands of each allele.



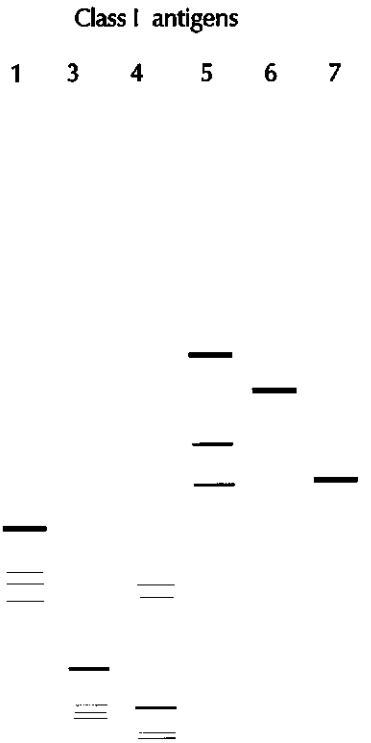
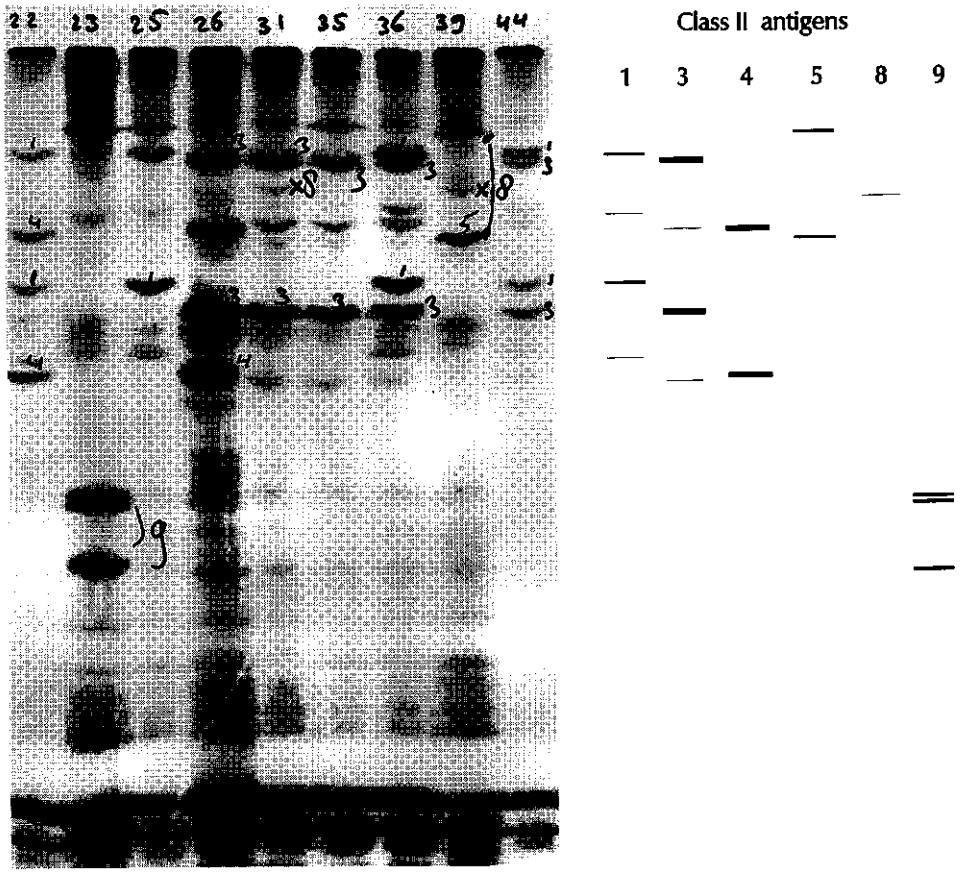


Figure 2. One-dimensional isoelectric focusing (1D-IEF) autoradiographic analysis of CLA class II antigens, precipitated from cell lysates from non-stimulated peripheral blood mononuclear cells (PBMC), using mAb human poly α-II, from a flock of 25 West African Dwarf goats; included is an interpretative drawing of the focussing bands of each allele.



Relation between CLA and clinical parameters

All animals showed a first peak of fever after approximately 4 days and parasites were detected in the blood. The infection followed a progressive course, without signs of recovery until treatment at day 35 of infection.

In Table 3 the mean voluntary DMI, PCV, body weight and rectal temperature of all animals is given. All animals showed intermittent fever and a large decrease in PCV with 40 % of initial PCV ($P < 0.001$). All animals showed a reduction of voluntary DMI, but the degree of reduction varied substantially between animals. On average, the DMI ratio was 0.54 during infection. No correlation was found between individual DMI ratio and PCV ratio ($P > 0.10$).

Table 3. Mean dry matter intake (in $g \cdot kg^{-0.75} \cdot d^{-1}$) before and during infection with *T. congolense*, and the ratio DMI [during infection / before infection], the packed cell volume before infection and after 3 weeks of infection, and the ratio PCV [after 3 weeks of infection / before infection], body weight (in kg) and rectal temperature (in °C) before and after 3 weeks of infection.

Parameter	1. Before infection		2. During infection		Ratio 2/1	
	Mean	sem	Mean	sem	Mean	sem
Number of animals	25		25			
Dry matter intake	68.2	2.6	36.3	2.9	0.54	0.04
Packed cell volume	39.8	0.8	23.9	0.8	0.60	0.01
Body weight	27.3	0.6	26.0	0.5		
Rectal temperature	38.7	0.35	39.3	0.97		

In Table 4 the mean DMI before and after infection, and the DMI ratio, as well as the body weight change and PCV ratio is presented per haplotype. No statistical analysis could be done, but the following trends were observed. Mean DMI before infection, calculated per haplotype, ranged between 65 and 74 $g \cdot kg^{-0.75} \cdot d^{-1}$.s The mean DMI per haplotype during infection, however, varied considerably (between 24 and 46 $g \cdot kg^{-0.75} \cdot d^{-1}$), as did DMI ratio (between 0.34 and 0.71). The 'A' haplotype showed the lowest DMI ratio, and the 'E' haplotype the highest ratio. Body weight changes during infection followed trends in DMI. The PCV ratio was hardly different among haplotypes.

In Table 5 the mean DMI before and after infection, the DMI ratio, the infection weight change and the PCV ratio per CLA genotype group are presented. DMI before infection tended to be affected by genotype ($P < 0.10$), whereas DMI after infection and DMI ratio were affected by genotype ($P < 0.05$). Genotype tended to affect body weight change ($P < 0.10$), but not PCV ratio.

Table 4. Dry matter intake before and during infection (in $g \cdot kg^{-0.75} \cdot d^{-1}$) and the ratio [DMI during infection / DMI before infection], body weight (BW) change during infection (in kg), and the ratio packed cell volume [after 3 weeks of infection / before infection] of West African Dwarf goats, infected with *T. congolense*; means per CLA haplotype.

CLA haplotypes	A	B	C	D	E	F
Number of animals	4	5	8	15	7	1
Dry matter intake						
before infection	74 ± 7	67 ± 10	65 ± 5	71 ± 3	66 ± 5	69
during infection	24 ± 10	39 ± 10	27 ± 4	40 ± 4	46 ± 3	42
ratio	0.34 ±0.13	0.60 ±0.11	0.45 ±0.08	0.56 ±0.04	0.71 ±0.03	0.62
BW change	-3.7 ±1.2	-1.3 ±1.2	-2.2 ±0.6	-0.9 ±0.4	0.0 ±0.3	0.5
PCV ratio	0.63 ±0.03	0.58 ±0.03	0.64 ±0.02	0.60 ±0.02	0.59 ±0.04	0.59

[1]: haplotype definitions refer to Table 2.

Table 5. Dry matter intake before and during infection (in $g \cdot kg^{-0.75} \cdot d^{-1}$), and the ratio DMI [during infection / before infection], body weight change during infection (in kg), and ratio packed cell volume [after 3 weeks of infection/ before infection] of West African Dwarf goats, infected with *T. congolense*; means per CLA genotype.

CLA Genotypes[1]	BD	BE	CC	CD	DD	DE	rmse[2]	P-value[3]
Number of animals	2	2	4	3	4	5		
Dry matter intake								
before infection	81	50	63	60	72	72	11	t
during infection	50	38	35	24	33	49	10	*
ratio	0.59	0.78	0.59	0.40	0.46	0.69	0.12	*
BW change	0.1	-0.4	-1.4	-2.2	-1.7	0.1	1.1	t
PCV ratio	0.56	0.55	0.61	0.67	0.55	0.60	0.07	ns

[1]: Genotype definitions refer to Table 2;
[2]: Root mean square error (sem = rmse/$\sqrt{n}$);
[3]: Significance of genotype effect; ns = not significant; t = tendency ($P < 0.10$); * = $P < 0.05$).

Discussion

Six different haplotypes were biochemically defined by both class I and II alleles. Because the WAD goat flock from which the animals were derived can genetically be seen as a subpopulation, and because the recombination distance between Class I and II is low it can be assumed that class I and II genes have cosegregated in the population. Consequently this resulted in 6 different MHC haplotypes in the studied population. In later studies a larger number of class I and class II alleles was found (unpublished results). This was explained by the introduction of unrelated breeding bucks in the following years.

In the present study, large variation in DMI and DMI ratio, and body weight change were observed. This corresponds with studies on *T. vivax* infected WAD goats (Verstegen *et al.*, 1991; Zwart *et al.*, 1991). Indications were found for segregation of feed intake parameters with the inherited MHC haplotypes. This effect may be linked to CLA polymorphism itself or, more likely, to other genes within the MHC locus, like the gene encoding for TNF-α or its regulators. It is not likely that CLA class I and II genes themselves exercised large impact on feed intake, as MHC molecules mainly play a role in presentation and recognition of specific antigens by T-cells (Nilsson, 1994). The specific immune response is, to our knowledge, adequately evaded by the parasite. Therefore, the TNF-α gene or related genes are a more likely candidate for the observed interaction. It was demonstrated that TNF-α plays a major role in the pathogenesis of trypanosome infection (Lucas *et al.*, 1993; Sileghem *et al.*, 1994).

The mean results per haplotype indicated that animals with haplotypes 'E', 'B' or 'D' seemed more tolerant to infection, and that animals possessing the haplotypes 'A' or 'C' were the more susceptible. However, it was not clear if the two haplotypes, combined in the same animal, showed additional effects on DMI, or that interaction between haplotypes occurred.

Therefore, animals were grouped per CLA genotype, which facilitated statistical analysis. It was found that the CLA genotype had an seffect on the DMI ratio ($P < 0.05$). Pairwise comparison showed that animals with either the genotype 'BE' or 'DE' showed a higher DMI ratio than animals with the 'CD' genotype ($P < 0.05$). Comparison between means per haplotype and means per genotype indicate that effects of combined haplotypes were probably not additive with respect to feed intake parameters. The results should be interpreted with caution, however, because of the low number of observations, from only one herd of (partly) related animals.

It is remarkable that no relation between CLA polymorphism and the degree of anaemia was found, although this is considered as one of the main characteristics of trypanotolerance. Moreover, a role for TNF-α in the induction of anaemia was hypothetized by Lucas *et al.* (1993) and Sileghem *et al.* (1994). However, the absence of a relation between PCV ratio and MHC polymorphism in our study is consistent with the observation that DMI ratio and PCV ratio were not related. It is possible that anaemia in trypanosome infection is also determined by other factors, such as interferon-τ (ILRAD, 1990).

Conclusions

A large variation between animals was found with respect to feed intake parameters during *T. congolense* infection. The results indicated that this variation was correlated to CLA class I and II polymorphism. CLA class I and II genes probably acted as a genetic marker for TNF-α gene polymorphism and therefore differences in TNF-α production. This may offer a genetic basis for differences in trypanotolerance within a trypanotolerant livestock breed.

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Chapter 4

The effect of *Trypanosoma vivax* infection on energy- and nitrogen metabolism, and serum metabolites and hormones in West African Dwarf goats at different feed intake levels

J.T.P. van Dam[1], D. van der Heide[2], W. van der Hel[1], T.S.G.A.M. van den Ingh[3], M.W.A. Verstegen[4], T. Wensing[5], and D. Zwart[1].

[1,2,4]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]Dept. of Animal and Human Physiology, Haarweg 10, 6709 PJ Wageningen;
[3]Dept. of Pathology, Utrecht University, PO Box 80.158, 3508 TD Utrecht, The Netherlands;
[4]Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen;
[5]Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht.

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J.T.P. van Dam[1], D. van der Heide[2], W. van der Hel[1], T.S.G.A.M. van den Ingh[3], M.W.A. Verstegen[4], T. Wensing[5], and D. Zwart[1].

[1,2,4]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]Dept. of Animal and Human Physiology, Haarweg 10, 6709 PJ Wageningen;
[3]Dept. of Pathology, Utrecht University, PO Box 80.158, 3508 TD Utrecht, The Netherlands;
[4]Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen;
[5]Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht.

Abstract

Effects of *Trypanosoma vivax* infection on nitrogen and energy metabolism and serum hormones and metabolites were measured using 24 castrated West African Dwarf bucks. In order to discriminate between the effect of infection and the effect of feed intake level on energy and nitrogen balance, feed quantity restriction was applied for isonutritional comparison; part of the animals were not infected and served as controls. Daily dry matter intake was measured, and energy and nitrogen balance for a 7-days period in week 2, 4 and 6 after infection. Weekly blood sampling for analysis of hormones and metabolites was done. Infected animals had a lower dry matter intake, compared with control animals, *viz.* 38.6 $\pm$ 3.2 and 16.1 $\pm$ 2.0 $g \cdot kg^{-0.75} \cdot d^{-1}$, respectively ($P < 0.001$). Intake of gross energy and nitrogen followed the same pattern. Metabolizability was not changed by infection and averaged 0.44. Heat production was increased by infection with on average 33 $kJ \cdot kg^{-0.75} \cdot d^{-1}$. Energy and nitrogen retention were negative for all groups; infection reduced energy retention and, during week 2 and 4 after infection, also nitrogen retention. The required metabolizable energy intake for maintenance was increased in infected animals (406 and 335 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for infected and control goats), based on linear regression of energy retention on metabolizable energy intake for infected and control animals. The efficiency with which energy mobilization from body stores was substituted by dietary metabolizable energy was estimated at 0.809 for both infected and control animals. The relationship between nitrogen retention and energy retention was not changed by infection. Therefore no indications were found for an increased catabolism of protein due to infection. Serum thyroxine and triiodothyronine were reduced by infection; serum metabolites and insulin levels reflected the negative energy balance in infected animals.

Introduction

Trypanosomiasis is a protozoan disease which is distributed over large parts of sub-Saharan Africa. In livestock trypanosomiasis causes high mortality and depressed productivity. The West African Dwarf (WAD) goat breed is known to withstand the deteriorating effects of trypanosome infection to a considerable degree (FAO, 1988; ILCA, 1986). This tolerance to infection consists of an ability to prevent anaemia as well as loss of productivity (Trail *et al.*, 1991).

Verstegen *et al.* (1991) and Zwart *et al.* (1991) observed a lower gross energy intake (GEI) and an increased heat production (HP), in mature WAD goats, infected with *Trypanosoma vivax*, compared with healthy goats. Because in this trial the animals were group housed, only a group estimate of HP and ER could be made, without information on individual variation. Moreover, feed intake differed between the infected and the control group.

Therefore, in the present trial the effect of *T. vivax* infection on heat production, energy- and nitrogen (N) metabolism and serum concentrations of metabolites and hormones, was measured in WAD goats, individually housed in small respiration chambers. To create a wide range of feed intake levels after infection, animals with a known high or low feed intake during trypanosomiasis were selected. Part of the infected and control animals received a restricted feed ration to enable isonutritional comparison. This enabled estimation of the efficiency of energy and N metabolism in infected and healthy goats, and the energy costs of infection.

Material and methods

Animals

Twenty-four castrated male goats with a mean age of 29 $\pm$ 0.7 months and a mean liveweight of 28 $\pm$ 0.7 kg were used. The animals had received anthelmintic treatment and ecthyma vaccination preceding the experiment.

Housing and feeding

Animals were housed in group pens with a bedding of wood shavings. Three weeks before infection the goats were moved to individual pens with the same bedding for individual measurement of feed intake. One week before infection animals were individually housed in one of the dummy chambers ("dummies"); these were replicas of the genuine respiration chambers (RC). In these dummies the animals were acclimated to the new housing and the complete social isolation which they would experience in

the RC's. This procedure was chosen because only two RC's were available; acclimation in the RC's itself would be too time-consuming. The dummies were opened once daily for measurements. The goats were housed in the dummies in the week before infection and week 1, 3 and 5 post infection (*p.i.*) In weeks 2, 4 and 6 *p.i.* they were individually housed in open-circuit indirect respiration chambers, described as chambers 3 and 4 by Verstegen *et al.* (1987).

Light was on between 07.00 h and 19.00 h. Temperature in both dummy and RC was kept at 20°C and relative humidity (RH) in the RC at 65 %. The RC's were not opened during the week; the daily feed ration could be supplied from outside the RC, by means of rubber gloves, attached to the inner wall of the RC's. Because only two RC's were available, the moment of infection for pairs of goats was scheduled after each other in time. The time span in which the respiration measurements were carried out totalled 36 weeks.

All animals received *ad libitum* pelleted lucerne preceding the experiment. During the experiment some animals received *ad libitum* pelleted lucerne, while others received a restricted pelleted lucerne ration, according to the experimental design. The dry matter (DM) of the feed was 926 ± 3.3 $g \cdot kg^{-1}$, while the ash and N concentration in the DM were 112 ± 1.1 $g \cdot kg^{-1}$ DM and 29.6 ± 0.27 $g \cdot kg^{-1}$ DM respectively. The GE concentration was 18.4 ± 0.07 $MJ \cdot kg^{-1}$ DM. Water and salt lick were freely available.

Infection

Animals were infected with *T. vivax* Y486, isolated in Nigeria by Leeflang *et al.* (1976). Blood of a previously infected goat and stored in liquid nitrogen, was defrosted and was inoculated into mice. These mice were bled after on average 6 days and mouse blood was administered intravenously to the goats at a dosage of approximately 1×10^6 parasites per animal. Control animals were sham-infected with saline. In one pair of goats infection did not establish after mouse blood administration; a second infection, 7 days later, however, succeeded. Results from the first respiration period of these two animals were removed from the dataset.

Experimental design

To create a wide range of feed intake levels after infection, the animals were selected as follows. In a previous experiment a group of 48 castrated WAD goats (mean weight 27.7 ± 1.0 kg and mean age 21.0 ± 1.4 months) had been infected with *T. congolense*. The animals received *ad libitum* pelleted lucerne. During a period of 4 weeks before and 5 weeks after this infection, animals were individually fed and feed residues were collected 3 times per week, to measure feed intake. Then all animals were medically treated with 7 mg Berenil/kg body weight (Hoechst Veterinär GmbH, München, BRD).

The average feed intake before and after infection was calculated per animal, as was the ratio feed intake [during infection / before infection]. Wassink *et al.* (1993) reported a high correlation between the dry matter intake ratio's of individual WAD goats during succeeding infections with *T. congolense* and *T. vivax*. Therefore it was expected that the feed intake response of WAD goats to *T. congolense* infection would give a reliable estimate of feed intake during future infections. So from the population of 48 goats, 8 animals with an expected high feed intake after infection were selected for the present trial (*i.e.*, low responders, LR; Ratio > 0.60), and 8 animals with an expected low feed intake after infection (high responders, HR; Ratio < 0.40). For the control group of 8 animals, a random selection was made from the remainder of the initial group of 48 animals.

To enable study of energy and N metabolism parameters at an isonutritional level, 4 control animals and 4 LR animals were randomly selected, to receive a restricted feed ration; the severity of restriction was established at the expected feed intake level of the HR group. This intake level was initially estimated at 50 % of maintenance requirements *viz.* 50 % of 51 g DM·kg$^{-0.75}$·d^{-1} (Verstegen *et al.*, 1991) which is approximately 26 g DM·kg$^{-0.75}$·d^{-1}, but was adjusted to 30 % of maintenance requirements (15 g DM·kg$^{-0.75}$·d^{-1}) after the first animal of each group had been subjected to infection, and overall feed intake had proved to be low. Because of this some variation in severity of feed restriction existed among animals within the LRR and CR group.

This procedure resulted in 5 experimental groups, *viz.* high responders (HR), low responders *ad libitum* (LRA), low responders restricted (LRR), control *ad libitum* (CA) and control restricted (CR). In Table 1 the experimental setup is depicted.

Table 1. Experimental setup.

	Infection		Control
	High Responders	Low Responders	
Ad libitum	n = 8; **HR**	n = 4; **LRA**	n = 4; **CA**
Restricted		n = 4; **LRR**	n = 4; **CR**

Measurements and calculations

Daily dry matter intake (DMI) was measured from one week before infection until euthanasia at the end of week 6 *p.i.* Body weight (BW) was measured at the start of each week using an electronic weighing scale. Body temperature was measured continuously by means of a temperature transmitter in the abdominal cavity of the goats. This

telemetric system was described by Van der Hel *et al.* (1993). Of continuous body temperature measurements one mean value per animal per day was calculated.

Energy and N balance measurements were carried out in week 2, 4 and 6 *p.i.* in the RC's, *viz.* Balance Periods (BP's) 1, 2 and 3. Therefore daily feed intake and weekly production of faeces and urine were measured. Other collected components of energy and nitrogen balance were water, used for cleaning of the chamber, condense water from the heat exchanger, and N, evaporated to the air, which was fixed in 25 % solution of sulphuric acid.

Dry matter and ash concentration (ISO 5984) were determined from offered and rest feed, and faeces. Gross energy (GE) concentration of offered feed, faeces, urine and cleaning water was determined using bombcalorimetry (IKA Analysentechniek GmbH, Heitersheim, BRD). Nitrogen was measured (ISO 5983-1991) in offered feed, faeces, urine, cleaning water, condense water and 25 % sulphuric acid solution. The composition in the DM of refused pelleted feed was assumed to be similar to the offered feed.

A mean HP per respiration week was calculated, using 9-min-interval data on O_2 consumption and CO_2 and CH_4 production and urinary N output, from 07.00h at day 2 of housing until 07.00h at day 7 of housing in the RC, according to the equation of Brouwer (1965). Within-day variation of heat production and the relation with body temperature and physical activity dynamics are reported elsewhere (Van Dam *et al.*, 1996a). MEI was calculated as the difference between GEI and faecal and urinary energy; ER was calculated as MEI minus HP. NR was calculated as nitrogen intake (NI) minus faecal and urinary nitrogen.

A blood sample was taken from the *vena jugularis* at the start of each week; a last sample was taken at the end of week 6 *p.i.* just before euthanasia. Serum levels of total Thyroxine (T4) and Triiodothyronine (T3) were determined using a homologous RIA technique; plasma glucose level and serum levels of ß-Hydroxy Buryrate (BHB), total protein (TP) and urea (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) and Non-Esterified Fatty Acids (NEFA) (NEFA C, Instruchemie B.V., Hilversum, The Netherlands) were determined enzymatically with commercially available kits. Protein spectrum was determined by electroforetic segregation on cellulose-acetate; after staining with Ponceau S, percentages of albumin, α-, ß-, and τ-globulin were measured.

Serum concentration of insulin was assessed using Radio Immuno Assay (Coat-a-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA, US). The time schedule and measurements are given in Table 2.

In whole blood parasitaemia was measured by determining the number of white blood cells (WBC) per ml blood and by establishing the WBC/trypanosome ratio in a thick smear stained with Giemsa; also Packed Cell Volume (PCV) was measured.

After BP 3 at the end of week 6 *p.i.* the animals were euthanized by injection, in accordance with Dutch welfare regulations. All infected and 4 control animals were submitted to *post mortem* gross and microscopic examination. In each animal the weight of the liver was assessed, and a fresh liver sample was stored in Phosphate Buffered Saline (pH 7.2) and was analyzed for triacylglycerol (TAG) with a commercial kit (Kit No. 405, Sigma Chemical Co., St. Louis, MO, US), to study whether infection and/or feed restriction would affect hepatic lipid metabolism.

Table 2. Time schedule and measurements[1].

Week *p.i.*	-3/-2	-1	1	2	3	4	5	6
Day *p.i.*	-7	0	7	14	21	28	35	42
Housing	ind.	dum.	dum.	RC	dum.	RC	dum.	RC
Feed ration	adl.	adl.	exp.	exp.	exp.	exp.	exp.	exp.
Procedure		inf.						PM
Feedintake	**********	**********	**********	**********	**********	**********	**********	**********
Body Temp.	**********	**********	**********	**********	**********	**********	**********	**********
Respiration trial			***BP1***		***BP2***		***BP3***	
Body weight	*	*	*	*	*	*	*	*
Blood traits	*	*	*	*	*	*	*	*

[1]: ind. individual; dum. = dummy chamber; RC = Respiration chamber; adl. = *ad libitum* ration; exp = experimental ration; inf. = infection; PM = *Post mortem* autopsy; BP 1,2,3 = Balance period 1, 2, 3.

Statistical model

Statistical analysis was carried out using SAS Statistical Package (SAS, 1990). The effect of experimental treatment, balance week and their interaction on the above described parameters were tested by means of F-test using a split-plot model [GLM procedure (SAS, 1990)], with week values within goats taken as repeated measurements:

$$Y_{ijk} = \mu + TR_i + AN(TR)_{ij} + BP_k + (TR \times BP)_{ik} + e_{ijk}; \quad [1]$$

where: Y_{ijk} = dependent variable; μ = overall mean; TR_i = fixed effect of treatment (i = 1..5); $AN(TR)_{ij}$ = random animal effect, nested within treatment group (j = 1..8 for HR or j = 1..4 for other treatments); B_k = fixed effect of balance period (k = 1..3); $(TR \times BP)_{ik}$ = effect of interaction between BP and Treatment; e_{ijk} = error term.

TR_i was tested against $AN(TR)_{ij}$ as error term; BP_k and $(TR \times BP)_{ik}$ were tested against e_{ijk}. When effects were not significant they were removed from the model.

Custom hypothesis tests were carried out on the effect of Infection treatment using Analysis of Variance; For this purpose, only data of *ad libitum* fed animals were used

(from experimental groups HR, LRA and CA). Covariance analysis, using model [1] with the addition of covariables (1) GEI, to test ME intake, (2) MEI, to test ER, and (3) ER, to test NR, was carried out on mean values per goat for the three balance periods, or in the case of NR, analysis was performed separately per BP.

Results

General course of infection

After infection all animals showed fever after approximately 5 days and were found blood positive for *T. vivax* parasites, except two animals; these were infected again one week later. Clinical signs included intermittent fever and severe anaemia. PCV dropped to an average 22 % in week 5, after which it stabilized. Infected animals showed intermittent fever, with average deep body temperature increased from 38.51 ± 0.04°C to 39.76 ± 0.07°C (control vs. infected goats; $P < 0.001$). Parasitaemia was high, on average $6.3 \times 10^9 \pm 1.4 \times 10^9$ trypanosomes·L^{-1} blood, but greatly fluctuated; towards the end of the infection period sometimes no parasites could be detected.

Two animals died before the end of the experiment. One animal of the LRA group died on day 39 as a result of *T. vivax* infection, and one animal of the HR group died on day 35, probably due to the combined effect of *T. vivax* and Clostridium enterotoxaemia infection. At day 42 the remaining animals were euthanized. Gross and microscopic *post mortem* examination of the infected animals revealed a marked reactive lymphoid hyperplasia of the lymph nodes and spleen. Several infected animals had lymphocytic infiltration of the endocardium, epicardium and myocardium. No renal or intestinal lesions were found in infected animals. The liver showed a non-specific reactive hepatitis; often a mild to moderate zonal fatty infiltration of the liver was seen. Control animals showed no abnormalities except two animals showing (some) zonal fatty infiltration of the liver.

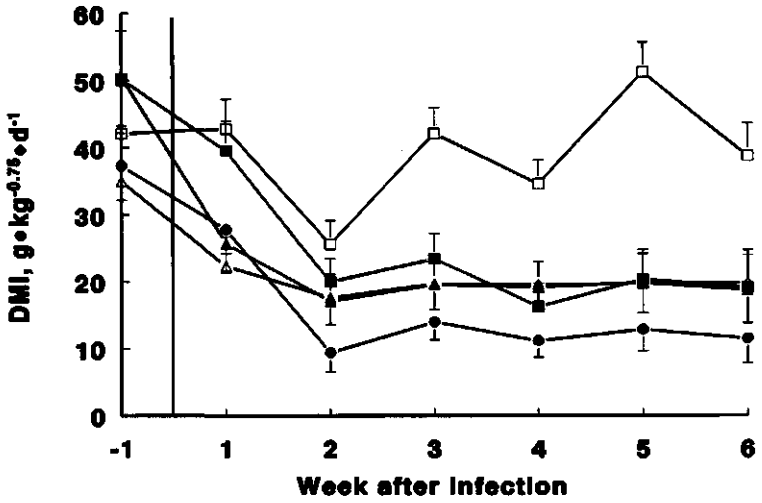
Liver weight of the experimental groups at autopsy was 743 ± 32, 676 ± 44, 764 ± 78, 559 ± 19, and 536 ± 25 g for respectively HR, LRA, LRR, CA and CR group. Infected *ad libitum* fed animals had a higher liver weight than *ad libitum* fed controls ($P < 0.01$). When expressed as liver weight·kg$^{-0.75}$ significance was even stronger (61.2 ± 2.0 vs. 42.3 ± 3.8 g·kg$^{-0.75}$ for infected and control goats respectively; $P < 0.001$).

Feed intake and body weight change

In Figure 1 the average daily DMI is depicted for the 5 treatment groups. Overall feed intake was relatively low. DMI of HR animals was not significantly different from DMI of LRA animals. Housing of CA goats in individual RC's significantly reduced DMI

compared with their DMI in the dummy (33.1 ± 2.3 and 44.6 ± 2.0 g DM·kg$^{-0.75}$·d^{-1}, respectively; P< 0.001). Average *ad libitum* DMI from week 1 *p.i.* until week 6 *p.i.* was significantly reduced in infected animals, compared with control animals, from 38.6 ± 3.2 to 16.1 ± 2.0 g·kg$^{-0.75}$·d^{-1} (P< 0.001).

Figure 1. Dry Matter intake (DMI) of West African Dwarf goats after infection with *Trypanosoma vivax* (HR —●—, LRA —■—, LRR —▲—, CA —□— and CR —△—; error bars indicate sem).



In Figure 2 the BW relative to week 0 is presented. All treatment groups lost weight during the infection period; this loss was up to 21 % in the HR group. A significant effect of infection on BW was found from 3 weeks *p.i.* onwards (P< 0.001). Daily BW change of *ad libitum* fed groups, calculated over the infection period, was more negative in *ad libitum* fed infected animals (-144 ± 14 g·d^{-1}, compared with -29 ± 23 g·d^{-1} for *ad libitum* fed control animals; P< 0.001).

Energy metabolism

In Table 3 energy metabolism data are presented. No significant effect of BP was found on the parameters involved; consequently one Least Square mean per treatment group for the whole infection period was estimated. Compared with *ad libitum* fed controls, GEI and MEI were reduced in *ad libitum* fed infected animals (P< 0.001), while HP was increased with 33 kJ·kg$^{-0.75}$·d^{-1} to 345 kJ·kg$^{-0.75}$·d^{-1} (P< 0.05) and the respiratory quotient (RQ) was lower (0.80 vs. 0.94; P< 0.001). This resulted in a lower ER in

infected animals of -271 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, compared with -113 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in control animals ($P < 0.001$).

Figure 2. Body Weight (BW) of West African Dwarf goats after infection with *Trypanosoma vivax*, as a percentage of Body Weight at week 0 (HR —●—, LRA —■—, LRR —▲—, CA —□— and CR —△—; error bars indicate sem).

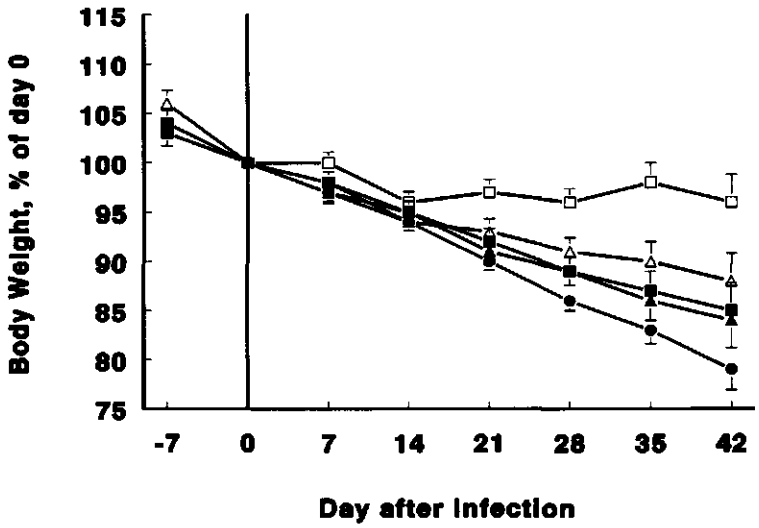


Table 3. Energy metabolism parameters of West African Dwarf goats after infection with *Trypanosoma vivax* (in $kJ \cdot kg^{-0.75} \cdot d^{-1}$).

Treatments[1]	HR	LRA	LRR	CA	CR	rmse[2]	— P-values[3] — Model	Inf.
No. animals	8	4	4	4	4			
GEI	162^{a}	291^{a}	288^{a}	520^{b}	299^{a}	97	***	***
MEI	46^{a}	102^{ab}	113^{ab}	199^{b}	123^{ab}	54	**	***
HP	342^{a}	349^{a}	340^{ab}	312^{ab}	292^{b}	15	*	*
RQ	0.78^{a}	0.83^{ab}	0.83^{ab}	0.94^{c}	0.85^{b}	0.03	***	***
ER	-296^{a}	-246^{ab}	-227^{ab}	-113^{c}	-168^{bc}	47	***	***

[1]: Treatment HR= High Responder; LRA= Low Responder *ad libitum* feeding; LRR= Low Responder Restricted feeding; CA= Control animals *ad libitum* feeding; CR= Control animals Restricted feeding;
[2]: Root mean square error (sem= $rmse/\sqrt{n}$);
[3]: Significancies of F-test of full model and of infection treatment (HR+LRA vs. CA); ns= not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
^{a,b,c}: *treatment means with common superscripts do not differ (P-level 0.05).*

Covariance Analysis of MEI, using model [1] with the addition of covariable GEI and one mean value per goat, showed a significant effect of GEI on MEI, with no effect of infection treatment. Therefore one regression equation was estimated:

$$MEI = -22.9\ (\pm\ 6.1) + 0.444\ (\pm\ 0.019) \times GEI\ (n = 24;\ r^2 = 0.96) \quad [2]$$

(MEI and GEI in $kJ \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets).

Both intercept and slope of the equation were not affected by infection.

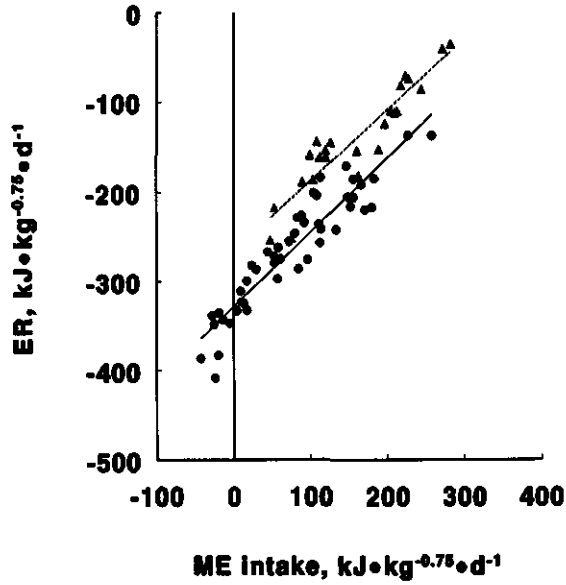
Analysis of Variance of ER, using model [1] with the addition of MEI as a covariable, with one mean value per goat, revealed a significant effect of MEI on ER. The estimated regression equation of ER on MEI ran as follows for infected and control animals (different intercept, $P < 0.001$; slopes not significantly different and estimated simultanuously) and is depicted in Figure 3:

$$\text{Infected: } ER = -328\ (\pm\ 8.3) + 0.809\ (\pm\ 0.081) \times MEI \quad [3]$$

$$\text{Control: } ER = -271\ (\pm\ 11.7) + 0.809\ (\pm\ 0.081) \times MEI \quad [4]$$

(ER and MEI in $kJ \cdot kg^{-0.75} \cdot d^{-1}$; $n = 24$; $r^2 = 0.93$; sem between brackets).

Figure 3. The relation between Energy Retention (ER) and Metabolizable Energy intake (MEI) after infection with *Trypanosoma vivax* (infected animals —●—, control animals - -▲- -).



Nitrogen metabolism

In Table 4 results on the N metabolism are given. Because BP had a significant effect on the parameters describing N metabolism, they are presented separately for each of the three BP's. N intake was affected by infection throughout the infection period ($P < 0.05$ in BP 1; $P < 0.001$ in BP 2 and BP 3). NR was reduced by infection in BP 1 and 2 ($P < 0.05$ respectively $P < 0.001$) but not any more in BP 3.

Addition of ER as a covariable to model [1] showed no effect of infection on NR; so model [1] was reduced to a linear regression model and resulted in the following equations:

BP 1: $NR = 0.171\ (\pm 0.049) + 0.00189\ (\pm 0.00021) \times ER$; $(n = 22; r^2 = 0.81)$;[5]
BP 2: $NR = 0.201\ (\pm 0.035) + 0.00188\ (\pm 0.00015) \times ER$; $(n = 24; r^2 = 0.88)$;[6]
BP 3: $NR = 0.158\ (\pm 0.038) + 0.00136\ (\pm 0.00017) \times ER$; $(n = 23; r^2 = 0.76)$;[7]
(NR in $g \cdot kg^{-0.75} \cdot d^{-1}$; ER in $kJ \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets).

Table 4. N metabolism parameters of West African Dwarf goats after infection with *Trypanosoma vivax* (in $g \cdot kg^{-0.75} \cdot d^{-1}$).

Treatments[1]	HR	LRA	LRR	CA	CR	rmse[2]	P-values[3] Model	P-values[3] Inf.
				BP 1				
No. of animals	6	4	4	4	4			
N intake	0.258^{a}	0.548^{ab}	0.470^{ab}	0.704^{b}	0.483^{ab}	0.198	*	*
urinary N	0.543	0.569	0.556	0.605	0.436	0.104	ns	ns
faecal N	0.112^{a}	0.226^{ab}	0.175^{ab}	0.298^{b}	0.173^{ab}	0.073	*	**
N retention	-0.402^{a}	-0.257^{ab}	-0.267^{ab}	-0.210^{b}	-0.131^{b}	0.090	**	*
				BP 2				
No. of animals	8	4	4	4	4			
N intake	0.308^{a}	0.452^{a}	0.527^{ab}	0.949^{b}	0.535^{ab}	0.199	**	***
urinary N	0.518	0.527	0.569	0.586	0.423	0.092	ns	ns
faecal N	0.134^{a}	0.206^{ab}	0.201^{ab}	0.358^{b}	0.196^{ab}	0.073	**	***
N retention	-0.351^{a}	-0.294^{ac}	-0.248^{ab}	-0.002^{b}	-0.089^{bc}	0.125	**	***
				BP 3				
No. of animals	7	4	4	4	4			
N intake	0.319^{a}	0.518^{ab}	0.512^{ab}	1.059^{b}	0.544^{ab}	0.272	**	***
urinary N	0.412^{a}	0.410^{a}	0.436^{a}	0.690^{b}	0.401^{a}	0.075	***	***
faecal N	0.135^{a}	0.180^{ab}	0.172^{a}	0.388^{b}	0.183^{ab}	0.095	**	***
N retention	-0.238	-0.085	-0.102	-0.025	-0.046	0.153	ns	ns

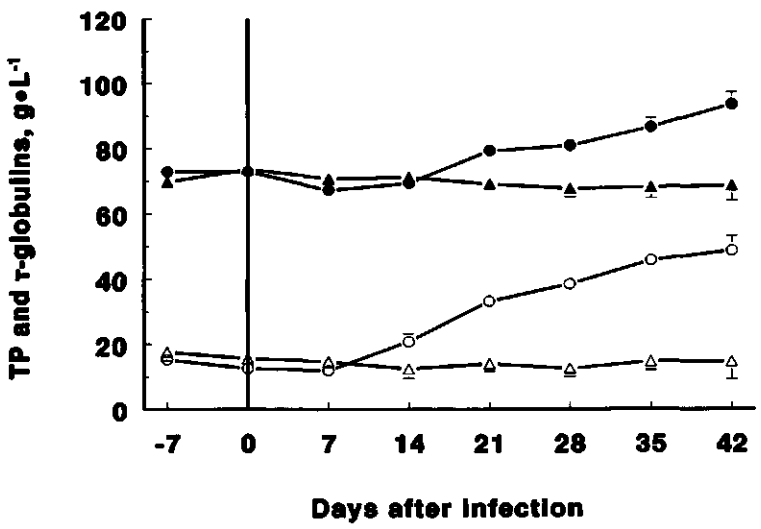
[1]: Treatment HR = High Responder; LRA = Low Responder *ad libitum* feeding; LRR = Low Responder Restricted feeding; CA = Control animals *ad libitum* feeding; CR = Control animals Restricted feeding;
[2]: Root mean square error (sem = rmse/$\sqrt{n}$);
[3]: Significancies of F-test of full model and of infection treatment (HR + LRA vs. CA); ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
[a,b,c]: treatment means with common superscripts do not differ (P-level 0.05).

Serum metabolite and hormone levels; liver TAG level

In Table 5 the serum levels of glucose, BHB and NEFA are given. Because within either the period before or after infection no effect of week number was found, results are presented as least square means before and after infection. Levels of glucose and BHB were not affected, but NEFA level was increased in infected animals ($P < 0.001$). The concentration of hepatic TAG at autopsy averaged 18.6 ± 3.3, 14.0 ± 3.2, 18.4 ± 2.4, 6.4 ± 1.9 and 14.5 ± 5.2 $g \cdot kg^{-1}$ wet weight for HR, LRA, LRR, CA and CR treatment respectively. A negative correlation between DMI during the infection period and TAG level was found ($n = 24$; $r = -0.69$; $P < 0.001$). The average NEFA concentration per animal over the infection period was negatively correlated with average DMI during the infection period ($n = 24$; $r = -0.66$; $P < 0.001$) and positively correlated with liver TAG level, measured at autopsy ($n = 24$; $r = 0.44$; $P < 0.05$).

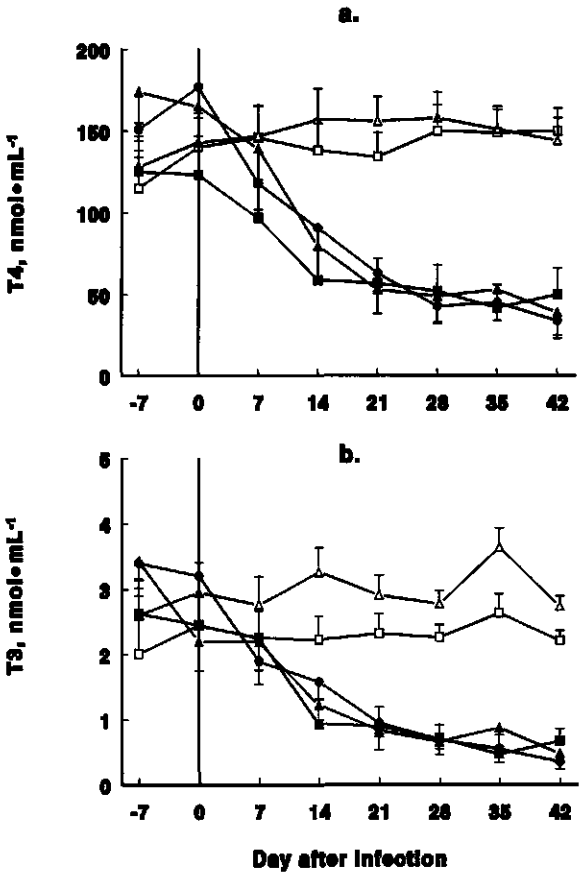
Serum urea level tended to be increased in infected animals, compared with control animals, in week 1 *p.i.* only (11.9 ± 1.0 vs. 8.1 ± 1.6 $mmol \cdot L^{-1}$; $P = 0.06$). One anomalous urea value was removed from the dataset. Serum concentration of insulin was significantly reduced ($P < 0.001$) in all groups during the experimental period compared with the pre-experimental concentration (10.0 ± 2.0 vs. 23.8 ± 2.0 $mi.u. \cdot L^{-1}$).

Figure 4. Serum levels of total protein (TP) and γ-globulins (GG) of West African Dwarf goats after infection with *Trypanosoma vivax* (—●— TP of infected animals, —▲— TP of control animals, —○— γ-globulins of infected animals, and —△— γ-globulins of control animals; error bars indicate sem).



In Figure 4 the average serum levels of TP and γ-globulins are given for the infected and control animals. Both TP and γ-globulins were increased in infected animals from week 3 *p.i.* onwards ($P < 0.001$). Albumin level was decreased in infected animals in week 5 and 6 *p.i.* ($P < 0.05$). In Figure 5 the serum concentration of total T4 and T3 are depicted for the 5 different treatment groups. Both T4 and T3 levels were decreased in infected animals from 3 weeks *p.i.* onwards ($P < 0.001$).

Figure 5. Serum levels of **a.** Thyroxine (T4) and **b.** Triiodothyronine (T3) of West African Dwarf goats (in nmol·mL^{-1}) after infection with *Trypanosoma vivax* (HR —●—, LRA —■—, LRR —▲—, CA —□— and CR —△—; error bars indicate sem).



Discussion

General course of infection

Infection followed a similar pattern as in previous studies with *T. vivax* infection in WAD goats (Akinbamijo *et al.*, 1992; Verstegen *et al.*, 1991; Van den Ingh *et al.*, 1976b) with hardly any signs of recovery to the end of the experimental period. Nevertheless most animals still had considerable body fat reserves at autopsy, due to luxuous body condition at the start of the experiment. Towards the end of the infection period, sometimes no parasites were detected due to the large fluctuation in parasite levels in trypanosomiasis (Van den Ingh *et al.*, 1976b) and also the relatively low sensitivity of the counting procedure.

The observed lymphocytic infiltration of the endocardium, epicardium and myocardium was also reported in cattle, infected with the same *T. vivax* strain (Van den Ingh *et al.*, 1976a). In the present study liver weight of infected animals was increased. Probably this was associated with the observed non-specific reactive hepatitis.

The observed severity of infection is not in agreement with the assumed trypanotolerance to infection of WAD goats (FAO, 1988). However, after an initially severe course of infection the animals may show spontaneous recovery, as observed in WAD goats by Osaer *et al.* (1994), about two months after infection with *T. congolense*.

Table 5. Glucose, NEFA and BHB concentration in serum of West African Dwarf goats before and after infection with Trypanosoma vivax (in mmol·L^{-1}).

Treatments[1]	HR	LRA	LRR	CA	CR	rmse[2]	P-values Model	P-values Inf
No. of animals	8	4	4	4	4			
				Before infection				
glucose	3.19	3.15	2.98	3.06	2.90	0.26	ns	ns
NEFA	0.256	0.199	0.175	0.283	0.209	0.156	ns	ns
BHB	0.129	0.126	0.148	0.234	0.131	0.149	ns	ns
				After infection				
glucose	2.89	2.97	2.87	3.02	2.90	0.51	ns	ns
NEFA	0.745^{a}	0.543^{ab}	0.714^{a}	0.201^{b}	0.381^{ab}	0.306	**	**
BHB	0.491	0.290	0.681	0.133	0.173	0.861	ns	ns

[1]: Treatment HR = High Responder; LRA = Low Responder *ad libitum* feeding; LRR = Low Responder Restricted feeding; CA = Control animals *ad libitum* feeding; CR = Control animals Restricted feeding;
[2]: Root mean square error (sem = rmse/$\sqrt{n}$);
[3]: Significancies of F-test of full model and of infection treatment (HR + LRA vs. CA); ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
[a,b,c]: treatment means with common superscripts do not differ (P-level 0.05).

Feed intake and body weight change

Overall DMI was extremely low, also in the CA treatment group, compared with pre-experimental level. Consequently the level of the chosen feed restriction for the LRR and CR group proved higher than the *ad libitum* DMI of the HR group. Nevertheless, of the LRR group only one animal had considerable left-overs in the first BP; in general the other restricted animals readily consumed their ration. From previous studies (Akinbamijo *et al.*, 1992; Zwart *et al.*, 1991) a higher DMI for both healthy and infected animals had been expected (with a mean DMI around 45 g $DM \cdot kg^{-0.75} \cdot d^{-1}$). As a result of low DMI all groups lost weight during the experimental period.

A possible reason for the very low DMI may be the effect of the social isolation that the animals were submitted to in the RC's, which was more complete than in the dummies; there the animals could still hear each other. Van Adrichem and Vogt (1993) and Bowers *et al.* (1993) found indications for stress in sheep, due to social isolation. Also the fatness of the animals may have led to a lower DMI compared with previous studies (Forbes, 1995).

The DMI reduction, relative to pre-infection level, of high and low responders (HR and LRA group) was not correlated with their DMI response in the previous infection (Van Dam *et al.*, 1996a); also no significant difference was found between mean DMI of the HR and LRA group. This conflicts with findings of Wassink *et al.* (1993). A possible reason for the low correlation between the two subsequent DMI responses to infection is the different housing systems in the two infection trials.

Energy metabolism

GEI was reduced in all treatments as compared to pre-experimental level. Metabolizability was estimated at 0.44, being the slope of equation [2]. This was about the same as reports of Verstegen *et al.* (1991). Equation [2] was not affected by infection, *i.e.*, no increase of faecal and/or urinary energy losses was found at a given GEI level. This indicates that digestion and renal function were not negatively affected by infection, and this was confirmed at autopsy.

Heat production was increased by infection with 33 $kJ \cdot kg^{-0.75} \cdot d^{-1}$. The intercepts of equation [3] and [4], which represent HP at ME = 0 were -328 and -271 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for infected and control animals respectively; these can be considered as a measure of Basal Metabolic Rate (BMR). Infection increased the intercept with 58 $kJ \cdot kg^{-0.75} \cdot d^{-1}$. The difference of intercept between infected and control animals is a more accurate estimate of HP increase due to infection, because it is corrected for feed intake level.

Ketelaars and Tolkamp (1991) estimated Fasting Heat Production (FHP) of goats at 275 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, which is in accordance with our estimated BMR for controls. Blaxter and Boyne (1982) estimated FHP for adult sheep on 240 $kJ \cdot kg^{-0.75} \cdot d^{-1}$.

The ME requirements for maintenance (ME_m) were calculated as the MEI at zero ER, and were 335 and 406 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for control and infected animals, respectively (increase of 22 % in infected animals). ME_m gives an indication of the energy cost of infection for animals around maintenance intake level. The slope of equations [3] and [4] represents an estimate of the efficiency with which MEI substitutes for depletion of body energy stores, k_m. This estimate was the same for infected and control animals, *viz.* 0.809. The efficiency with which MEI is converted in body growth (k_f, for intake levels above maintenance) could not be estimated in the present trial, due to the low feed intake.

Verstegen *et al.* (1991) estimated ME_m at 375 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for control animals and 464 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for infected animals (25 % increase). However, their calculation of ME_m was based on assumed efficiencies with which MEI substitutes for body energy stores. NRC (1981) reported an ME_m for healthy goats of 424 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, based on 10 original estimates from literature; Zemmelink *et al.* (1991) reported an ME_m of 384 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, in two experiments with 24 young healthy WAD goats.

Our estimate of k_m of 0.809 is higher than the average k_m of 0.66 for pelleted feeds with a metabolizability of 0.44, as reported by ARC (1980), but is in agreement with the assumed k_m of 0.80 of Verstegen *et al.* (1991).

The low ME_m for control goats and the relatively high k_m in the present experiment may have been induced by feed restriction. Olthoff *et al.* (1989) showed that a period of feed restriction of sheep prior to a respiration study led to a lower ME_m and an increased k_m. In our experiment possibly both the experimental feed restriction of LRR and CR goats, and the intake reduction due to isolated housing may have triggered a lower ME_m.

Nitrogen metabolism

The intake level of N followed the feed intake pattern. Apparent N digestibility was estimated at about 0.61 (Table 4); this is slightly higher than Verstegen *et al.* (1991) reported. No effect of infection was found; this agrees with studies of Akinbamijo *et al.* (1992) and Verstegen *et al.* (1991).

The regression equations of NR on ER (equations [5-7]) were not different for infected and control animals. Overall N utilization in relation to ER was very efficient with an estimated positive NR of on average 0.153 $\pm$ 0.038 $g \cdot kg^{-0.75} \cdot d^{-1}$ at ER = 0 and a slope of 0.0016 $\pm$ 0.00016 g $NR \cdot kJ^{-1}$ ER (average values per goat; n = 24; r^2 = 0.82). Verstegen *et al.* (1991) found a slope between 0.0009 and 0.0013 g $NR \cdot kJ^{-1}$ ER, which is much lower. Possibly the low feed intake level induced a more efficient N metabolism at a given ER. Also Akinbamijo *et al.* (1992) showed that N metabolism became more

efficient when animals received a restricted feed ration. Our observed values should therefore be treated with caution.

Concluding, infection did not reduce efficiency of N utilization at a given ER. The general conception that infection induces catabolism rather than lipolysis, as reported by Beisel (1985) is not confirmed in this experiment.

Serum metabolite and hormone levels; liver TAG level

Serum urea was increased by infection only in week 1 *p.i.* This may have been caused by a slight increase of protein catabolism (Payne, 1989); this was also found incidentically by Verstegen et *al.* (1991). Taking into account the N balance data, however, this increase was of minor importance.

NEFA level tended to be increased in the HR and LRR group, compared with the CA group; this indicates an increased lipolysis. This corresponded with the reduced feed intake and the low insulin levels, relative to pre-infection.

Liver TAG level was increased in animals with low DMI. This was probably the only experimental factor of influence, without a direct effect of infection on TAG level. Our TAG levels were comparable to those of dairy goats, either restricted or fed *ad libitum* in a 2-months period before parturition (Van den Top et *al.*, 1995). They also observed a negative relationship between feed intake and liver TAG level. Veenhuizen et *al.* (1991), however, found much higher hepatic TAG levels in dairy cows with experimentally induced ketosis (8 to 10 % of wet weight).

NEFA level was positively correlated with liver TAG level. This was also found by Veenhuizen et *al.* (1991) and Van den Top et *al.* (1995). The increased hepatic TAG level probably resulted from an increased hepatic uptake of NEFA. No evidence was found for impairment of liver function by infection with respect to lipid metabolism; on average mobilization of energy substrates was not hampered and ketogenesis was limited.

Serum TP level was increased in infected animals, mainly due to increase of the γ-globulin fraction; this is a common finding in trypanosomiasis where antibodies are produced to the variable antigen types (VAT's) of each successive wave of parasitaemia (Morrison et *al.*, 1985).

T3 and T4 levels were decreased due to infection. Abebe and Eley (1992) and Mutayoba and Gombe (1989) found the same for T4. This is a consistent finding in trypanosomiasis, despite the fact that HP was increased after infection (Zwart et *al.*, 1991). Abdullah and Falconer (1977) found a positive correlation between feed intake and serum T4 concentration in goats. In the present study T3 level of the CR group tended to be higher than T3 of the CA group, despite a lower intake of the former group.1

Conclusions

West African Dwarf goats, infected with *Trypanosoma vivax*, showed a reduced DMI and increased heat production. Consequently, energy and N balance were reduced in infected animals to levels below zero retention, and ME requirements for maintenance were increased. However, metabolizability and the estimated k_m were not changed by infection. No indications were found for an increased catabolism of protein due to infection.

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Chapter 5

Heat production, body temperature, and body posture in West African Dwarf goats infected with *Trypanosoma vivax*

J.T.P. van Dam[1], J.W. Schrama[1,2], W. van der Hel[1], M.W.A. Verstegen[2] and D. Zwart[1].

[1,2]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]: Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen.

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J.T.P. van Dam[1], J.W. Schrama[1,2], W. van der Hel[1], M.W.A. Verstegen[2] and D. Zwart[1].

[1,2]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]: Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen.

Abstract

The relationships between heat production, body temperature, and body posture (standing/lying) were studied in goats suffering from trypanosomiasis. Sixteen goats were selected and infected with 1×10^6 *Trypanosoma vivax* parasites and 8 goats served as controls. In weeks 2, 4, and 6 after infection heat production, body posture, and body temperature were measured at 15-minute intervals. Heat production was higher ($P < 0.01$) in infected animals compared with control animals (342, respectively 306 $kJ \cdot kg^{-0.75} \cdot d^{-1}$), body temperature was also higher ($P < 0.001$) in infected goats (39.78°C, respectively 38.51°C). The standing related energy costs per day were lower in infected animals (27 respectively 36 $kJ \cdot kg^{-0.75} \cdot d^{-1}$). Infected animals, therefore, masked part of the energy costs of infection by reducing the standing time. The heat production of infected animals was increased by 21 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ per 1°C fever (7 % increase). During periods of standing, body temperature increased with time, whereas during lying periods, it decreased. The number of standing periods was increased in infected animals. It was discussed whether postural behaviour is influenced by thermoregulatory mechanisms.

Introduction

The protozoa *Trypanosoma vivax*, which in sub-Saharan Africa is transmitted by flies of the genus *Glossina Spp.*, is a major constraint to animal production. In tsetse infested areas all domestic species are at risk of infection. The disease causes intermittent fever, anorexia, and anaemia. The dynamics of the fever reflect the immune response to subsequent peaks of parasite sub-populations in the blood (Stephen, 1986). Zwart *et al.* (1991) showed that heat production (HP) was increased in goats infected with *T. vivax*.

Heat production partly results from physical activity (Blaxter, 1989). Van Diemen *et al.* (1995) demonstrated that about 24 % of the total heat production of pigs was related to activity. Animal behaviour may be changed by infection and consequently thereby also heat production. Normally animals are less active during disease (Hart, 1985). Van

Diemen *et al.* (1995) showed that exposure to *Pasteurella multocida* toxin tended to decrease activity-related HP in young pigs.

Apart from the indirect effect of activity, total heat production can be affected by fever due to infection. An increase in body temperature (BT) will accelerate chemical processes (Blaxter, 1989). According to the theoretical Van 't Hoff/Arrhenius relationship, heat production increases by 10 % per °C increase in BT (the 'energy cost of fever'). However, in clinical studies in which fever is induced, large variations in the energy cost of fever are found (Baracos *et al.*, 1987).

Therefore, in the present study it was investigated how a *Trypanosoma vivax* infection in West African Dwarf goats affected heat production, body temperature, and physical activity. From these results the metabolic cost of trypanosome infection was estimated and it was studied if physical activity, measured as body posture (standing/lying behaviour), affected this relation.

Material and methods

Experimental design and housing

In the present study, data on heat production, posture, and body temperature were used from a previously reported experiment on the effect of *T. vivax* infection on energy and nitrogen balances (Van Dam *et al.*, In press). From a group of 24 castrated West African Dwarf goats, 16 animals were randomly selected and were infected intravenously with *T. vivax* Y486 stabilate (Leeflang *et al.*, 1976), with about 1×10^6 parasites per animal. The remaining 8 animals served as controls and were injected with saline. The moment of infection was defined as the start of the experimental period. From both treatment groups 4 animals were assigned randomly to a restricted lucerne ration of $17 g \cdot kg^{-0.75} \cdot d^{-1}$. All other animals had *ad libitum* access to lucerne. Every day, animals were given feed between 08.00h and 09.00h. The animals were housed individually in 'dummy' chambers, to allow them to adapt to the respiration chambers, in the week before infection, as well as in the 1st, 3rd, and 5th week post infection (p.i.) In weeks 2, 4, and 6 p.i., goats were housed individually in one of two open-circuit, indirect climatic respiration chambers, with an iron mesh floor (Verstegen *et al.*, 1987). Goats were tethered but could move freely to stand up and lie down. Lights were on between 7.00h and 19.00h and ambient temperature was maintained at 20°C. In the respiration chambers the relative humidity was maintained at approximately 65 %.

Measurements

After 1 week of adaptation in the dummy chamber, and 1 day in the respiration chamber, measurements were started. The consumption of O_2 and production of CO_2 and CH_4 were measured for each goat during successive 9-minute intervals. From this gaseous exchange, HP was calculated according to Brouwer (1965). BT was recorded in succeeding intervals of about 7.5 minutes with a temperature transmitter using a telemetric system as described by Van der Hel *et al.* (1993). Therefore, 3 weeks before infection, a temperature transmitter was implanted surgically in the abdominal cavity of each goat. Implantation was carried out after laparotomy in the left side under complete halothane anaesthesia. No health problems occurred after surgery. Within 3 days after surgery, feed intake returned to normal levels and 1 week after surgery the wound was healed.

The goat's body posture (standing or lying) was registered with a photo-electric cell (Telemecanique, XUG-F04031, Technische Unie, Arnhem, The Netherlands), as described by Schrama *et al.* (1993). Posture was recorded every minute within each 9-minute period associated with the measurement of HP. The body posture during a specific 9-minute period was defined as 'standing', if the goat had stood for more than 50 % of this interval; otherwise it was defined as 'lying'.

Calculations

Measurements during the feeding period (08.00h to 09.00h) were excluded. Total heat production (HP_{tot}) was the average of all 9-minute HP values during day 2 to 7 of each week that the animals were housed in the respiration chamber. Heat production during standing (HP_{st}) and during lying (HP_{ly}) were obtained by averaging the 9-minute HP values during standing and lying respectively. The energy cost of standing (ECS) was the difference between HP_{st} and HP_{ly}. Total daily body temperature (BT_{tot}), BT during standing (BT_{st}), BT during lying (BT_{ly}), and the difference between BT_{st} and BT_{ly} were calculated as described for the HP variables. Time spent standing (f_{st}) was obtained from the daily percentage of 9-minute periods spent standing. The extra daily amount of energy expenditure due to standing ($HP_{f \times ECS}$) was obtained by multiplication of ECS by the time spent standing. Furthermore, the number of standing periods (N_{st}) and the average duration of a standing (T_{st}) and of a lying (T_{ly}) period were derived from the 9-minute body posture measurements.

These HP, BT, and posture variables were calculated for each goat separately per respiration period. Zwart *et al.* (1991) demonstrated in goats that the effect of *T. vivax* on HP varied within a day. Therefore, HP_{tot}, time spent standing, and HP_{ly} (heat production corrected for standing) were calculated separately for the light (07.00h to 19.00h) and dark phases of the respiration week (19.00h to 07.00h).

For the study on the relationship between BT and HP the separate datasets on BT and HP were merged into one dataset with a mean BT, body posture, and HP, per 15-minute period. The relation between HP and BT, representing the energy cost of fever (ECF), was assessed by linear regression of the 15-minute data for HP on BT per goat for either standing or lying periods.

The average BT for successive 30-minute intervals after the onset of either a standing or lying period was calculated, to study the time-related change due to body posture. Therefore BT was related to time after the onset of either standing or lying, by linear regression. Data recorded 200 minutes after postural changes were excluded, because of very low frequency.

Statistical analysis

Statistical analysis was carried out using the SAS statistical package (1990). Preliminary testing of the parameters described above revealed no effect of experimental feed ration (restricted versus *ad libitum*) nor an interaction with other treatments, and thus this variable was removed from the statistical model. The effects of infection, respiration week, and their interaction on the above described parameters of HP, BT, and posture were tested by means of F-test, using a split-plot model [GLM procedure (SAS, 1990)], with week values within goats taken as repeated measurements:

$$Y_{ijk} = \mu + INF_i + e_{1;ij} + WEEK_k + (INF \times WEEK)_{ik} + e_{2;ijk} \quad [1]$$

in which Y_{ijk} = Parameter studied; μ = overall mean; INF_i = fixed effect of infection (i = 1,2); $e_{1,ij}$ = error term 1 which represents the random effect of goat nested within infection treatment i (for i = 1, j = 1,..,8; for i = 2, j = 1,..,16); $WEEK_k$ = fixed effect of respiration week (time after infection) (j = 1,2,3); $(INF \times WEEK)_{ik}$ = interaction effect between infection and week; $e_{2;ijk}$ = error term 2. The effect of infection was tested against error term 1, whereas the effect of week and the interaction was tested against error term 2.

For different reasons, the data for 3 infected animals and 1 control animal were omitted from the dataset.

Results

In Table 1 mean HP_{tot}, HP during standing (HP_{st}) and lying (HP_{ly}), energy cost of standing (ECS), time spent standing (f_{st}), and the standing related HP ($HP_{f \times ECS}$) are given for infected and control animals. HP_{tot} was increased ($P < 0.01$) with 36 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in

infected animals. HP_{st} tended to be increased in infected animals ($P < 0.10$) by 35 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, whereas HP_{ly} was increased ($P < 0.01$) in infected animals by 45 $kJ \cdot kg^{-0.75} \cdot d^{-1}$. Both ECS and f_{st} were slightly lower in infected goats (ns), leading to a significant difference in $HP_{f \times ECS}$ of 9 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ between control and infected animals.

Table 1. Average heat production (HP_{tot}; $kJ \cdot kg^{-0.75} \cdot d^{-1}$) and heat production during standing (HP_{st}) and lying (HP_{ly}), the energy cost of standing (ECS), the time spent standing (f_{st}, %), and the standing related heat production ($HP_{f \times ECS}$) in infected and control WAD goats.

	Infected n = 13	sem	Control n = 7	sem	P-value[a]
HP_{tot}	342	6.8	306	9.3	**
HP_{st}	393	9.9	358	13.5	tend
HP_{ly}	315	7.0	270	9.6	**
ECS	78	5.5	88	7.5	ns
f_{st}	36	2.7	43	3.7	ns
$HP_{f \times ECS}$	27	1.8	36	2.5	*

[a]: ns = not significant, $P > 0.10$; tend = tendency, $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

In Table 2 the number of standing periods (N_{st}) and the duration of lying periods (T_{ly}) and standing periods (T_{st}) are given for infected and control goats. Infected animals changed position more often within each 24-hour period than control animals did ($P < 0.001$). Consequently both T_{ly} ($P < 0.001$) and T_{st} ($P < 0.01$) were reduced in infected animals compared with control animals.

Table 2. Number of standing periods per day (N_{st}) and duration of standing (T_{st}; minutes) and lying periods (T_{ly}) of infected and control WAD goats.

	Infected n = 13	sem	Control n = 7	sem	P-value[a]
N_{st}	22.8	1.2	13.8	1.6	***
T_{st}	25.9	4.8	51.0	6.6	**
T_{ly}	41.9	2.9	67.9	4.0	***

[a]: ** $P < 0.01$; *** $P < 0.001$.

In Table 3, values on HP_{tot}, HP_{st}, HP_{ly}, and f_{st} during either the light phase (07.00 - 19.00 h) or dark phase (19.00 - 07.00 h) are presented, as are differences between light and dark phases. HP_{tot} during the dark phase was increased in infected animals by 54

$kJ \cdot kg^{-0.75} \cdot d^{-1}$ ($P < 0.001$) compared with controls. During the light phase, however, the increase in HP_{tot} due to infection was only 20 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (ns). Consequently, the difference in HP_{tot} between the light and dark phase was smaller in infected goats ($P < 0.001$).

Table 3. Heat production (HP_{tot}; $kJ \cdot kg^{-0.75} \cdot d^{-1}$), HP corrected for standing (HP_{st}), HP corrected for lying (HP_{ly}), and frequency of standing (f_{st}; %) measured during light phase and dark phase and the difference between light period and dark phase values in infected and control WAD goats.

	Infected n= 13	sem	Control n= 7	sem	P-value[a]
HP_{tot} light phase	352	7.9	332	10.8	ns
HP_{tot} dark phase	333	6.2	279	8.5	***
difference	19	4.4	53	6.0	***
HP_{st} light phase	391	10.4	360	14.2	ns
HP_{st} dark phase	396	9.5	344	12.9	**
difference	-5	5.0	15	6.9	*
HP_{ly} light phase	318	7.8	272	10.6	**
HP_{ly} dark phase	315	6.8	270	9.2	**
difference	3	3.3	1	4.5	ns
f_{st} light phase	48	4.1	71	5.6	**
f_{st} dark phase	23	2.3	14	3.2	*
difference	26	3.4	57	4.6	***

[a]: ns= not significant, $P > 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Moreover, there were differences in the time spent standing during the light and the dark phase, *i.e.*, in infected animals, f_{st} was higher during the dark phase ($P < 0.01$), whereas it was reduced during the light phase ($P < 0.05$) compared with controls. HP_{ly} was increased by 45 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in infected animals, irrespective of the light or dark phase. HP_{st} was increased by infection during the dark phase ($P < 0.01$) but not during the light phase. The difference between HP_{st} during the dark and light phase was greater in control animals than in infected animals ($P < 0.05$).

In Table 4, results on BT are given. Large within-day variations in BT were found in infected animals, with BT changes up to 0.40°C per hour. BT_{tot}, and BT during lying (BT_{ly}) and standing (BT_{st}) were increased by infection throughout the experiment. Over the whole infection period, BT_{tot} was 39.78°C and 38.51°C for infected and control animals, respectively; infection BT_{tot} was increased by 1.27°C ($P < 0.001$). BT_{st} was significantly higher than BT_{ly} in infected animals in week 2 p.i. ($P < 0.01$) and 4 p.i. ($P <$

0.05), but not in week 6 p.i. No difference was found between BT_{st} and BT_{ly} in control animals.

Table 4. Average body temperature (BT_{tot}; °C), body temperature during standing (BT_{st}) and lying (BT_{ly}), and the difference between BT_{st} and BT_{ly} in infected and control WAD goats in week 2, 4 and 6 after infection.

	Infected n = 13	sem	Control n = 7	sem	P-value[a]
Week 2 p.i.					
BT_{tot}	40.04	0.07	38.51	0.09	***
BT_{st}	40.24	0.07	38.56	0.09	***
BT_{ly}	39.87	0.07	38.49	0.09	***
difference	0.37	0.05	0.07	0.07	**
Week 4 p.i.					
BT_{tot}	39.81	0.08	38.51	0.10	***
BT_{st}	40.01	0.10	38.53	0.13	***
BT_{ly}	39.73	0.07	38.50	0.10	***
difference	0.28	0.07	0.03	0.09	*
Week 6 p.i.					
BT_{tot}	39.49	0.13	38.52	0.17	***
BT_{st}	39.61	0.13	38.54	0.18	***
BT_{ly}	39.46	0.13	38.51	0.18	***
difference	0.15	0.08	0.03	0.11	ns

[a]: ns = not significant, $P > 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5. Increase in heat production per 1 °C BT increase (energy cost of fever, ECF; $kJ \cdot kg^{-0.75} \cdot d^{-1} \cdot °C^{-1}$) during standing and lying and the difference between standing and lying, in infected and control WAD goats .

	Infected n = 13	sem	Control n = 7	sem	P-value[a]
Energy Cost of fever (ECF)					
during standing	18.8	3.0	32.9	4.1	*
during lying	22.8	2.7	12.2	3.6	*
difference	-4.1	3.8	20.8	5.1	**

[a]: * $P < 0.05$; ** $P < 0.01$.

In Table 5, estimates for ECF, calculated by linear regression analysis per animal per infection week, are presented. ECF during standing was higher in control animals, whereas ECF during lying was higher in infected animals (both $P < 0.05$). However, the estimates for ECF of control animals had a very low r^2 of 0.02 and may not have been

very accurate, whereas the average r^2 for infected animals was about 0.20. The difference between ECF during lying and ECF during standing in infected animals was not different from zero; in controls however, ECF during standing was significantly higher than ECF during lying ($P < 0.01$).

Within a standing or a lying period, BT was not constant with time (Figure 1). BT increased with time during standing periods but decreased with time during lying periods. The following regression equations were estimated for infected and control animals, either standing or lying (BT in °C; TIME in h, sem between brackets):

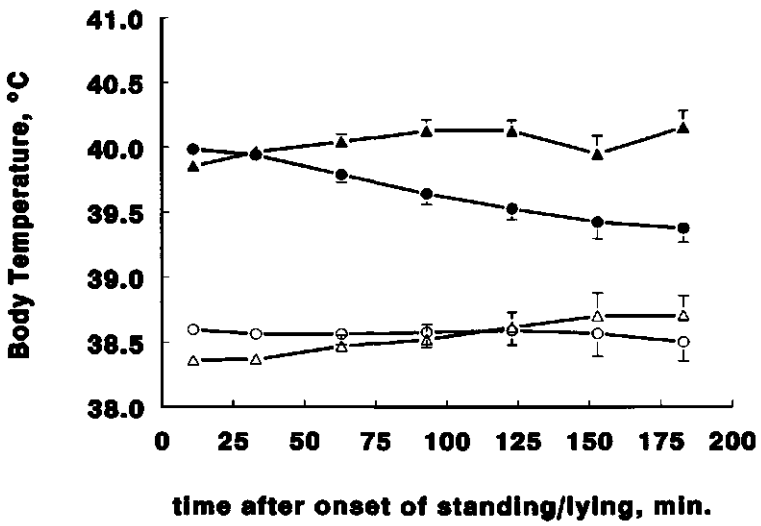
Inf., st.: $BT = 39.92(\pm 0.052) + 0.066(\pm 0.0216) * TIME$; $n = 13$; $r^2 = 0.61$ [2]

Inf., ly.: $BT = 39.94(\pm 0.055) - 0.164(\pm 0.0218) * TIME$; $n = 13$; $r^2 = 0.90$ [3]

Cont., st.: $BT = 38.35(\pm 0.021) + 0.118(\pm 0.0086) * TIME$; $n = 7$; $r^2 = 0.97$ [4]

Cont., ly.: $BT = 38.63(\pm 0.024) - 0.045(\pm 0.0087) * TIME$; $n = 7$; $r^2 = 0.81$ [5]

Figure 1. Body temperature as affected by time after onset of either a standing or lying period in infected and control WAD goats The line —▲— represents infected animals (standing); —●— represents infected animals (lying); —△— represents control animals (standing); —○— represents control animals (lying).



The decrease in BT per hour in lying infected animals was larger ($P < 0.001$) than that in lying control animals. However, the increase in BT per hour during standing was not significantly different between infected and control animals. For HP no trends with time were observed after the onset of either a standing or lying period.

Discussion

In this experiment, data on HP, BT, and body posture were collected continuously, with mean values for every 15 minutes. Thus short-term variation could be monitored and relations between HP, BT, and body posture were studied.

Metabolic costs of infection

The average HP was increased by 36 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in infected animals (12 % increase; Table 1). This is in accordance with the increase of 14 % reported elsewhere for *T. vivax* infected goats (Verstegen *et al.*, 1991).

In the present study, time spent standing (f_{st}) tended to decrease in infected animals. Also ECS was somewhat lower in infected animals, leading to a significantly decreased HP_{fxECS}. This means that infected animals masked part of the increased metabolic demands by reducing their standing-related energy costs.

The energy cost of standing was estimated at respectively 78 and 88 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in infected and control goats (increase of 25, respectively 29 %, relative to HP_{ly} of control animals). Schrama *et al.* (1993) observed a difference between standing and lying of 114 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (T_a at 18°C) in young calves (27 % increase). Ortigues *et al.* (1994) reported a value of 107 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (23-27 % increase) for young calves, and Purwanto *et al.* (1993) reported 106 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ for dairy heifers on a low feed intake (21 % increase). The absolute estimates of ECS, therefore, were lower than those of other reports; however, when expressed as a percentage of total HP, the results of the present study corresponded well with those of other studies.

The difference in total HP (HP_{tot}) during the dark and light phase (Table 3) was also observed by Zwart *et al.* (1991). Differences between dark and light phase, however, were fully explained by different f_{st}. Consequently HP_{ly} was the same for the dark and light phase, and a mean difference of 45 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ between infected and control animals, irrespective of the dark or light phase, was estimated. HP_{st}, however, was higher during the light phase than during the dark phase in control animals. This may be because the animals were more active during daytime standing periods (feeding, explorative behaviour). Ortigues *et al.* (1994) also observed differences in activity during standing between the day and night phase. The higher activity could possibly account for the slightly higher ECS of control animals.

Many studies report a positive relationship between fever and metabolic rate. The average rate of chemical reactions, and consequently heat production, is thought to increase by approximately 10 % per °C temperature increase (Van 't Hoff/ Arrhenius relationship; Blaxter, 1989). In the present study, the metabolic cost of infection can be estimated as the increase in HP_{tot} of 36 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, divided by the increase in BT. This

was (12 % HP increase / 1.27°C fever) = 9.3 % per °C fever. However, this underestimated the energy cost of fever because part of the HP increase was masked by the reduced standing related HP. Therefore, the difference between HP_{ly} of infected and of control goats may be a better estimate of the metabolic costs of infection. This amounts to a metabolic cost of infection of 45 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (16.7 % increase compared with control animals; Table 3), with an increase of BT_{ly} of 1.19°C in infected animals (Table 4). Thus the HP increase per degree centigrade was 14.0 %. Du Bois (1921) estimated the metabolic costs of 1°C fever, caused by a variety of different diseases in humans, at 13 %. However, periods of shivering were left out of his calculation; during shivering HP may be doubled (7). Baracos *et al.* (1987) observed a large variation in the increase in HP, from 13 to 35 % per °C fever.

However, not all energy costs involved in infection can be attributed to fever *per se*. Additional energy costs comprise thermoregulatory heat production, *i.e.*, shivering periods (Baracos *et al.*, 1987), and other metabolic costs, *i.e.*, higher protein turnover, immune response, and tissue repair (Beisel, 1985). As the occurrence of shivering in infected goats was not monitored, the results on HP were related to BT by linear regression to obtain a more accurate estimate of increase of HP due to the direct effect of fever (Table 5). Within-animal variations in HP and BT were positively correlated with each other, with an average increase in HP per 1°C difference in BT of 21 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (average of regression equations for standing and lying periods; Table 5). The regression estimate is relatively low compared with that of other reports and the theoretical relationship of Van 't Hoff/ Arrhenius. The regression estimate explains approximately 60 % of the observed differences in HP_{ly} between infected and control animals, with the remaining 40 % of energy costs of infection probably being accounted for by thermogenesis and/or other metabolic costs (Beisel, 1985).

Body temperature and body posture

Both average BT_{tot} and the short-term variation in BT_{tot} were increased by infection (Table 4). Fluctuating fever is a typical symptom of trypanosomiasis, which reflects the response to successive waves of parasitaemia (Stephen, 1986). The body temperature set point in the hypothalamus is then changed under the influence of pyrogenic stimuli released during infection (Baracos *et al.*, 1987; Kluger, 1989). The animal then responds to the increase in the BT set point by increasing its body temperature. This increase in BT (hyperthermia) can be achieved by increasing HP without changing heat loss, or it may be achieved by reducing heat loss (eg. by vasoconstriction and higher tissue insulation) without changing HP (Simon, 1993).

In the present experiment in week 2 and 4 p.i., BT during standing and during lying was different in infected animals (Table 4). Moreover, analysis of repeated measurements

within a standing or lying period revealed an increase in BT with time while animals were standing, and a decrease with time while lying (Figure 1; equations [2] to [5]). Apparently during standing, the balance between heat production and heat loss was positive, while during lying it was negative. The decrease in BT during lying may have been induced by the absence of bedding material and the use of an iron mesh floor, which may have increased conductive heat loss. Mount (1967) demonstrated, in a study with newborn piglets, that conductive heat loss was affected strongly by the type of floor.

The time-related alterations in BT during standing and lying periods may have implications for thermoregulation, and it is possible that animals change their posture due to thermal distress. The higher frequency of posture change in infected animals, and the more even distribution of standing periods over the dark and light phase, supports this hypothesis, because the temperature set point changes frequently in infected animals. Also Schrama *et al.* (1993) postulated that body posture might be thermoregulatorely induced. Diseased animals may change their behaviour for thermoregulatory purposes (Hart, 1985). Van Diemen *et al.* (1995) showed that the activity of pigs infected with *Pasteurella multocida* was reduced.

Thermoregulatory mechanisms are aimed at bringing the actual BT to the set point temperature with a minimum of energy costs. The manipulation of body posture might well fit in this strategy. However, other factors, such as the needs of the animal with respect to eating/drinking or movement/rest, might also play a role in the induction of postural change.

Conclusions

Both HP and BT were increased in goats infected with *Trypanosoma vivax*. The daily energy costs of standing were reduced in infected animals. This masked part of the increased HP due to infection. The largest part of the energy costs of infection were explained by fever, according to the Van 't Hoff/ Arrhenius relationship. The frequency of change of body posture was increased in infected animals; BT increased with time during a standing period, whereas it decreased with time during a lying period.

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Chapter 6

Effect of fibrous feed quality on the course of *Trypanosoma vivax* infection in West African Dwarf goats. I. Organic matter intake, body weight change and efficiency of nitrogen metabolism

J.T.P van Dam, P. Hofs, B.J. Tolkamp, and D. Zwart.

Dept. of Animal Husbandry, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands.

Submitted.

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J.T.P van Dam, P. Hofs, B.J. Tolkamp, and D. Zwart.

Dept. of Animal Husbandry, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands

Abstract

In an experiment studying the interaction between diet quality and the course of trypanosome infection, 29 West African Dwarf goats were randomly allotted to either a diet of pelleted lucerne with a high N content (n = 14) or chopped grass straw with a low N content (n = 15). Nine lucerne fed animals and ten grass straw fed animals were infected with *Trypanosoma vivax* to study its effects on feed intake and efficiency of N utilization during the first 6 weeks of infection. Infection reduced organic matter intake from 54.9 (± 2.1) to 37.7 (± 1.7) $g \cdot kg^{-0.75} \cdot d^{-1}$. Lucerne fed animals had a higher organic matter intake than grass straw fed animals. The relative decrease of digestible organic matter intake (DOMI) due to infection was the same in animals fed lucerne or grass straw diet (36 and 35 %). Retention of N was lower in infected animals and in animals fed grass straw. By relating N retention to DOMI the efficiency of N utilization, corrected for feed intake level, could be estimated. No effect of infection or diet type on the efficiency of N utilization was detected. One overall regression equation was estimated:
N Retention = -0.450 (± 0.038) + 0.0167 (± 0.0015) × DOMI (n = 29; r^2 = 0.86). Serum urea concentration was higher in lucerne fed animals than in grass straw fed animals; within the former group, infected animals showed a lower urea concentration post infection than control animals. Serum creatinine concentration was higher in grass straw fed animals than in lucerne fed animals. From the former group, infected animals had a lower creatinine concentration post infection than controls. It was concluded that infection affected feed intake, but that efficiency of N utilization was not changed by infection.

Introduction

Trypanosomiasis is a protozoan disease, which is endemic in large parts of sub-Saharan Africa (ILCA, 1986). Parasitic infections often induce an increase of N losses in the host due to intestinal or renal damage (Holmes, 1987). In addition Seed and Hall (1985) reported a higher protein turnover and increased catabolism in trypanosome infection.

On the other hand, trypanosomiasis often leads to feed intake reduction, which in turn causes an increase of catabolic processes and consequently N losses (Verstegen *et al.*, 1991). Therefore, in order to correctly estimate direct effects of trypanosome infection on N losses of the host, differences in feed intake level should be corrected for.

Regression analysis of nitrogen retention (NR) on digestible organic matter intake (DOMI), *viz.* $NR = b_0 + b_1 \times DOMI$, gives information about the relation between net protein and net energy availability to the tissues (Ketelaars and Tolkamp, 1991; Oosting *et al.*, 1995). They found that both b_0 and b_1 were not affected by fibrous feed type or metabolizability of diets, if animals were offered *ad libitum* diets. Hereby the efficiency of nitrogen utilization could be estimated, corrected for energy intake level, irrespective of fibrous feed type. Akinbamijo *et al.* (1992) studied the relation between NR and DOMI in West African Dwarf goats, infected with *T. vivax* and fed lucerne pellets, a feed which is rich in crude protein (CP). They found no indications for a changed relation between NR and DOMI in infected animals. It cannot be excluded, however, that a diet which has a lower CP level than lucerne, might well have aggravated the N losses due to infection, leading to a different relation between NR and DOMI.

In the present work, therefore, the effect of fibrous feed quality, as reflected in the N content of the feed, on the course of infection with *T. vivax* in West African Dwarf goats was studied. The variables of study were feed intake and body weight change, and the efficiency of N utilization, measured as the relationship between NR and DOMI.

Material and methods

The experiment was evaluated and approved of by the University Ethical Committee on Animal Welfare.

Animals

Twenty-nine castrated male WAD goats were used averaging 23 (± 0.8) kg BW and 12 (± 0.1) months of age. They were derived from the university flock of WAD goats, which had been established some 15 years ago (Montsma, 1986). The experimental animals had never been exposed to trypanosome infections before. Prior to the experiment they received an anthelmintic treatment and were vaccinated against ecthyma.

Feeds and housing

Two different feeds were used *viz.* pelleted lucerne and chopped grass straw. Lucerne had a high N content, whereas grass straw had a low N content. The composition of the two feeds is given in Table 1. Salt lick and water were freely available. Animals were housed in group pens nine weeks before infection to adapt to the experimental diets. Four weeks before infection (*ante infectio; a.i.*) they were randomly placed on individual digestibility cages. During the whole experiment ambient temperature was kept at 20°C and lights were on from 07.00h to 19.00h.

Table 1. Composition of the experimental feeds: dry matter (DM; in $g \cdot kg^{-1}$ fresh feed), and organic matter (OM) and crude protein (CP; both in $g \cdot kg^{-1}$ dry matter).

Diet	DM	OM (in DM)	CP (in DM)
pelleted lucerne	933	863	172
chopped grass straw	929	936	68

Infection

The animals were infected intravenously with approximately 1×10^5 *T. vivax* parasites from strain Y486, isolated by Leeflang *et al.* (1976). This was defined as day 0 of the experiment.

Experimental design

Fourteen animals were randomly allocated to an *ad libitum* ration of pelleted lucerne, and 15 animals were allocated to an *ad libitum* ration of chopped grass straw. Before the moment of infection they were assigned to infection or control treatment as follows: per diet group animals were sub-divided in groups of 3 animals with approximately the same body weight and feed intake. Per group two animals were randomly allotted to the infection and 1 animal to the control group. Accordingly, a 2×2 scheme was adopted using four different experimental groups *viz.* Infected animals fed Lucerne (IL; n = 9), Control animals fed Lucerne (CL; n = 5), Infected animals fed Grass straw (IG; n = 10) and Control animals fed Grass straw (CG; n = 5) group. Also during the infection period, all animals had *ad libitum* access to feed. This was necessary because the b_0 in the equation $NR = b_0 + b_1 \times DOMI$ tends to increase under the influence of feed restriction, which would complicate the comparison between treatments (Akinbamijo *et al.*, 1992); Blaxter (1989) reported a decrease in energy maintenance requirements due to food restriction. As a consequence, the employed experimental design did not allow for an iso-nutritional comparison of infected and control goats.

Sample procedures and sample preparation

In Table 2 the time schedule and measurements are presented. Daily feed intake was recorded from 1 week *a.i.* until week 6 after infection (*post infectio*; *p.i.*) in early morning by collection of refused feed of the previous day and offering fresh feed, at an excess of 40 % over daily intake (based on individual intake data from the preceding week). Body weight was measured weekly at the start and at the end of a nitrogen balance trial. Rectal temperature was measured daily before feeding from 1 week *a.i.* until the end of the experiment, and blood samples were taken weekly; these measurements are reported elsewhere, as well as the *post mortem* micro- and macroscopic examination after week 6 *p.i.* (Van Dam *et al.*, submitted).

Table 2. Time schedule and measurements

Week	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	
Housing	group pen					individual digestibility cages										
N balance trials									#1		#2		#3		#4	
Procedure[1]										I						PM
Feedintake	group intake					daily individual										
Body Temp.									from here						to here	
Blood traits and body weight									x	x	x	x	x	x	x	x

[1]: I: infection with *T. vivax*, PM: Post mortem examination.

Four N balance trials were carried out in wk 1 *a.i.* and wk 2, 4 and 6 *p.i.* All N balance trials lasted 7 days. Per N balance trial the amounts of offered feed, refused feed, faeces and urine were measured. For analysis of composition, samples were taken from offered feed (one composite sample per N balance period per diet), refused feed, faeces and urine (one composite sample per N balance per animal). Sulphuric acid was added to urine for keeping the pH of urine low in order to prevent NH_3 escape. Formalin was added to the faeces to avoid fermentation.

Once per balance period a 20-h sample of urine was collected for urea and creatinine determination. Once per week a blood sample was taken for measurement of serum urea concentration.

N content of feed offered, feed residues, fresh faeces and urine were determined using Kjeldahl technique (ISO 5983-1991); DM and ash content of offered feed, feed residues and faeces were determined (ISO 5984). Organic matter intake (OMI) was calculated as the organic matter (OM) offered minus OM refused; digestible organic matter intake (DOMI) was calculated as OMI minus organic matter in faeces. NR was calculated as the difference between N intake (NI) and N losses via faeces (FN) and urine

(UN). Data on OMI, DOMI, NI, FN and UN were expressed per kg metabolic weight ($kg^{0.75}$) per day.

Urea and creatinine concentration in serum and urine were determined with a Synchron 5 autoanalyzer using Beckman reagents (Beckman Instruments GmbH, München, Germany).

Statistical model

Statistical analysis of the data was carried out using SAS Statistical Package (SAS, 1990). The effect of infection, diet, week number either before or after infection, and their interaction on the above described intake and N metabolism variables were tested by means of F-test using a split-plot model [GLM procedure (SAS, 1990)], with week values within goats taken as repeated measurements:

$$Y_{ijkl} = \mu + D_i + I_j + (D \times I)_{ij} + e_{1;ijk} + WK_l + (WK \times I)_{jl} + e_{2;ijkl} \quad [1]$$

where:

Y_{ijkl} = dependent variable; μ = overall mean; D_i = effect of Diet ($i = 1,2$); I_j = effect of Infection ($j = 1,2$); $(D \times I)_{ij}$ = effect of interaction between Diet and Infection; $e_{1;ijk}$ = Error term 1: randomized effect of Animal nested within D×I subgroup; D_i, I_j, and $(D \times I)_{ij}$ were tested against error term 1; WK_l = effect of Week number/ Balance trial ($l = 1,...,3$ post-infection N balance trials or $l = 1,...,6$ post-infection weeks); $(WK \times I)_{jl}$ = effect of interaction between Week number (or balance trial) and Infection; $e_{2;ijkl}$ = error term; WK_l and $(WK \times I)_{jl}$ were tested against error term 2.

Class factors which did not significantly contribute to the model were excluded. The relation between NR and NI, and the relation between NR and DOMI, were studied. Therefore, results on NR were pooled per animal, and were tested using model [1] with the addition to the model of either NI or DOMI as a covariable.

Results

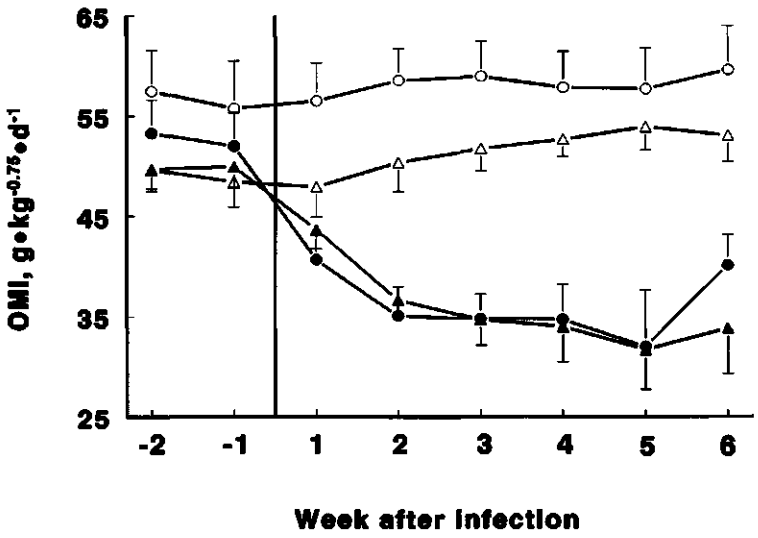
General course of infection

Four animals died before the end of the experiment or were euthanized when moribund of the effects of trypanosome infection, *viz.* two animals of the IL group on day 32 and 33 *p.i.* and two animals of the IG group on day 30 and 40 *p.i.* From the animal that died on day 40 *p.i.*, the results of the N balance period 4 were included in the study. From results on packed cell volume and parasite count (reported by Van Dam *et al.*, submitted) it was concluded that the disease was fully established in all infected animals.

Feed intake and body weight change

In Figure 1 OMI is depicted from week 1 *a.i.* until week 6 *p.i.* The average intake per animal per day, calculated for the whole infection period, was reduced in infected animals, compared with control animals, *viz.* 37.7 (± 1.7) and 54.9 (± 2.1), respectively ($P < 0.001$). The mean OMI over the total infection period per treatment was not significantly affected by fibrous feed quality. The apparent increase of mean OMI of the IL group in week 6 *p.i.* was mainly caused by the death of two animals with a very low feed intake.

Figure 1. Organic matter intake (OMI) after infection with *Trypanosoma vivax* of goats, fed different diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.



Daily body weight change per $kg^{0.75}$, averaged over the whole infection period, was for the IL group -3.0 (± 1.2) $g \cdot kg^{-0.75} \cdot d^{-1}$, for the CL group 4.2 (± 0.9) $g \cdot kg^{-0.75} \cdot d^{-1}$, for the IG group -11.6 (± 1.4) $g \cdot kg^{-0.75} \cdot d^{-1}$ and for the CG group -0.5 (± 0.5) $g \cdot kg^{-0.75} \cdot d^{-1}$. Infection reduced body weight gain ($P < 0.001$). Animals fed grass straw had a lower mean body weight gain than lucerne fed animals ($P < 0.001$). No statistical interaction between diet quality and infection treatment was detected ($P > 0.10$).

Table 3. Body weight (BW; in kg), organic matter intake (OMI) and digestible organic matter intake (DOMI; both in $g \cdot kg^{-0.75} \cdot d^{-1}$), organic matter digestibility (OMD), N intake (NI), N excretion in faeces (FN) and urine (UN), N retention (NR; all in $g \cdot kg^{-0.75} \cdot d^{-1}$) and N digestibility (ND), before and after infection with *Trypanosoma vivax* of goats, fed different diets; least square means of pre-infection N balance trial and combined post-infection N balance trials.

	LS Means of treatments[1]					P-values[2]		
Variable	IL	CL	IG	CG	rmse[3]	diet	inf	int
	Pre-Infection Period							
No. of animals	9	5	10	5				
BW	22.1	24.0	22.1	22.0	3.8	ns	ns	ns
OMI	53.1	56.5	50.3	48.8	8.5	ns	ns	ns
DOMI	32.6	34.3	26.0	25.6	4.6	***	ns	ns
OMD	0.615	0.609	0.518	0.522	0.020	***	ns	ns
NI	1.646	1.776	0.615	0.562	0.240	***	ns	ns
FN	0.733	0.821	0.426	0.394	0.148	***	ns	ns
ND	0.556	0.545	0.312	0.304	0.053	***	ns	ns
UN	0.818	0.856	0.135	0.146	0.087	***	ns	ns
NR	0.094	0.098	0.054	0.023	0.045	**	ns	ns
	Infection Period							
Number animals	9	5	10	5				
BW, kg	21.8	25.1	19.5	21.8	6.2	ns	ns	ns
OMI	36.8	59.4	33.9	51.9	12.9	ns	***	ns
DOMI	23.6	36.6	17.9	27.5	7.9	***	***	ns
OMD	0.641	0.616	0.509	0.530	0.037	***	ns	ns
NI	1.131	1.867	0.429	0.597	0.299	***	***	***
FN	0.472	0.843	0.312	0.408	0.181	***	***	**
ND	0.580	0.553	0.271	0.314	0.068	***	ns	ns
UN	0.695	0.892	0.284	0.148	0.106	***	ns	***
NR	-0.034	0.131	-0.162	0.042	0.157	**	***	ns

[1]: IL = Infected animals fed Lucerne, CL = Control animals fed Lucerne, IG = Infected animals fed Grass straw, CG = Control animals fed Grass straw;
[2]: Significance of treatments; inf: infection, int: interaction; ns = not significant; ** = $P < 0.01$; *** = $P < 0.001$;
[3]: Root mean square error (sem = rmse/$\sqrt{n}$).

In Table 3 intake of OM, DOM and N, as well as FN, UN, and N retention per $kg^{0.75}$ per day are presented, with mean values *a.i.* and *p.i.*, and mean body weight before and after infection. All presented traits, except BW and OMI, were affected by diet quality throughout the experiment. In lucerne fed animals DOMI, N intake, and urinary and faecal N losses were higher than in grass straw fed animals ($P < 0.001$). Also N retention was higher in lucerne fed animals ($P < 0.01$). In post-infection N balance trials, OMI, DOMI, N intake and N retention were lower in infected animals of both diet groups, compared with control animals ($P < 0.001$). DOMI decrease due to infection was 36 % in lucerne fed animals and 35 % in grass straw fed animals.

N Metabolism

In Figures 2a to 2d the relation between NR and NI is presented in the four successive N balance trials. NI affected NR ($P < 0.001$), but the relation between NR and NI was different for either the grass straw or the lucerne fed goats ($P < 0.05$). No time effect was found on the relation between NR and NI, so data were pooled per animal for the balance trials *p.i.* For grass straw fed animals one linear regression equation ($P < 0.05$) was estimated:

$$NR = -0.536\ (\pm\ 0.077) + 0.904\ (\pm\ 0.154) \times NI; \quad [2]$$

($n = 15$; $r^2 = 0.73$; NR and NI in $g \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets).

For lucerne fed animals two linear equations which pivoted at NI = 1.34 $g \cdot kg^{-0.75} \cdot d^{-1}$ had a higher r^2 than one linear regression:

$$\text{For } NI < 1.34: \quad NR = 0.071\ (\pm\ 0.076) + 0.512\ (\pm\ 0.121) \times (NI - 1.34); \quad [3]$$

$$\text{For } NI > 1.34: \quad NR = 0.071\ (\pm\ 0.076) + 0.125\ (\pm\ 0.107) \times (NI - 1.34); \quad [4]$$

($n = 14$; $r^2 = 0.81$; NR and NI in $g \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets).

Both the intercepts of equation [3] and [4], and the slope of equation [4] were not different from zero ($P > 0.25$).

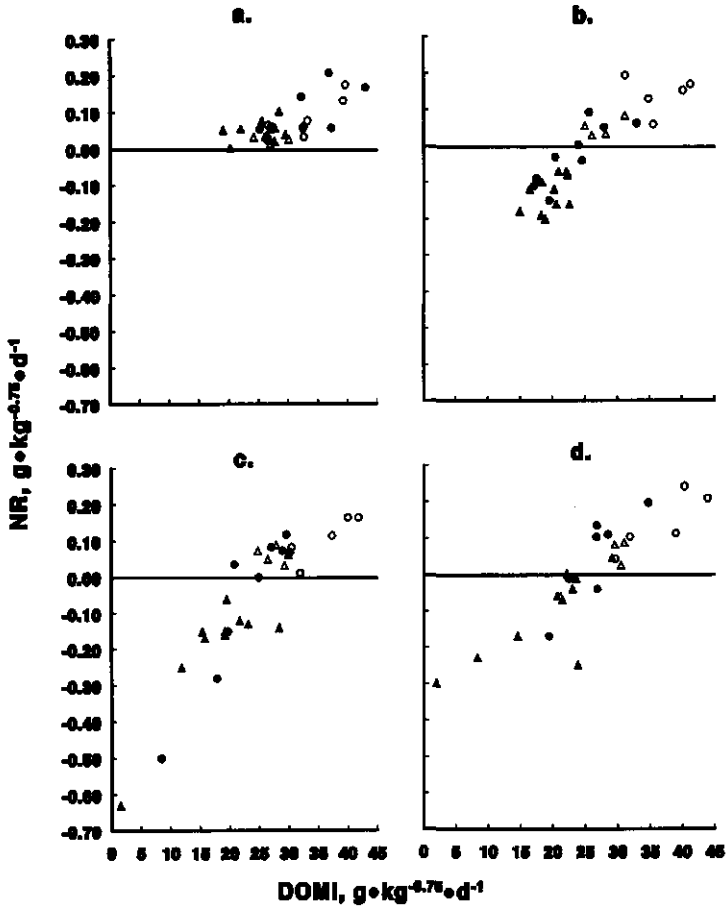
In Figures 3a to 3d the relation between N retention and DOMI for the four different N balance trials is shown. Covariance analysis of N balance trials *p.i.* demonstrated that the class factors diet and infection (with interaction) and time effect (N balance number) did not have a significant effect on this relationship. Consequently the statistical model was reduced to a simple regression model with DOMI as the only factor, and data per animal *p.i.* were pooled. The estimated regression equation was as follows:

$$NR = -0.450\ (\pm\ 0.038) + 0.0167\ (\pm\ 0.0015) \times DOMI; \quad [5]$$

($n = 29$; $r^2 = 0.86$; NR and DOMI in $g \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets).

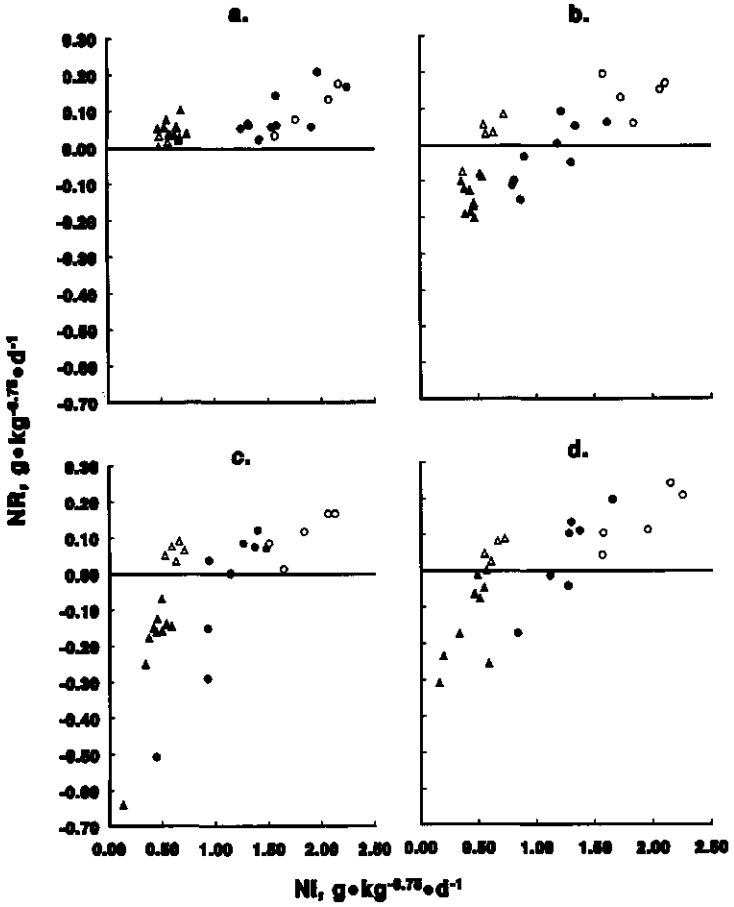
Figure 4 shows serum concentrations of urea. Throughout the infection period, urea concentration was higher in the lucerne fed animals ($P < 0.001$), except in week 0. In week 3 and 4 *p.i.* interaction between diet and infection was observed (a reduction of urea concentration in the IL group but not in the IG group ($P < 0.05$). Figure 5 shows serum concentrations of creatinine. Creatinine concentration was higher in grass straw fed animals throughout the infection ($P < 0.01$); infection decreased creatinine concentration from week 2 *p.i.* onwards ($P < 0.05$).

Figure 2. The relation between N retention (NR) and N intake (NI) after infection with *Trypanosoma vivax* of goats, fed different diets in four subsequent N balance trials: **a.** wk 1 *a.i.*; **b.** wk 2 *p.i.*; **c.** wk 4 *p.i.*; **d.** wk 6 *p.i.* ● represents infected animals fed lucerne, ○ control animals fed lucerne, ▲ infected animals fed grass straw, and △ control animals fed grass straw.



Urinary excretion of urea and creatinine was increased in lucerne fed animals throughout the experiment ($P < 0.001$; Table 4). In week 2 *p.i.* infection slightly increased urea and creatinine excretion ($P < 0.05$). In week 4 and 6 *p.i.* urea excretion was decreased in infected, compared with control lucerne animals ($P < 0.001$).

Figure 3. The relation between N retention (NR) and digestible organic matter intake (DOMI) after infection with *Trypanosoma vivax* of goats, fed different diets in four subsequent N balance trials: **a.** wk 1 *a.i.*; **b.** wk 2 *p.i.*; **c.** wk 4 *p.i.*; **d.** wk 6 *p.i.* ● represents infected animals fed lucerne, ○ control animals fed lucerne, ▲ infected animals fed grass straw, and △ control animals fed grass straw.



Discussion

Feed intake and body weight change

Average OMI *p.i.* was reduced in both infection groups, compared with control groups (Figure 1). This may be caused by cytokines, like Tumour Necrosis Factor (TNF) and interleukin-1, which are produced by activated mononuclear cells during infection (Sileghem *et al.*, 1994; Van Miert, 1995), and often play a role in the induction of anorexia (Van Miert *et al.*, 1992, Plata-Salaman *et al.*, 1988).

Table 4. Urinary excretion of urea and creatinine (in $g \cdot d^{-1}$), after infection with *Trypanosoma vivax* of goats, fed different diets; least square means of the four subsequent balance trials.

	LS means of treatments[1]					P-values[2]		
Variable	IL	CL	IG	CG	rmse[3]	diet	inf	int
N balance trial 1 (week 1 *a.i.*)								
urea	1.57	1.73	0.24	0.24	0.24	***	ns	ns
creatinine	0.050	0.054	0.021	0.031	0.009	***	ns	ns
N balance trial 2 (week 2 *p.i.*)								
urea	1.88	1.85	0.58	0.26	0.21	***	*	ns
creatinine	0.068	0.050	0.033	0.029	0.010	***	*	ns
N balance trial 3 (week 4 *p.i.*)								
urea	1.33	2.05	0.72	0.26	0.36	***	ns	***
creatinine	0.045	0.057	0.032	0.025	0.012	***	ns	*
N balance trial 4 (week 6 *p.i.*)								
urea	1.44	1.83	0.52	0.22	0.21	***	ns	***
creatinine	0.036	0.044	0.020	0.026	0.012	**	ns	ns

[1]: IL = Infected animals fed Lucerne, CL = Control animals fed Lucerne, IG = Infected animals fed Grass straw, CG = Control animals fed Grass straw;
[2]: Significance of treatments; inf: infection, int: interaction; ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$;
[3]: Root mean square error (sem = rmse/$\sqrt{n}$).

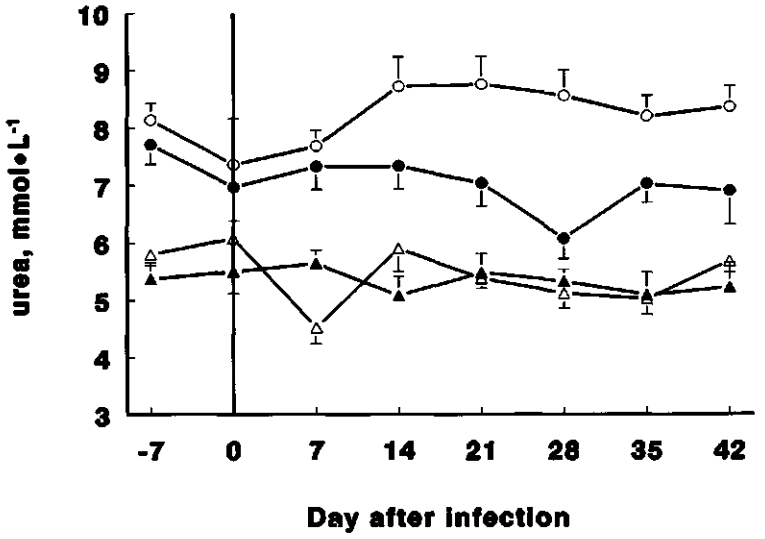
DOMI was affected both by infection and diet type. The percentage decrease of DOMI due to infection, however, was not different for lucerne and grass straw fed animals, *i.e.*, no significant statistical interaction between infection and feed quality was observed with respect to DOMI.

The offered diets were homogenized, and the animals were not able to select for the better parts of the diet. This was concluded from the fact that composition of the offered and residual feed was not significantly different from each other. Under farm conditions, however, it may well be possible that West African Dwarf goats, being good browsers, select the better parts of their diet, thus improving the nutritive value of the ingested feed (Bosman *et al.*, 1995). Kyriazakis *et al.* (1994) demonstrated that growing sheep, suffering from an intestinal nematode infection, compensated for reduced feed intake due to infection, by showing a higher preference for feed with a high N content, compared with healthy controls, when offered free choice between diets with low and high N content.

Infection decreased body weight gain. The grass straw diet resulted in lower body weight gain, compared with lucerne fed animals. No statistical interaction between infection and diet was detected. This corresponds with results from Blackburn *et al.* (1991), who observed no significant interaction between the effect of two planes of dietary energy and the effect of 3 levels of *Haemonchus contortus* infection on weight gain of goats. In their study, however, a tendency for a larger reduction of liveweight

gain of infected animals, compared with controls, was observed at the low feeding plane. Katunguka-Rwakishaya *et al.* (1993) concluded that *T. congolense* infection had a more negative impact on body weight gain in sheep fed low protein diet, compared with sheep fed a high protein diet. Also Fagbemi *et al.* (1990) found evidence for an interaction between the effect of 3 planes of nutrition and the effect of *Trypanosoma brucei* infection on liveweight gain in growing pigs, although they found the largest reduction due to infection at a medium plane of nutrition, as compared with low and high plane.

Figure 4. Serum concentration of urea after infection with *Trypanosoma vivax* of goats, fed different diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.

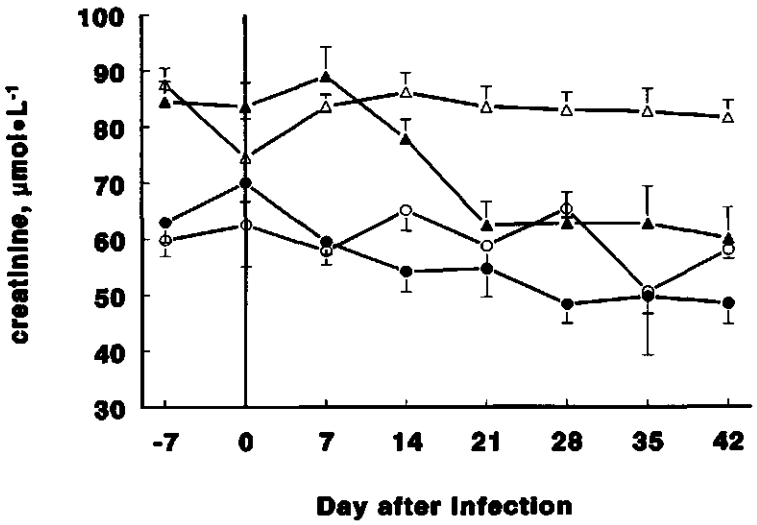


N Metabolism

The digestibility of OM and N was not affected by infection. Post mortem examination of intestines and kidneys revealed no lesions (Van Dam *et al.*, submitted), so it is unlikely that protein leakage to faeces and urine has occurred. Still, from Figure 2C, it can be derived that some animals showed a very low N retention at a relatively low NI level in week 4 *p.i.* These were severely diseased animals; the animals to which the three most extreme NR values belonged, succumbed to the infection in week 5 *p.i.* This moribund state in week 4 *p.i.* may have led to increased protein catabolism, because it was shown

at autopsy that the fat deposits of these animals were depleted (Van Dam *et al.*, submitted).

Figure 5. Serum concentration of creatinine after infection with *Trypanosoma vivax* of goats, fed different diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.



The results on N excretion and N retention were affected by intake level of individual animals (Table 3). Because mean DOMI in infected and control groups was different, this complicated the comparison of mean NR among groups. Therefore, NR was related to the intake variables DOMI and NI by linear regression, to quantify the effect of infection on NR, independent of intake level. Digestible organic matter intake was considered as the best intake variable to be related to NR, because it had been demonstrated by Oosting *et al.* (1995) and Ketelaars and Tolkamp (1991) that the relation between NR and DOMI is not affected by type of roughage diet or by diet metabolizability in small ruminants, fed *ad libitum* diets. The diets that were used in their study included wheat straw, grass straw and pelleted lucerne. Oosting *et al.* (1995) postulated that the mechanism behind the constant relation between NR and DOMI is, that voluntary intake in ruminants is established at a level at which net protein (represented by NR) and net energy (represented by DOMI), available to the tissues, are balanced, irrespective of diet type. Although DOMI does not provide a direct estimate of net energy, the conversion of DOMI into net energy is thought fairly constant for a wide range of diet types and diet

metabolizabilities, in *ad libitum* fed ruminants (Tolkamp and Ketelaars, 1994). However, it cannot be excluded that during fever, the relation between DOMI and net energy is changed; Verstegen *et al.* (1991) reported increased ME maintenance requirements due to fever.

In our experiment no effect of infection or diet type was observed on the relationship between NR and DOMI. The slope of the overall equation of 0.0167 (± 0.0015) $g \cdot kg^{-0.75} \cdot d^{-1}$ is not different from 0.0144 (± 0.0014) $g \cdot kg^{-0.75} \cdot d^{-1}$, estimated by Ketelaars and Tolkamp (1991) for WAD goats, neither from 0.0154 (± 0.0026) $g \cdot kg^{-0.75} \cdot d^{-1}$, reported by Oosting *et al.* (1995). Elliott and Topps (1964) reported a similar coefficient for sheep, *i.e.*, 0.0146 (± 0.0008) $g \cdot kg^{-0.75} \cdot d^{-1}$; data from their study were converted from total digestible nutrient (TDN) system to DOMI, by accepting that 1 g TDN contains 0.95 g of DOMI. The range of DOMI values of the different treatments, however, only partly overlapped each other, which complicated comparison among treatments.

The intercepts of regression equations, *i.e.*, NR at DOMI = 0, for either infected or control animals, were not different. The intercept of the overall equation [5] of -0.450 (± 0.038) $g \cdot kg^{-0.75} \cdot d^{-1}$ was identical to Oosting *et al.* (1995) but lower than in the study of Ketelaars and Tolkamp (1991), who reported -0.378 (± 0.045) $g \cdot kg^{-0.75} \cdot d^{-1}$. Elliott and Topps (1964) presented data for sheep, from which an even higher intercept of -0.292 (± 0.030) $g \cdot kg^{-0.75} \cdot d^{-1}$ could be derived.

DOMI maintenance requirements were derived from equation [5] at NR = 0; this averaged 26.9 $g \cdot kg^{-0.75} \cdot d^{-1}$, which is identical to estimates of Ketelaars and Tolkamp (1991) with 26.3 $g \cdot kg^{-0.75} \cdot d^{-1}$ DOMI, and NRC (1981) with 26.8 $g \cdot kg^{-0.75} \cdot d^{-1}$ DOMI, but somewhat higher than estimates of 24.3 $g \cdot kg^{-0.75} \cdot d^{-1}$ DOMI by Zemmelink *et al.* (1991).

In conclusion, we found no evidence for a changed relation between NR and DOMI, due to infection or to diet quality. This confirms the observations of Akinbamijo *et al.* (1992). A few moribund animals showed very high protein catabolism (Figure 3C); the pooled regression equations for infected animals fed either grass straw or pelleted lucerne, however, were not altered significantly by these few observations.

The relation between NR and NI was different for lucerne and grass straw animals. This is probably caused by different N concentration in the two diets. For lucerne fed animals a segmented model with two linear regressions had a higher r^2 than one linear regression estimate. It is not clear, whether the relation between NR and NI was changed due to different efficiencies below and above maintenance level, or that the steeper line below maintenance was caused by infection, as most infected animals had a low NI. Lobley (1992) also reports a biphasic reponse of alterations in protein dynamics observed between fasted and *ad libitum* intake conditions for healthy ruminants.

Findings on daily urea excretion (Table 4) in the urine showed the same picture as urinary N from Kjeldahl analysis (Table 3). Urinary urea and creatinine excretion were

in line with serum urea and creatinine concentrations. This is an indication that kidney function was intact, which was also confirmed at autopsy (Van Dam *et al.*, submitted). The feeding of lucerne diet resulted in a higher urea concentration in blood and a higher excretion in urine, compared with grass straw diet. This agrees with findings of Cheema *et al.* (1991), who observed increased serum urea concentration in lambs, after protein supplementation of the diet. The effect of infection was relatively small in this experiment, compared with the effect of diet type. Nevertheless, urea levels were increased in both serum and urine in infected animals in wk 1 *p.i.* only. This may either have been caused by a short-lived increase of protein catabolism due to infection, or by a passing glomerulonephritis (Van den Ingh *et al.*, 1976). Variation in creatinine excretion indicates differences in muscle mass of the animal and may therefore give information about possible muscle breakdown (Kaneko, 1989). The decrease of serum creatinine concentration in infected animals may support this; however, the difference in creatinine concentration between lucerne fed animals and grass straw fed animals, which was already present before infection, must have been caused by dietary factors.

Conclusions

The results obtained from this study provide little evidence for the suggestion that an interaction exists between trypanosome infection and feed quality, with respect to feed intake, body weight change and N metabolism, during the acute phase of infection. Intake, expressed as DOMI, was reduced by infection with the same percentage in both diets, whereas the relation between N retention and DOMI was not significantly different between treatments and not different from literature. This implies that improving feed quality under practical conditions may offset (part of) the negative effect of infection on productivity.

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Chapter 7

Effect of fibrous feed quality on the course of *Trypanosoma vivax* infection in West African Dwarf goats. II. Metabolic profile, packed cell volume, and pathology of disease

J.T.P van Dam[1], D. van der Heide[2], T.S.G.A.M. van den Ingh[3], T. Wensing[4], and D. Zwart[1].

[1,2]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1] Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2] Dept. of Human and Animal Physiology, Haarweg 10, 6709 PJ Wageningen;
[3] Dept. of Veterinary Pathology, Utrecht University, PO Box 80.158, 3508 TD Utrecht, The Netherlands.
[4] Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht.

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Effect of fibrous feed quality on the course of *Trypanosoma vivax* infection in West African Dwarf goats. II. Metabolic profile, packed cell volume, and pathology of disease

J.T.P van Dam[1], D. van der Heide[2], T.S.G.A.M. van den Ingh[3], T. Wensing[4], and D. Zwart[1].

[1,2]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1] Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2] Dept. of Human and Animal Physiology, Haarweg 10, 6709 PJ Wageningen;
[3] Dept. of Veterinary Pathology, Utrecht University, PO Box 80.158, 3508 TD Utrecht, The Netherlands.
[4] Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht.

Abstract

Effects of trypanosome infection and fibrous feed quality on the metabolism of trypanotolerant West African Dwarf goats were measured. Goats were allotted to either a diet of lucerne pellets (n = 14) or a diet of chopped grass straw (n = 15); 5 animals per feed group served as controls and the other animals were infected with *Trypanosoma vivax* parasites. Before and after infection, blood samples were taken weekly, and analyzed for packed cell volume and parasitaemia, and for serum metabolites and hormones concentrations. Six weeks after infection *post mortem* analysis was carried out to study pathology of disease. Infected animals showed reduced feed intake, increased plasma non-esterified fatty acids concentration, and decreased serum insulin concentration. Liver triacylglycerol concentration was increased in all grass straw fed animals, and some infected goats fed lucerne diet. Infection drastically reduced serum concentration of thyroxine and triiodothyronine. Infection caused an increased weight of the liver and prescapular lymph nodes in animals from both diet treatments, but lymph nodes were more enlarged in infected animals fed lucerne. Pathological findings were typical for *T. vivax* infection in goats, irrespective of diet quality. Packed cell volume was reduced by infection in both feed groups to values below 20 per cent point. Serum γ-globulin concentration was increased in infected animals, but more in those fed lucerne than in those fed grass straw. It was concluded, that by feeding a better quality diet, nutritional status of infected West African Dwarf goats was improved. This was reflected in the serum concentrations of some metabolites and hormones. However, in general no indications for an interaction between infection and fibrous feed type with respect to nutritional status were found. Feed quality did not change the nature and severity of pathological variables, measured at autopsy after 6 weeks of infection.

Introduction

Trypanosoma vivax causes nagana disease in livestock. The disease causes anorexia, anaemia and cachexia, and eventually death (Stephen, 1986). In sub-Saharan Africa, several local breeds of cattle, goats and sheep show a milder course of infection; this is called trypanotolerance (ILCA, 1979). Also the West African Dwarf (WAD) goat breed can be considered as trypanotolerant (Osaer *et al.*, 1994). This tolerance, however, is not absolute; the outcome of disease is a delicate balance between immune response and parasite performance. External factors like body condition and nutrition may affect the course of infection too (Ferguson, 1988). Literature on the extent of interaction between external factors and the course of infection, however, is scarce.

Feed quality, one of these external factors, is highly variable in tropical regions. The WAD goat is often not able to maintain itself on poor quality tropical grasses (Ademosun *et al.*, 1988). In the present experiment it was studied whether, and if so, how fibrous feed quality affects the course of *T. vivax* infection in WAD goats. Therefore, the variables feed intake, serum metabolites and hormones (which signify trends in energy metabolism) packed cell volume and other pathological variables have been monitored during the first 6 weeks of an induced infection with *Trypanosoma vivax* in WAD goats, fed either a high or a poor quality fibrous feed.

Material and methods

Material and methods are described in more detail by Van Dam *et al.* (submitted).

Animals, feeding and housing

Twenty-nine castrated male adult WAD goats with an average body weight of 23 (± 0.78) kg and an average age of 12 (± 0.03) months were used. Before the experiment they received an anthelmintic treatment and were vaccinated for ecthyma. Two different feeds were used *viz.* pelleted lucerne and chopped grass straw. Lucerne contained 27.5 g $N \cdot kg^{-1}$ dry matter, whereas grass straw contained 10.9 g $N \cdot kg^{-1}$ dry matter. The animals had *ad libitum* access to experimental diets during both the adaptation and the experimental period. Salt lick and water were freely available.

Infection

The way that animals were infected is decribed elsewhere (Van Dam *et al.*, submitted). Fly density in the stable was kept low by applying UV lamps and insecticide, in order to prevent cases of mechanical transmission of trypanosomiasis.

Experimental design

Animals were allocated to either trypanosome Infection, fed Lucerne (IL; n = 9), Control, fed Lucerne (CL; n = 5), Infection, fed Grass straw (IG; n = 10) and Control, fed Grass straw (CG; n = 5) treatment. Animals from the IL and IG group were infected at day 0 and were followed for 6 weeks, after which they were euthanized by administration of T61 (Hoechst Veterinär GmbH, München, BRD) in the jugular vein.

Sample procedures and sample preparation

Daily dry matter intake (DMI) was measured by daily offering *ad libitum* feed to animals and collecting refusals after 24 h. Dry matter (DM) content of offered and refused feed was measured from weekly samples per animal, as described by Van Dam *et al.* (submitted), and DMI was calculated as the difference between daily offered DM and refused DM.

Rectal temperature was measured every morning, just before feeding. Blood samples were taken weekly from 2 weeks *ante infectio* (*a.i.*) until 6 weeks *post infectio* (*p.i.*). Blood was collected from the jugular vein in evacuated tubes (Venoject vacuum tubes, Terumo, Leuven, Belgium). Blood samples, with the addition of heparin, were processed for analysis of packed cell volume (PCV) and parasitaemia. The PCV was assessed by means of spinning heparinized capillaries containing heparinized blood, for 3' in a micro-hematocrit centrifuge. Parasitaemia was measured by determination of the white blood cell count, and establishment of the WBC/ trypanosome ratio in a thick smear stained with Giemsa.

Furthermore, a number of clinical biochemical variables in the blood was quantified. In blood, collected with NaF/ K-oxalate coated tubes, plasma glucose concentration was measured (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). In blood, collected with Li-heparin/ paraoxon coated tubes, plasma non-esterified fatty acids (NEFA) concentration was measured (NEFA C, Instruchemie B.V., Hilversum, The Netherlands). Furthermore serum samples were analyzed for β-hydroxy butyrate (BHB) concentration (Boehringer Diagnostica). Serum total protein (TP) concentration, and protein spectrum were measured, according to the method, described by Wensing *et al.* (1989), and from this the concentrations of albumin and γ-globulin were calculated. Serum insulin concentration was measured using a radio immuno assay kit (Coat-a-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA). The serum concentration of thyroxine (T4) and triiodothyronine (T3) was measured using homologous RIA technique.

Immediately after euthanization, a fresh liver sample was harvested and deep frozen in liquid nitrogen. From these samples, triacylglycerol (TAG) was measured using a commercial kit (Kit No. 405, Sigma Chemical Co., St. Louis, MO, USA). Liver glycogen was measured, as described by Van den Top *et al.* (1995). Gross and microscopic *post*

mortem examination was done. Additionally the *post mortem* weight of liver, thyroid gland, adrenals and prescapular lymph node were measured.

Statistical model

Preliminary analysis showed that, for either pre-infection or post-infection period, no time effect in the repeated measurements of plasma glucose and NEFA and serum BHB was present. Therefore these data were pooled per animal, for either pre- or post-infection period, and were subjected to statistical analysis. For the variables DMI, PCV, parasitaemia, TP, albumin, γ-globulin, insulin, T3 and T4, which had been weekly measured in each animal, an effect with time after infection was detected. These data were therefore analyzed per measuring week. Data on hepatic TAG and glycogen, and post *mortem* weights of liver, adrenals, thyroids and prescapular lymph nodes were only measured once.

The following statistical model was used to test effects of treatments (General Linear Models procedure, SAS, 1990):

$$Y_{ijk} = \mu + D_i + I_j + (D \times I)_{ij} + e_{ijk} \quad [1]$$

where: Y_{ijk} = dependent variable; μ = overall mean; D_i = effect of Diet ($i = 1,2$); I_j = effect of Infection ($j = 1,2$); $(D \times I)_{ij}$ = effect of interaction between Diet and Infection; e_{ijk} = error term. Least square (LS) means were calculated and differences between treatments were tested using the F-test. Correlations were calculated between different variables, using individual data that were pooled over the post-infection period.

Results

General course of infection

After day 4 *p.i.* all infected animals showed intermittent fever with temperature peaks reaching over 42°C. The mean rectal temperature from day 5 *p.i.* onwards was 39.9 (± 0.07)°C, 38.6 (± 0.08)°C, 39.6 (± 0.06)°C and 37.9 (± 0.08)°C in IL, CL, IG and CG animals, respectively. The group means were all significantly different from each other (at least $P < 0.05$).

One week after infection, parasites were detected in the blood of all infected animals. In each infection group two animals died before the end of the experiment or were euthanized when moribund. The two animals belonging to the IL group died on days 32 and 33, whereas the two animals from the IG group died on days 30 and 40.

Dry matter intake, metabolites and hormones

Dry matter intake was decreased in infected animals throughout the infection period ($P < 0.001$; Figure 1). Grass straw DMI was lower than lucerne DMI ($P < 0.05$). No statistical interaction between infection and feed quality was detected ($P > 0.10$).

Because no effect of week number *p.i.* on glucose, BHB and NEFA was observed, repeated measurements, either before or after infection were pooled per animal, and least squares means per treatment were calculated (Table 1). Results per animal *p.i.* were pooled, and LS means per treatment were estimated. Glucose concentration was not affected by diet or infection. Before infection BHB concentration was slightly lower and NEFA concentration slightly higher ($P < 0.05$) in groups, which were selected for infection. Lucerne diet decreased ($P < 0.05$) and infection increased ($P < 0.001$) NEFA concentration during the infection period.

Figure 1. Daily dry matter intake (DMI) during infection with *Trypanosoma vivax* and two experimental diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw.

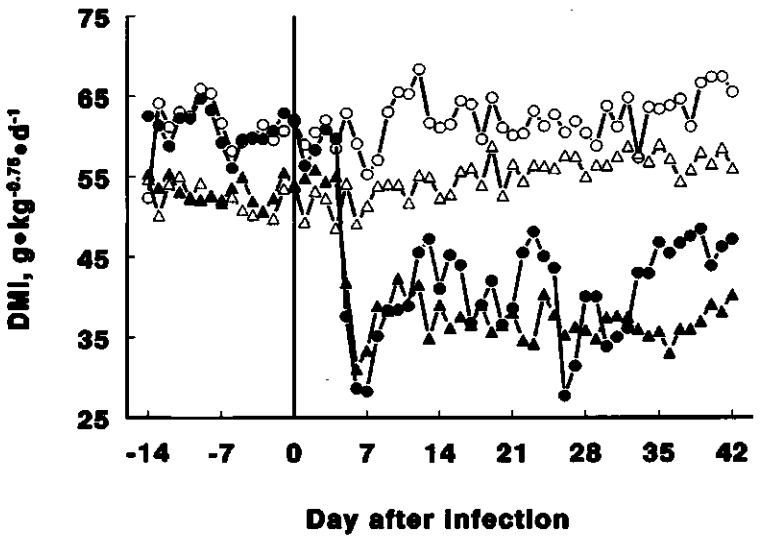


Table 1. Serum glucose, beta-hydroxy butyrate (BHB) and non-esterified fatty acids (NEFA) concentrations (all in mmol·L^{-1}), during infection with *Trypanosoma vivax* and two different diets; least square means of pre-infection and post-infection period.

	LS Means of treatments[1]					P-values[2]		
	IL	CL	IG	CG	rmse[3]	diet	inf	int
	Pre-Infection Period[4]							
No. of animals	9	5	10	5				
glucose	3.36	3.34	3.13	3.09	0.46	ns	ns	ns
BHB	0.141	0.157	0.115	0.133	0.041	ns	*	ns
NEFA	0.145	0.115	0.177	0.158	0.066	ns	*	ns
	Infection period[4]							
No. of animals	9	5	10	5				
glucose	3.12	3.25	3.06	3.05	0.66	ns	ns	ns
BHB	0.318	0.170	0.171	0.113	0.478	ns	ns	ns
NEFA	0.267	0.108	0.366	0.192	0.211	*	***	ns

[1]: IL = Infected animals fed Lucerne, CL = Control animals fed Lucerne, IG = Infected animals fed Grass straw, CG = Control animals fed Grass straw;
[2]: Significance of treatments; inf = infection, int = interaction; ns = not significant; * = $P < 0.05$; *** = $P < 0.001$;
[3]: Root mean square error (sem = rmse/$\sqrt{n}$);
[4]: pooled per animal.

Serum concentrations of total T3 and T4 are presented in Figure 2. Infection reduced both serum T3 and T4 concentration ($P < 0.001$). From wk 1 *p.i.* statistical interaction between diet and infection was observed with respect to serum T4 concentration, whereas in wk 3, 5 and 6 *p.i.* this was also noted for serum T3 concentration ($P < 0.05$).

Serum insulin concentration was lowest in the IG group throughout infection and was highest in the CL group (Figure 3). No explanation is available that could account for the sudden depression of control group insulin levels in wk 4. During the entire experimental period animals from the lucerne diet treatment had a higher insulin concentration than animals from the grass straw treatment ($P < 0.01$). *T. vivax* infection led to a reduction of serum insulin concentration, except in week 4 *p.i.*

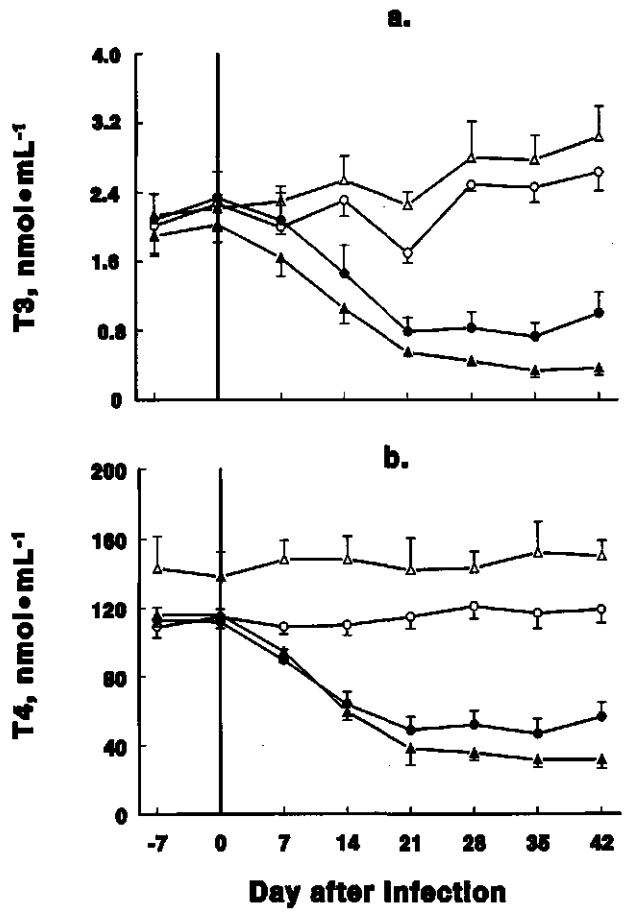
In Table 2 the hepatic TAG and glycogen concentrations are presented. Animals fed grass straw had a higher TAG content than animals fed lucerne ($P < 0.001$). No effect of treatments on the hepatic glycogen content was observed.

Packed cell volume and serum proteins

The PCV of infected animals (Figure 4) initially showed a sharp decrease in the first weeks of infection but stabilized after wk 3 *p.i.* at a much lower level than the control groups ($P < 0.001$). The CG group showed a lower PCV than the CL group from wk 1

p.i. onwards ($P < 0.001$); also significant interaction between diet and infection was observed after week 1 *p.i.* ($P < 0.01$).

Figure 2. Serum concentration of **a.** Triiodothyronine and **b.** Thyroxine during infection with *Trypanosoma vivax* and two experimental diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.



Serum TP concentration increased in infected animals during the course of infection ($P < 0.001$). Average concentrations at six weeks *p.i.* were 98 (± 3.1), 71 (± 3.6), 77 (± 2.7) and 67 (± 3.6) $g \cdot L^{-1}$ TP in IL, CL, IG and CG group respectively. The serum γ-globulin concentration increased in infected animals during the course of infection ($P < 0.001$; Figure 5). From week 4 *p.i.* onwards, IL animals had a higher γ-globulin

concentration than IG animals ($P < 0.05$). Statistical interaction between the effect of infection and the effect of diet on γ-globulin was observed from week 4 *p.i.* onwards (at least $P < 0.05$).

Table 2. Post mortem hepatic concentrations of triacylglycerol (TAG) and glycogen (both in g·kg^{-1} liver), 6 weeks after infection with *Trypanosoma vivax* and two different diets; least square means per treatment.

	LS Means of treatments[1]					P-values[2]		
	IL	CL	IG	CG	rmse[3]	diet	inf	int
No. of animals	6	5	8	5				
TAG	29	24	62	70	25	***	ns	ns
Glycogen	26	22	18	28	10	ns	ns	ns

[1]: IL = Infected animals fed Lucerne, CL = Control animals fed Lucerne, IG = Infected animals fed Grass straw, CG = Control animals fed Grass straw;
[2]: Significance of treatments; inf: infection, int: interaction; ns = not significant; *** = $P < 0.001$;
[3]: Root mean square error (sem = rmse/√n).

Figure 3. Serum concentration of insulin during infection with *Trypanosoma vivax* and two experimental diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.

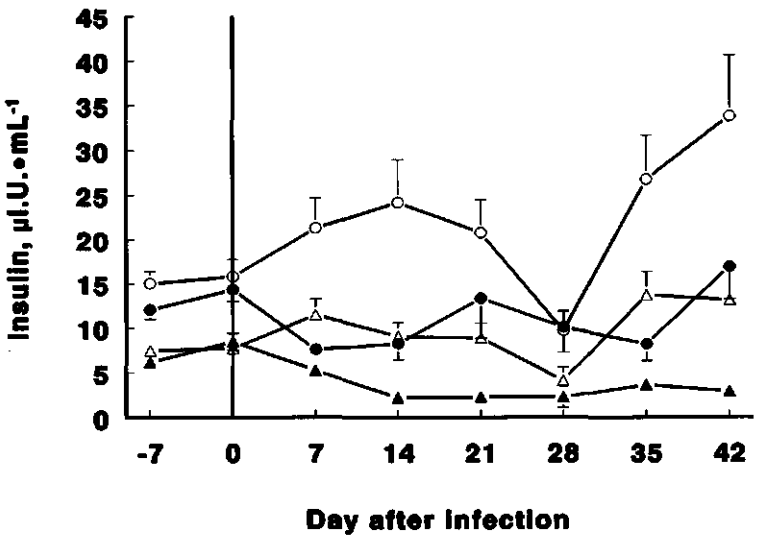


Figure 4. Packed Cell Volume during infection with *Trypanosoma vivax* and two experimental diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.

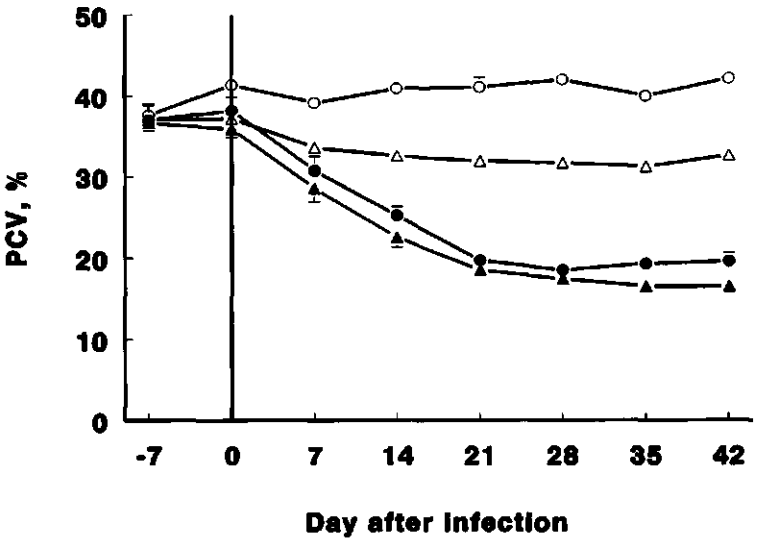


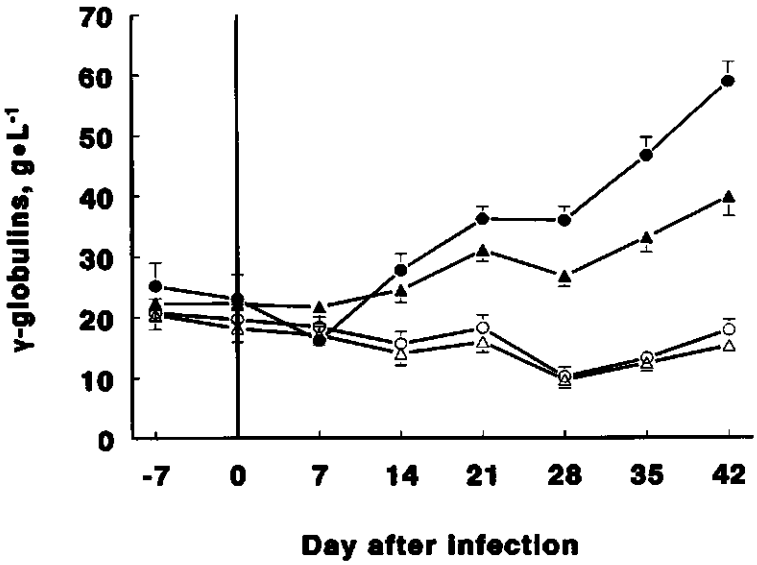
Table 3. Weights of liver, thyroid gland, adrenals and prescapular lymph node (all in g), 6 weeks after infection with *Trypanosoma vivax* and two different diets; least square means per treatment.

	LS means of treatments[1]					P-values[2]		
	IL	CL	IG	CG	rmse[3]	diet	inf	int
No. of animals	9	3	10	3				
liver	604	436	488	291	110	*	**	ns
liver·kg$^{-0.75}$	61	40	57	29	8	ns	***	ns
lymph node	14.6	4.0	7.3	3.3	5.0	ns	**	ns
thyroid gland	2.1	1.8	1.5	1.4	0.5	*	ns	ns
adrenals	2.1	1.7	2.2	1.4	0.4	ns	**	ns

[1]: IL = Infected animals fed Lucerne, CL = Control animals fed Lucerne, IG = Infected animals fed Grass straw, CG = Control animals fed Grass straw;
[2]: Significance of treatments; inf: infection, int: interaction; ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$;
[3]: Root mean square error (sem = rmse/$\sqrt{n}$).

Serum albumin concentration decreased in the course of infection, in infected animals. Thus average albumin concentration was lower in infected animals than in control animals from week 2 *p.i.* onwards ($P < 0.05$). At 6 weeks *p.i.*, albumin concentration was 25 (± 0.8), 38 (± 1.0), 27 (± 0.7) and 37 (± 1.0) g·L^{-1} in the IL, CL, IG and CG group, respectively.

Figure 5. Serum concentration of γ-globulins during infection with *Trypanosoma vivax* and two experimental diets. The line —●— represents infected animals fed lucerne, —○— control animals fed lucerne, —▲— infected animals fed grass straw, and —△— control animals fed grass straw; error bars indicate sem.



Post mortem examination

Parasitaemia level and relative decrease of PCV were not correlated, neither were parasitaemia level and DMI.

Gross and microscopic examination was carried out on all infected and six control animals. PM autopsy revealed in infected animals a typical picture of subacute to chronic *T. vivax* infection with marked hyperplasia of lymphoid tissues; often a mononuclear myocarditis (11 cases out of 19), associated with the presence of extravascular trypanosomes, was observed. In one animal a mononuclear meningo-encephalitis was detected, also associated with extravascular trypanosomes. Most infected animals showed a non-specific reactive hepatitis.

Fatty livers were encountered in all infected and non-infected grass straw animals and some goats from the IL group with a low DMI. The animals that died during the trial, virtually had no fat deposits left. No indications were found for specific mineral or vitamin deficiencies from gross or microscopic examination. No intestinal or renal lesions, that could be attributed to trypanosomiasis, were detected. In 5 animals, however, two of which were in the control groups, minor intestinal lesions were detected, which were probably caused by paratuberculosis.

The PM weight of some organs and tissues is shown in Table 3. The liver weight per $kg^{0.75}$ was increased in infected animals ($P < 0.001$). The total liver weight showed the same effect of infection ($P < 0.01$). Animals fed lucerne showed an increased total liver weight compared with animals fed grass straw ($P < 0.05$). The right prescapular lymph node, and the adrenals were enlarged in infected animals ($P < 0.01$). The thyroid gland was enlarged in animals fed lucerne ($P < 0.05$).

Discussion

General course of infection

Infection followed a severe course, with > 20 % of the infected animals dying within 6 weeks. The severity and pattern of the fever were typical for *T. vivax* infection in WAD goats (Zwart *et al.*, 1991, Van den Ingh *et al.*, 1976). Rectal temperature was slightly lower in IG animals, compared with IL animals. The difference in rectal temperature between control animals fed either lucerne or grass straw was remarkable. Possibly CG animals had a lower morning rectal temperature, because of a lower feed intake than CL animals (Akinbamijo *et al.*, 1996).

Dry matter intake, metabolites and hormones

Dry matter intake in both fibrous feed groups was reduced by infection (Figure 1). This led to body weight loss implying a negative energy balance and lypolysis (Van Dam *et al.*, submitted). As a consequence the concentrations of circulating NEFA and BHB increased at low DMI levels as expected (Payne, 1989; Van den Top *et al.*, 1995). Increased plasma NEFA concentration coincided with a higher hepatic TAG concentration in IG animals, but not in IL animals (Table 2). Van den Top *et al.* (1995) postulated that hepatic TAG accumulation is correlated with high plasma NEFA concentration. However, in the present trial animals from the CG treatment had a higher hepatic TAG concentration but a lower plasma NEFA concentration than the IL treatment. Possibly, besides hepatic NEFA uptake, TAG accumulation is also dependent on diet quality.

Serum glucose and BHB concentrations were not changed by treatments. Apparently serum NEFA is a more sensitive indicator of nutritional status of the animal than serum glucose or BHB under the present experimental design. Van den Top *et al.* (1995), however, concluded that serum glucose, NEFA and BHB were all good indicators of a negative energy balance in *peri partum* goats, glucose being decreased and NEFA and BHB being increased. Our findings on serum BHB were highly variable in underfed animals; especially some moribund animals showed extremely high values in the terminal phase. However, due to large variation among infected animals, group means

of BHB were not significantly affected by treatments. Wolkers (1993) observed hardly any increase of NEFA and BHB in underfed red deer, and postulated that ketogenesis does not play the same role in the different ruminant species. In the present experiment no effects of treatments on liver glycogen were observed. This agrees with findings of Van den Top *et al.*, (1995) who observed no effect of undernutrition on liver glycogen level in goats around parturition.

Insulin concentrations showed large variation both between and within treatments (Figure 3). Significant effects of both diet and infection were observed in most post-infection weeks. In general animals fed lucerne had higher serum insulin concentrations than animals fed grass straw. Infection was found to reduce serum insulin concentration. The changes in insulin were found to correspond with the observed trends in DMI and plasma NEFA concentration in these treatment groups. Plasma glucose concentration is positively related to insulin level, and is the most important factor that controls insulin release (Hardy, 1981). However, only small differences in glucose concentration were observed between treatments, in spite of a lower DMI in infected animals (Table 1; Figure 1). This may be explained by an increased gluconeogenesis in underfed animals.

T3 and T4 levels were severely depressed in infected animals (Figure 2). Mutayoba and Gombe (1989) postulated that thyroid gland function is impaired during trypanosome infection, which may explain the serum T3/T4 reduction in our trial. Reincke *et al.* (1993) also found indications for impaired thyroid function; he observed increased TSH levels but reduced T3 and T4 levels in infected human subjects. Microscopic examination of thyroids in our experiment, however, revealed moderate activity of follicular epithelium, which means that T3/T4 production was not impaired. Another possible explanation for decreased T3/T4 during infection was given by Beisel (1985), who described an increase in the rates of thyroid hormone uptake and degradation by peripheral tissues and blood neutrophils during infection, as well as an increased clearance rate by the liver. Enomoto *et al.* (1990) showed that mouse serum T4 levels were reduced after administration of the cytokine interleukin 1-α (IL-1-α), which is also produced during trypanosome infection, and the thyroid rendered unresponsive to thyroid stimulating hormone (TSH). The same clinical signs were observed in an infection trial by Reincke *et al.* (1993), which was accompanied by an increase of Tumor Necrosis Factor-α (TNF-α). Sweep *et al.* (1992) concluded that chronic infusion of a subanorectic dose of TNF-α in rats caused a reduction of serum T3 and T4 concentration. In their experiment, thyroid function, responsiveness to TSH and peripheral thyroid hormone metabolism, however, were not affected by TNF-α. They postulated that the blood levels of T4 Binding PreAlbumin (TBPA) were reduced by TNF-α, which limited the binding capacity in the blood, thus leading to lower serum T3/T4 concentrations. This may also

have been the case in the present experiment. It is not clear, if at cellular level T3 availability was changed.

Concluding, serum metabolites and hormones reflected the nutritional status of individual goats. Because infection led to a reduced DMI, also metabolites and hormones concentrations were altered. Probably the sharp reduction of serum concentrations of T3 and T4 were a direct effect of trypanosome infection.

Packed cell volume and serum proteins

The decrease of the PCV in both infection groups (Figure 4) to values below 20 % was larger than in previous studies with *T. vivax* Y486 in dwarf goats (Verstegen *et al.*, 1991; Akinbamijo *et al.*, 1992) but corresponded with studies on *T. congolense* infection in WAD goats of Adah *et al.* (1993) and Osaer *et al.* (1994). However, the N'Dama, an extensively studied cattle breed, is able to minimize PCV reduction after infection (Trail *et al.*, 1991). Probably WAD goats are not as tolerant as N'Dama cattle.

Lucas *et al.* (1993) suggested an important role for TNF in the induction of anaemia during trypanosomiasis, by hyperactivation of macrophages and a subsequent increase of erythrophagocytosis, which may have been the case in the present experiment. TNF-α, as well as other cytokines like Interferon-γ (IFN-γ) and IL-1, may also play a role in parasite control and immunosuppression (Lucas *et al.*, 1993). Lomo *et al.* (1995) observed that anaemia due to *T. congolense* infection in rabbits was related to the decrease of serum T3 and T4 concentration; treatment of infected animals with replacement doses of L-thyroxine reduced parasitaemia levels and partly prevented the occurrence of anaemia. The mechanism behind this was not clear, however. Also in the present trial anaemia coincided with reduced serum T3/T4 concentration.

In the present study no indications were found for an effect of diet quality on the decrease of PCV. Agyemang *et al.* (1990) showed that high quality supplement to the diet did not change the PCV response to infection. This finding corresponds with the view held by Murray and Dexter (1988) who stated that anaemia during trypanosomiasis is mainly caused by immunological mechanisms. In contradiction to this, Trail *et al.* (1991) reported that BW gain, which is a reflection of the amount of ingested nutrients, showed a positive correlation with the ability to control anaemia. However, their conclusions were based on a long term field study in which chronic, instead of acute infections were often encountered.

The difference in PCV between CG and CL group was remarkable, approximately 10 % points during the whole experiment. This may be explained by the low protein intake of the CG group, which averaged 3.68 ($\pm$ 0.48) $g \cdot kg^{-0.75} \cdot d^{-1}$. The NRC (1981) mentions maintenance requirements of 4.15 $g \cdot kg^{-0.75} \cdot d^{-1}$.

Serum TP level was increased by infection. This agreed with results from a study of Akinbamijo *et al.* (1992) with lucerne fed goats, infected with *T. vivax*. In an infection trial with *T. congolense* in Scottisch Blackface sheep, however, infected animals tended toward a lower TP level, compared with healthy sheep (Katunguka-Rwakishaya *et al.*, 1995). The TP concentration at wk 6 *p.i.* of the IG group was lower than the TP level of the IL group. This also may have been caused by dietary protein deficiency (Payne, 1989). The serum concentration of γ-globulins was increased in both infected groups (Figure 5), which explains the increased TP level, despite the slightly lower albumin concentration in infected animals. The decreased albumin concentration in infected goats was supported by results from a study of Katunguka-Rwakishaya *et al.* (1993); they found in sheep, that both *T. congolense* infection and a low protein diet led to a reduction of the serum albumin concentration.

Post mortem examination

The weight of the right prescapular lymph node was used as a reflection of the stimulation of the immune system by the *T. vivax* infection. It was evident that infected animals had larger lymph nodes (Table 3). It cannot be excluded that the detected difference between IL and IG results from inadequate nitrogen sources for massive lymphocellular proliferation in grass straw fed animals, but most other pathological variables were not different between the IL and the IG group. The higher γ-globulines in lucerne fed animals apparently did not result in a more successful immune response.

Liver weight of infected animals was higher than that of controls. This corresponds with findings of Anosa and Isoun (1983) and Losos and Mwambu (1979). Probably hepatic TAG accumulation has not accounted for most of the liver weight increase in the infected animals. That animals from the CG group showed increased TAG but no liver weight increase supports this suggestion. Therefore, the liver weight increase may be a direct pathological effect of trypanosome infection, resulting from the observed non-specific reactive hepatitis.

Fatty infiltration in the liver mainly develops in animals with large fat depots brought to negative energy balance (Payne, 1989). This may have occurred in grass straw animals, with a DMI around or below maintenance requirements for a prolonged period, and for some animals from the IL group with low DMI.

Conclusions

Fibrous feed quality differences did not interact with the metabolic changes induced by trypanosome infection. Dry matter intake was reduced by the infection, and changes in the serum concentrations of some relevant clinical biochemical variables, which are typical for ruminants in a negative energy balance, were detected. The serum γ-globulin concentration, and the weight of prescapular lymph nodes was more increased in infected animals fed lucerne, than in infected animals fed grass straw. In animals fed grass straw, hepatic triacylglyceride content was increased. Serum thyroxine and triiodothyronine concentrations were decreased by the infection.

Therefore, improving the quality of the diet does not abate most of the studied variables during the acute phase of trypanosome infection; only the nutritional status of the animal is improved.

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Chapter 8

The effect of previous growth retardation on energy and nitrogen metabolism of goats, infected with Trypanosoma vivax

J.T.P. van Dam[1], J.W. Schrama[1,2], A. Vreden[1], M.W.A. Verstegen[2], T. Wensing[3], D. van der Heide[4], and D. Zwart[1].

[1,2,4]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]: Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen;
[3]: Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht, The Netherlands;
[4]: Dept. of Human and Animal Physiology, Haarweg 10, 6709 PJ Wageningen.

Submitted.

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J.T.P. van Dam[1], J.W. Schrama[1,2], A. Vreden[1], M.W.A. Verstegen[2], T. Wensing[3], D. van der Heide[4], and D. Zwart[1].

[1,2,4]: Wageningen Institute of Animal Sciences, Wageningen Agricultural University;
[1]: Dept. of Animal Husbandry, PO Box 338, 6700 AH Wageningen, The Netherlands;
[2]: Dept. of Animal Nutrition, PO Box 338, 6700 AH Wageningen;
[3]: Dept. of Large Animal Medicine, Utrecht University, PO Box 80.152, 3508 TD Utrecht, The Netherlands;
[4]: Dept. of Human and Animal Physiology, Haarweg 10, 6709 PJ Wageningen.

Abstract

The effect of growth retardation, resulting from feed restriction for a prolonged period, on the course of infection with *Trypanosoma vivax* was studied. Therefore, 12 male castrated West African dwarf goats were subjected to a restricted feeding regimen of 55 $g \cdot kg^{-0.75} \cdot d^{-1}$ pelleted lucerne for on average 17 weeks. Twelve other animals were fed pelleted lucerne *ad libitum*, resulting in a normal growth pattern. After this period, all animals were fed pelleted lucerne *ad libitum*, and six animals of each previous feeding regimen treatment were infected with *Trypanosoma vivax*. The other animals served as controls. In week 2 and 4 *p.i.* energy and nitrogen balances were measured. In the week before infection and during infection also blood biochemical and clinical parameters were measured. Two weeks before, and 4 weeks after infection, a liver biopsy was taken for measurement of triacylglycerol. Infection caused intermittent fever and anaemia. The first peak of fever persisted longer in infected animals with normal growth than in infected animals with retarded growth. Gross energy and metabolizable energy intake, and energy retention were reduced in infected animals. Metabolizable energy requirements for maintenance were increased by infection. Results suggested that in animals with retarded growth, maintenance requirements were less increased by infection than in animals with normal growth. Plasma NEFA and glucose concentrations were increased in infected animals, whereas serum T3/T4 concentrations were decreased. Plasma urea concentration and liver triacylglycerol were not affected by treatments. No interaction of growth retardation with infection with respect to blood biochemical parameters was found, apart from plasma NEFA in week 2 *p.i.*. Nitrogen retention was not significantly affected by treatments. Concluding, minor indications were found for an interaction of growth retardation as applied in this study, with trypanosome infection in West African Dwarf goats, with respect to energy and nitrogen metabolism.

Introduction

Trypanosomiasis, a protozoan disease of (sub-)humid regions of Africa, causes anorexia, anaemia and intermittent fever in domestic animals (Van den Ingh *et al.*, 1976; Zwart *et al.*, 1991); energy requirements for maintenance of West African Dwarf goats are increased by approximately 25 % during the acute phase of the infection (Verstegen *et al.*, 1991). As a consequence of what is called trypanotolerance, several local breeds of goats, sheep and cattle, however, are able to survive from the disease (Murray & Morrison, 1981).

Malnutrition often interacts with the severity of disease (Beisel, 1985). Also the degree of trypanotolerance is affected by nutritional status of the host animal (Murray, 1988). In tropical countries malnutrition frequently occurs due to shortage of good quality roughage. In small ruminants malnutrition was found to be related to increased mortality due to trypanosome infection (Reynolds & Egwuruke, 1988). Van Dam *et al.* (1996a) fed fibrous diets with a high or a low nutritional quality for 3 months to trypanotolerant West African Dwarf goats. They observed no interaction between trypanosome infection and fibrous feed quality with respect to N retention, *i.e.*, the negative effect of infection was not greater in animals, fed a poor quality diet compared with animals fed a good quality diet. However, in both feed groups, animals were in a good body condition at the start of infection, meaning that they could mobilize (part of their) body reserves during infection.

In the present trial, it was therefore investigated, how dietary limitations (by offering maintenance feed to growing animals) for a prolonged period would affect the course of a subsequent infection with *T. vivax* with respect to energy and nitrogen metabolism.

Material and methods

Experimental design

1. Feed restriction period

At the start of the feed restriction period, pairs of goats with similar body weight were selected from a group of castrated West African Dwarf goats with a mean age of 3.3 (± 0.1) months. From each pair of animals, 1 animal was randomly allocated to restricted feeding, (retarded growth group, R); the other was to receive *ad libitum* ration (normal growth group, N). The initial mean body weight of the retarded growth group was 13.34 (± 0.72) kg, and of the normal growth group 13.24 (± 0.45) kg. The mean duration of the feed restriction period was 16.5 (± 0.7) weeks. During this period, the applied feed

ration of restricted animals was 55 g fresh feed per kg metabolic body weight per day ($g \cdot kg^{-0.75} \cdot d^{-1}$; approximately maintenance level).

2. infection period

After the feed restriction period all animals received *ad libitum* pelleted lucerne for a period of 2 weeks, preceding the moment of infection. This period of 2 weeks was meant for adaptation of the restricted animals to the *ad libitum* feeding regimen which would be applied for all animals during infection. Then half of the animals of both feed groups (n = 6) were randomly selected, to be infected with trypanosomes. Therefore, a 2×2 factorial design was used, *i.e.*, infected normal growth (IN; n = 6), infected retarded growth (IR, n = 6), control normal growth (CN; n = 6) and control retarded growth (CR; n = 6).

The goats from the IN and IR group were infected with 1×10^6 parasites from strain *Trypanosoma vivax* Y486, isolated by Leeflang *et al.* (1976). Control animals were sham infected by intravenous injection of 2 mL saline.

During the infection period all animals received *ad libitum* pelleted lucerne. At the day of infection (day 0) the mean body weight of the animals with retarded growth was 15.60 (± 0.84) kg, and the mean body weight of the animals with normal growth was 21.59 (± 0.87) kg; mean body weight change over the feed restriction period was 17 and 64 $g \cdot d^{-1}$ for animals with retarded and animals with normal growth, respectively.

After day 28 *p.i.* animals were euthanized by intravenous administration of 5 mL of T61 (Hoechst Veterinär GmbH, München, Germany), according to animal welfare regulations.

Feeding and housing

Throughout the experiment, a diet of pelleted lucerne was offered to the animals. The average dry matter content of the feed was 924 $g \cdot kg^{-1}$, with 175 g crude protein and 16.2 MJ gross energy per kg dry matter. Before infection animals were housed individually in balance cages for at least 3 weeks.

The time schedule of housing and measurements is presented in Table 1. In week 1 and 3 *p.i.* animals were housed in a dummy chamber to adapt the goats to housing conditions in the climatic respiration chamber. They were housed in one of two identical climatic respiration chambers (described by Verstegen *et al.*, 1987) in week 2 and 4 *p.i.* In each chamber two goats were housed. This was done to prevent stress due to social isolation (Carbonaro *et al.*, 1992). The two animals were separated by a wired fence, in order to facilitate the individual measurement of feed intake. The space allowance per goat was 1.00 × 0.40 × 0.97 m (l × w × h) in both the dummy and respiration chambers. The light period was between 7.00 a.m. and 7.00 p.m. Temperature was

maintained at 20°C. In the respiration chambers relative humidity was maintained at 65 %. The allocation of different treatments over the two chambers was balanced. Because only 4 animals were housed in the respiration chamber at the same time, animals were infected in groups of 4, after each other. The treatment sequence over time was balanced.

Table 1. Measurements and time schedule for each pair of goats[1].

day after infection	-14	-7	0	7	14	21	28
housing	indiv. pen		| dum	| RC	| dum	| RC	|
treatment	B		inf				eut;B
feed intake	+ +						
rectal temperature			+ +				
body weight	+	+	+	+	+	+	+
blood samples		+	+	+	+	+	+
energy balance measurements				|————|		|————|	

[1]: dum. = dummy chamber; RC = respiration chamber; B = Biopsy; inf. = day of infection; eut. = day of euthanization.

Measurements and calculations

From day 0 (day of infection) onwards, rectal temperature was measured daily just before morning feeding to monitor fever during infection. Blood samples were collected on day -7, 0, 7, 14, 21 and 28 in the morning after feeding. Blood was extracted from the jugular vein, using Venoject vacuum tubes (Terumo, Leuven, Belgium). From heparinized blood, packed cell volume (PCV) was measured by means of a microhaematocrit centrifuge. Also from heparinized blood, parasitaemia was measured by assessment of the number of leucocytes per mL in a Coulter Counter, and the number of parasites relative to leucocytes in a thick smear stained with Giemsa, and was expressed as the number of parasites per mL.

Both in the restriction period and in the infection period the body weight was measured every week. Daily body weight gain over the infection period (day 0 to day 28) was calculated per animal per day and per $kg^{0.75}$ per day.

From day -14 (*i.e.*, 14 days before infection) onwards, individual daily feed intake was measured by offering *ad libitum* lucerne pellets in early morning, and subsequent collection of feed residues the next day. Dry matter content of offered feed and feed residues was measured according to the ISO 5984 instructions, in composite samples, collected per animal per week; from this daily dry matter intake (DMI) per kg metabolic weight was calculated.

Fourteen days before infection, from all animals a liver biopsy was taken (method described by Van den Top *et al.*, 1995). After euthanization of the animals at 28 days

p.i., another liver sample was taken by incision of the thoracic wall. Liver samples were stored in saline and analyzed for triacylglycerol (TAG) with kit no. 405 (Sigma Chemical Co., St. Louis, MO, USA) to monitor the effects of treatments on liver fat metabolism.

When housed in the climatic respiration chambers, the following parameters were measured to calculate the energy and nitrogen balance for pairs of goats for a 7-day period. The O_2 consumption and CO_2 and CH_4 production were measured during successive intervals of 9 minutes. For each interval, heat production was calculated from these gaseous exchanges, using the equation of Brouwer (1965). Faeces and urine, and the water that was used to clean the chamber, were collected and weighed at the end of the balance period and a representative sample was taken and analyzed for N (ISO 5983). Gross energy was measured, using bomb calorimetry (IKA Analysentechniek GmbH, Heitersheim, BRD). In faeces samples also dry matter and ash content were analyzed (ISO 5984). The weekly amount and composition (dry matter, ash, nitrogen and gross energy) of offered and refused feed were measured. Gross energy (GE) was calculated as the amount of ingested energy in feed. Metabolizable energy (ME) was calculated as GE minus the energy in faeces, urine, expired CH_4 and the energy trapped in cleaning water. Energy retention (ER) was calculated as ME minus the produced heat.

For each observation the ME maintenance requirements (ME_m) were calculated. It was assumed that energy above maintenance had been deposited with a partial efficiency of 0.6, whereas energy mobilization from the tissues could be prevented by offering 1.25 kJ ME·kJ^{-1} body energy loss (partial efficiency of 0.8; ARC, 1980).

Nitrogen retention (NR) was calculated as the difference between N intake and N losses via faeces and urine, and was expressed in g·kg$^{-0.75}$·d^{-1}. Furthermore, NR was corrected for N that was evaporated from faeces and urine to the air. Protein gain was calculated as the product of retained protein (NR × 6.25) and the energy content of 1 g deposited protein, (23.7 kJ). Fat gain was calculated, being the difference between ER and protein gain. NR was expressed in g·kg$^{-0.75}$·d^{-1}. The ME, HP, ER, ME_m, fat gain and protein gain were expressed in kJ·kg$^{-0.75}$·d^{-1}.

In the respiration chambers, for pairs of goats, physical activity was measured continuously using Doppler-radar activity meters (Radar MD5, Suther, Vierpool, Amsterdam). The visual movements of the animals housed in the respiration chambers were converted into counts, per interval of 9 minutes which corresponded with a HP measurement interval. The relation between number of activity counts and HP was assessed per goat pair per respiration week by linear regression. These regression estimates were used to calculate activity related heat production, HP_{act}. The difference between HP and HP_{act} was the HP, corrected for activity, HP_{cor}.

The following blood parameters which can be associated with energy metabolism were measured. From blood containing Li-heparin and paraoxon, plasma non esterified

fatty acids (NEFA) concentration was analyzed enzymatically (NEFA C, Instruchemie B.V., Hilversum, The Netherlands). From blood containing NaF and K-oxalate as anti-coagulants, plasma glucose concentration was measured enzymatically (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Serum triiodothyronine (T3) and thyroxine (T4) were measured using a homologous radio immuno assay (RIA); serum insulin concentration was measured by means of RIA (Coat-a-Count Insulin, Diagnostic Products Corporation, Los Angeles, USA). Serum urea, being an indicator of nitrogen metabolism, was was measured enzymatically (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany).

After euthanization, gross and microscopic examination was done.

Statistical model

The results were analyzed, using the General Linear Models (GLM) procedure of SAS Statistical package (SAS, 1990). From preliminary analysis of the results, it was concluded that both respiration chamber number, as well as the time sequence in which the animals entered the experiment did not affect the data. Therefore, these factors were not included in the statistical model.

For energy and nitrogen balance traits, each pair of goats represented an experimental unit; for all other traits, the experimental unit was the individual animal. Measurements before and after infection were analyzed separately.

Treatment effects were tested using 2-way analysis with repeated measurements; the effect of time after infection was taken up in the model:

$$Y_{ijkl} = \mu + I_i + G_j + (I \times G)_{ij} + e_{1,ijk} + T_l + e_{2,ijkl} \qquad [1]$$

where: Y_{ijkl} = dependent variable; μ = overall mean; I_i = fixed effect of Infection (j = 1,2); G_j = fixed effect of Growth pattern in pre-infection period (i = 1,2); $(I \times G)_{ij}$ = fixed effect of interaction between Infection and Growth pattern; $e_{1,ijk}$ = error term 1 which represents the random effect of goat nested within infection × growth pattern treatment (k = 1,..,6); T_l = fixed effect of Time after infection (l = 1,..,4 weeks; l = 1,..,28 days); $e_{2,ijkl}$ = error term 2.

The I_i, G_j and $(I \times G)_{ij}$ effects were tested against error term 1; T_l was tested against error term 2.

Preliminary analysis showed that HP, body temperature, DMI, and the blood traits PCV, parasitaemia, NEFA, insulin, T4, T3 were affected by the time after infection. For these traits, the factors $e_{1,ijk}$ and T_l were removed from the model and the results at different moments of measurement were analyzed separately. This reduced model was

also applied for the single measurents: hepatic TAG before and after infection, and body weight change during the restriction and infection period.

For all energy and nitrogen balance parameters, except HP, no time effect was observed, and model [1] was used with the exclusion of the T_i factor.

To study partial efficiency with which ME is deposited, the relation between data on ER and ME, pooled per animal, was studied using linear regression. Also the relation between NR and ER was studied, using data pooled per animal.

Results

General course of infection

All infected animals developed intermittent fever about 4 days after inoculation of the *T. vivax* parasites (Figure 1). The mean rectal temperature from day 4 until day 28 *p.i.* was 39.89 (± 0.083)°C and 38.60 (± 0.020)°C for infected and control goats, respectively. The first peak of fever persisted for a longer time in the animals with normal growth, compared with animals with retarded growth ($P < 0.05$). After this, fever fluctuated to the same extent in both infected animals with normal growth and infected animals with retarded growth.

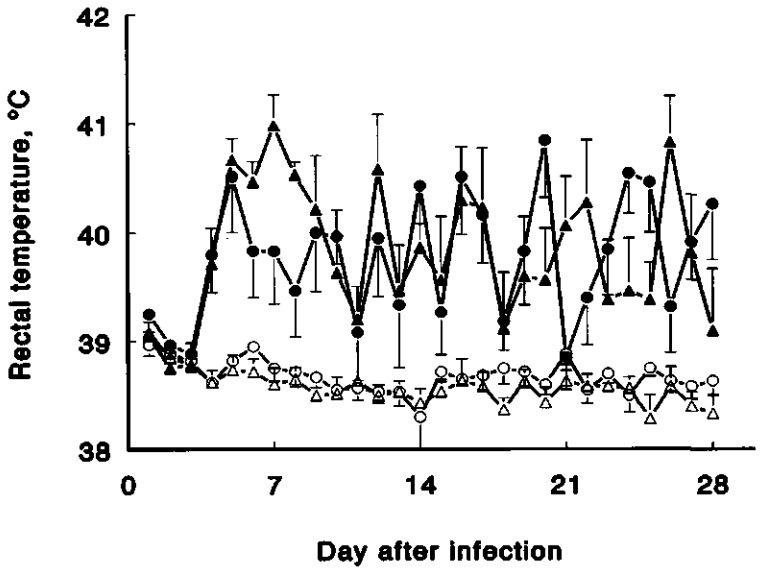
Packed cell volume gradually decreased in all infected animals with time after infection ($P < 0.001$) to an average 17 % in week 4 *p.i.* (control animals had a mean PCV of 38 %). Also interaction between infection and growth retardation with respect to PCV was observed in week 1 and 2 *p.i.* ($P < 0.01$), *i.e.*, control animals with retarded growth had a PCV that was 5 percent points lower than the PCV of control animals with normal growth, but this difference was not present between infected animals with either retarded growth or normal growth.

All infected animals showed parasites in the blood, but parasitaemia followed an irregular course; toward the end of the infection period some animals had undetectable parasite levels. Parasitaemia was not significantly different between the animals with retarded growth and the animals with normal growth ($P > 0.10$).

Dry matter intake and body weight gain

In Figure 2 the mean daily dry matter intake of animals belonging to the different treatments is presented; 2 mean values per week were calculated (3- and 4-day means). Infection reduced DMI in days 5 to 22 p.i. (at least $P < 0.05$); from day 23 onwards, no effects were detected ($P > 0.10$). No interaction between growth retardation and infection was observed ($P > 0.10$).

Figure 1. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on morning rectal temperature (in °C). The line —▲— represents infected goats (normal growth), —●— infected goats (retarded growth), —△— control goats (normal growth), and —○— control goats (retarded growth); error bars indicate sem.



Body weight gain during the infection period was affected by growth retardation, *i.e.*, animals from the group with retarded growth before infection, gained more weight after infection, both per day, and per unit metabolic weight per day, compared with the animals with normal growth ($P < 0.05$; Table 2).

Energy and nitrogen balance

Results on energy balance parameters, protein gain, and derived maintenance requirements are presented in Table 3. Infection reduced GE and ME ($P < 0.05$). Heat production tended to be increased in infected goats ($P < 0.10$). The metabolizability (ME/GE) was not changed by treatments ($P > 0.10$), and averaged 0.56, 0.57, 0.54, and 0.56 for IN, IR, CN, and CR treatment, respectively. Energy retention was decreased by infection ($P < 0.001$). Moreover, ER was lower in animals with a normal growth pattern, compared with animals with retarded growth ($P < 0.05$). There was no interaction between growth retardation and infection with respect to these parameters ($P > 0.10$).

Figure 2. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on dry matter intake (in $g \cdot kg^{-0.75} \cdot d^{-1}$). The line —▲— represents infected goats (normal growth), —●— infected goats (retarded growth), —△— control goats (normal growth), and —○— control goats (retarded growth); error bars indicate sem.

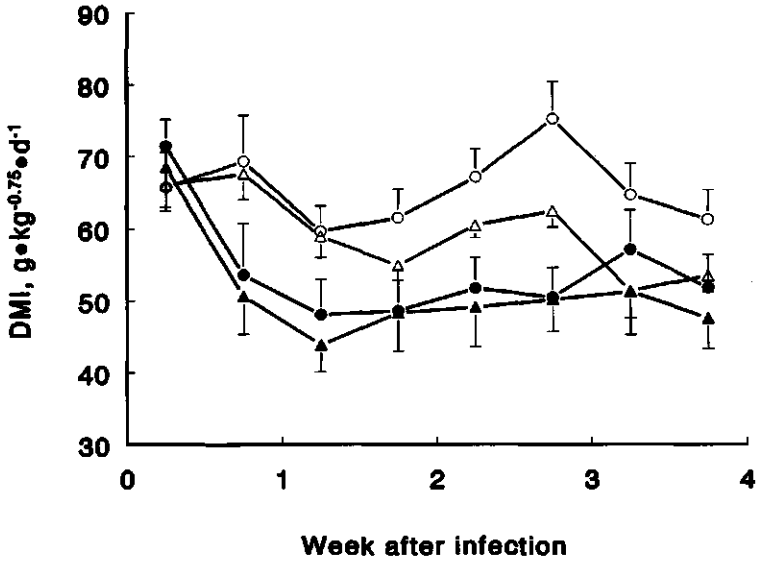


Table 2. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on body weight gain over the infection period, expressed in $g \cdot d^{-1}$, and in $g \cdot kg^{-0.75} \cdot d^{-1}$.

Infection:	Infected		Control			F-statistic[2]		
Growth pattern:	Normal	Retarded	Normal	Retarded	rmse[1]	I	G	I×G
observations	6	6	6	6				
in $g \cdot d^{-1}$	-19.3	0.8	-13.4	14.4	27.1	ns	*	ns
in $g \cdot kg^{-0.75} \cdot d^{-1}$	-1.83	0.39	-1.36	2.05	3.06	ns	*	ns

[1]: Root mean square error (sem = rmse/√n);
[2]: level of significancy of Infection (I), Growth pattern (G) or interaction (I×G) effect; ns = not significant, $P > 0.10$; * = $P < 0.05$.

Table 3. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on gross energy intake (GE), metabolizable energy intake (ME), heat production (HP), energy retention (ER), protein gain, fat gain and ME for maintenance (ME_m; all parameters in $kJ \cdot kg^{-0.75} \cdot d^{-1}$).

Infection:	Infected		Control			F-statistic[2]		
Growth pattern:	Normal	Retarded	Normal	Retarded	rmse[1]	I	G	I×G
observations	3	3	3	3				
replicates	2	2	2	2				
GE	885	923	1017	1141	69	*	ns	ns
ME	498	530	552	639	42	*	ns	ns
H	492	480	427	457	16	t	ns	ns
ER	6^{a}	50^{ab}	125^{bc}	183^{c}	36	***	*	ns
Protein gain	13	22	20	33	16	ns	t	ns
Fat gain	-7^{a}	28^{a}	105^{b}	150^{b}	27	***	*	ns
ME_m	483^{a}	452^{ab}	373^{b}	376^{b}	22	***	ns	ns

[1]: Root mean square error (sem = rmse/√n);
[2]: Level of significancy of Infection (I), Growth pattern (G) or interaction (I×G) effect; ns = not significant, P > 0.10; t = tendency, P < 0.10; * = P < 0.05; *** = P < 0.001;
[a,b,c]: Values with common superscripts do not differ (P < 0.05).

Nitrogen digestibility was not changed (P > 0.10) by treatments and averaged 0.543. The energy deposited in body protein tended to be higher in animals with a normal growth pattern (P < 0.10). Fat gain was decreased in infected animals (P < 0.001) and was increased in animals with retarded growth (P < 0.05). The calculated ME_m was increased (P < 0.001) by 25 % in infected animals. A tendency toward a different ME_m was observed between infected animals either with normal or with retarded growth (483, respectively 452 $g \cdot kg^{-0.75} \cdot d^{-1}$; P = 0.25).

A positive relation between ER and ME intake was observed (Figure 3). However, no differences among treatments were detected (P > 0.10). A positive relation was also observed between NR and ER (Figure 4) without effect of treatments (P > 0.10).

Heat production was affected by time after infection. Therefore results on HP, HP_{cor} and HP_{act} were analysed for week 2 and 4 *p.i.* separately (Table 4). In week 4, but not in week 2 *p.i.*, HP was higher in infected animals. If HP was corrected for physical activity (HP_{cor}), a stronger significant effect of infection occurred both in week 2 *p.i.* (P < 0.05) and in week 4 *p.i.* (P < 0.01), compared with the effect on HP. Activity related heat production (HP_{act}) tended to decrease in infected animals (P < 0.10). No effect of growth retardation or the interaction between infection and growth retardation was found for HP traits (P > 0.10).

Figure 3. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on the relation between energy retention (ER) and metabolizable energy intake (ME; both in $kJ \cdot kg^{-0.75} \cdot d^{-1}$). The symbol ▲ represents infected goats (normal growth), ● infected goats (retarded growth), △ control goats (normal growth), and ○ control goats (retarded growth).

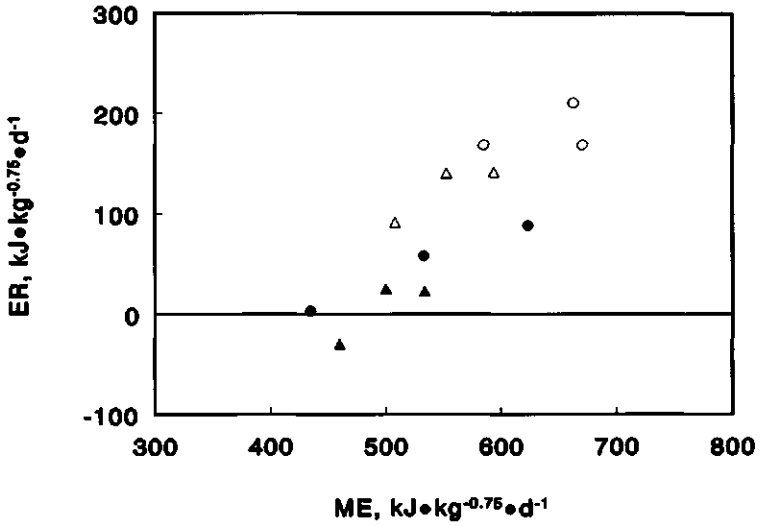
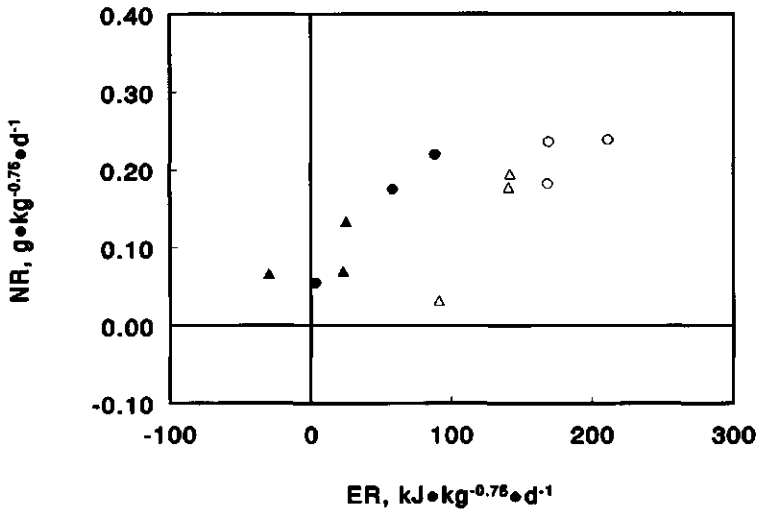


Figure 4. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on the relation between N retention (NR; in $g \cdot kg^{-0.75} \cdot d^{-1}$) and energy retention (ER; in $kJ \cdot kg^{-0.75} \cdot d^{-1}$). The symbol ▲ represents infected goats (normal growth), ● infected goats (retarded growth), △ control goats (normal growth), and ○ control goats (retarded growth).



Serum and hepatic metabolic parameters

Plasma glucose concentration was increased by infection ($P < 0.001$). A tendency toward higher values in goats with normal growth was observed, compared with goats with retarded growth ($P < 0.10$). No effect of the time after infection was detected ($P > 0.10$). Mean glucose levels were 3.796 (± 0.059), 3.683 (± 0.057), 3.492 (± 0.061), and 3.358 (± 0.057) mmol·L^{-1}, for IN, IR, CN, and CR treatment, respectively.

Compared to controls, serum insulin concentrations (Figure 5) were lower in infected animals in week 1 *p.i.* ($P < 0.05$) and tended to be lower in week 2 *p.i.* ($P < 0.10$). In week 2 *p.i.* growth retardation tended to affect serum insulin ($P < 0.10$). Also a tendency toward an interaction between growth retardation and infection was observed ($P > 0.10$). After week 2 *p.i.* no effect of treatments was detected ($P > 0.10$).

Table 4. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on heat production (HP), heat production, corrected for physical activity (HP_{cor}), and heat production, attributed to physical activity (HP_{act}) in week 2 and 4 after infection (in kJ·kg$^{-0.75}$·d^{-1}).

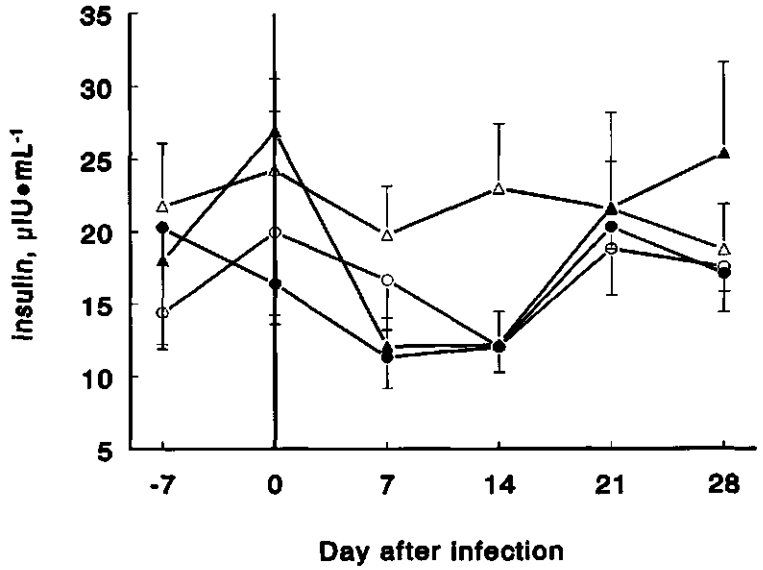
Infection:	Infected		Control			F-statistic[2]		
Growth pattern:	Normal	Retarded	Normal	Retarded	rmse[1]	I	G	I×G
	week 2 *p.i.*							
observations	3	3	3	3				
HP	489	461	433	449	38	ns	ns	ns
HP_{cor}	437	405	361	379	37	*	ns	ns
HP_{act}	52	56	71	70	17	ns	ns	ns
	week 4 *p.i.*							
observations	3	3	3	3				
HP	495	500	421	464	36	*	ns	ns
HP_{cor}	448	442	354	389	36	**	ns	ns
HP_{act}	47	57	67	75	16	t	ns	ns

[1]: Root mean square error (sem = rmse/√n);
[2]: Level of significancy of Infection (I), Growth pattern (G) or interaction (I×G) effect; ns = not significant, $P < 0.10$; t = tendency, $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

Plasma concentration of non esterified fatty acids (NEFA; Figure 6) were increased in infected animals ($P < 0.001$). NEFA concentration tended to be higher in IN animals than in IR animals ($P < 0.10$). Plasma NEFA concentration was positively correlated with plasma glucose (Pearson's correlation $r = 0.44$; $P < 0.01$).

The T4 concentration was lower in infected animals. Mean values for infected and control goats were 72 (± 3) and 152 (± 4) mmol·L^{-1}, respectively ($P < 0.001$). Also T3 concentration in infected animals was lower with mean values of 1.26 (± 0.08) and 2.10 (± 0.08) mmol·L^{-1} for infected and control goats, respectively ($P < 0.001$). No effect of growth retardation was detected ($P > 0.10$).

Figure 5. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on serum insulin concentration (in $\mu IU \cdot mL^{-1}$). The line —▲— represents infected goats (normal growth), —●— infected goats (retarded growth), —△— control goats (normal growth), and —○— control goats (retarded growth); error bars indicate sem.



Serum urea concentration was not affected by post infection week number, infection or growth retardation ($P > 0.10$). The average concentration was 7.6 ($\pm$ 1.3) $mmol \cdot L^{-1}$.

Hepatic triacylglycerol (TAG) concentration (Table 5) was higher in animals with a normal growth pattern at day 14 before infection ($P < 0.05$). At day 28 after infection, only a tendency toward a higher TAG concentration in goats with a normal growth pattern was observed ($P < 0.10$). Overall TAG concentration on day 28 after infection was higher than on day -14 ($P < 0.001$). Liver TAG was positively correlated with plasma NEFA concentration (Pearson's correlation $r = 0.53$; $P < 0.001$).

Pathology

Gross and microscopic examination after euthanization revealed hyperplasia and a plasmacellular reaction of lymph nodes, hyperplasia of the spleen, and mononuclear infiltration of several organs and tissues, including the brain of some animals. The thyroid showed active epithelial cells, in a few cases cuboidal cells, with many follicles present. The liver of many infected animals showed mild to moderate fat accumulation. The bone marrow of many infected animals was activated and showed extended erythropoiesis and to a smaller extent myelopoiesis.

Figure 6. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on plasma non esterified fatty acids concentration (in mmol·L^{-1}). The line —▲— represents infected goats (normal growth), —●— infected goats (retarded growth), —△— control goats (normal growth), and —○— control goats (retarded growth); error bars indicate sem.

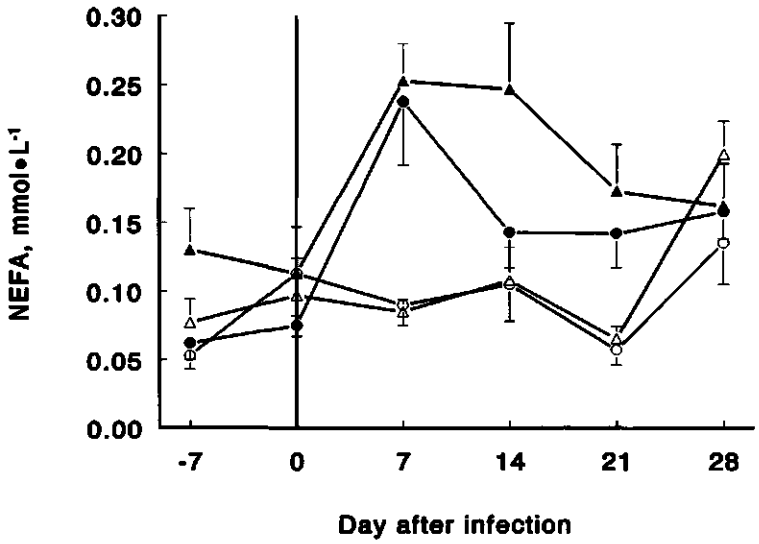


Table 5. The effect of *Trypanosoma vivax* infection and different growth patterns of goats, on liver triacylglycerol (TAG) concentration before and 28 days after infection (in mg·g^{-1}).

Infection:	Infected		Control			F-statistic[2]		
Growth pattern:	Normal	Retarded	Normal	Retarded	rmse[1]	I	G	I×G
observations	6	6	6	6				
day -14	15.9	14.0	15.0	12.6	2.14	ns	*	ns
day 28	22.6	19.3	23.3	20.2	4.18	ns	t	ns

[1]: Root mean square error (sem = rmse/√n);
[2]: Level of significance of Infection (I), Growth pattern (G) or interaction (I×G) effect; ns = not significant, P< 0.10; t = tendency, P< 0.10; * = P< 0.05.

Discussion

Effect of T. vivax infection

In this study, infection affected most studied variables with respect to energy and nitrogen metabolism, and pathology of disease. Intake of GE and N was reduced by infection, and this led to changes in retention and in the blood metabolic profile, which are typical for suboptimal nutrition; however, most infected animals still showed a positive energy balance.

The following pathological findings were present. All infected animals developed anaemia to the same extent, irrespective of growth retardation. The PCV level after 4 weeks of infection was very low, compared with reports of Verstegen *et al.* (1991) and Akinbamijo *et al.*, 1992) on WAD goats, and reports of (Paling *et al.*, 1991) on infected N'Dama's. Parasitaemia extremely fluctuated with time, which is a normal phenomenon in trypanosome infection (Stephen, 1986). Also other pathological findings at autopsy revealed the typical picture of *T. vivax* infection (Van den Ingh *et al.*, 1976).

Feed intake was reduced; DMI was about 20 % lower in infected animals; this is a smaller decrease than the 35 % intake decrease reported by Van Dam *et al.* (1996a). Metabolizability of GE, and digestibility of N were not changed. This corresponds with previous studies (Verstegen *et al.*, 1991; Akinbamijo *et al.*, 1992; Van Dam *et al.*, 1996c). It means that kidneys and intestines were intact, which was confirmed at autopsy. Moreover, no indications for a decrease of NR at a given ER level (Figure 4) were found.

The observed reduction in intake has obviously affected the decrease in serum insulin concentration. The increased plasma NEFA concentration demonstrates that this decrease in insulin has induced lipolysis (Payne, 1989). Wassink *et al.* (1993) reported a negative correlation coefficient r of -0.76 between NEFA and DMI in *T. vivax* infected WAD goats. This is in agreement with the present study. That liver TAG at day 28 *p.i.* was not increased may imply that the lipolysis as induced by the reduction of energy retention, was mild to moderate. Van den Top et al. (1995) found increased liver TAG in peri-partum goats, probably due to negative energy balance and substantially increased NEFA supply from the blood to the liver. The obtained data neither offered evidence for increased hepatic TAG under influence of immunological products like TNF, described by Feingold *et al.* (1990), although post mortem analysis indicated some zonal fat accumulation in livers of infected goats.

The increase of plasma glucose in infected animals was unexpected, given the results in previous studies (Akinbamijo *et al.*, 1992; Van Dam *et al.*, 1996c). In animals with a negative energy balance the glucose level is often decreased (Payne, 1989). In our study most animals showed a positive energy balance, however. Serum T3/T4 was decreased

in the infected animals; this corresponds with earlier findings (Abebe & Eley, 1992; Van Dam *et al.*, 1996*c*). No effect of growth retardation was detected, however.

Heat production in infected animals was increased by about 10 %. This is lower than reports of Verstegen *et al.* (1991), who reported 16 % increase in WAD goats due to *T. vivax* infection. Heat production due to physical activity, measured with Doppler-radar activity meters, tended to be reduced in week 4 *p.i.* in infected animals. Van Dam *et al.* (1996*b*) reported a reduction of standing time of WAD goats, due to *T. vivax* infection. Lying down costs less energy due to a lower muscle tone and an increased thermal insulation (Hart, 1985). We did not monitor postural behaviour in this study, however.

Interaction between nutritional history and infection

The ME_m was increased by 25 % in the infected group. This can be referred to as the metabolic costs of infection. Both the absolute level of ME_m and the increase due to infection agrees with findings of Verstegen *et al.* (1991). Baracos *et al.* (1987) attributed the increment of ME_m to increase of basal metabolic rate due to fever, and to other metabolic costs, like mounting of the immune response and increased protein turnover.

However, ME_m increase due to infection tended to be lower in animals with retarded growth, *i.e.*, 20 % increase in IR animals compared with 29 % in IN animals. Thus both infected groups showed a higher ME_m than controls, but it appeared that goats with retarded growth lost less energy due to infection than goats with normal growth.

It is not clear, what caused the lower ME_m in IR animals, compared with IN animals. This could possibly be related to differences in the severity of the fever (Baracos *et al.*, 1987; Van Dam *et al.*, 1996*b*). However, rectal temperature of IN and IR goats in week 2 and 4 *p.i.* was not different (39.84°C in IN goats and 39.86°C in IR goats). Other factors which are known to affect HP and consequently ME_m, like physical activity (Blaxter, 1989) and feed uptake (ARC, 1980) were not significantly different between IN and IR animals, either.

Figure 3 shows the relation between ER and ME. Regression analysis of this relation can also provide an estimate for ME_m, *i.e.*, the point of intersection with the X-axis; however, no statistical effects of treatments were observed, which is at least partly explained by the low number of observations. The same problem prevented statistical analysis of the relation between NR and ER, although it can be derived from Figure 4, that NR was at least not decreased at a given ER in infected animals. This implies that maintenance requirements for nitrogen probably were not increased by infection, as was also reported by Verstegen *et al.*, (1991) and Van Dam *et al.* (1996*a*).

The lower ME_m of IR animals compared with IN animals, together with a slightly higher GE, led to a somewhat higher ER of IR animals. This was also reflected in the

higher NEFA concentration at day 14 *p.i.* of the IN group, compared with the IR group, indicating a higher lipolysis in IN animals (Payne, 1989).

Apart from the tendencies found for ME_m and ER, only minor (if any) carry-over effects of previous growth check on the course of infection were observed. Katunguka-Rwakishaya *et al.* (1995) reported a more severe anaemia and greater growth retardation under influence of *T. congolense* infection, in sheep on a low energy intake level compared with those on a high energy intake level. Reynolds & Ekwuruke (1988) observed an increased mortality of *T. vivax* infected WAD sheep if fed at a low plane of nutrition. This corresponds with the view of Murray (1988) that shortage of nutrients negatively affects the immune response during infection.

An experiment with chicken, however, provided no evidence that nutritional stress in early life has an effect on disease resistance (Zulkifi et *al.*, 1994).. Kim & Lovell (1995), reported reduced resistance of 1-year old catfish to infection with *Edwardsiella ictaluri*, if they had experienced a period of starvation. However, they observed the opposite for 2-year old catfish: they showed increased survival if subjected to starvation prior to infection. The latter is in line with observations of Murray & Murray (1979) who reported a sharp increase of mortality of mice after infection with *Listeria monocytogenes* when mice were force-fed, compared with their anorectic congeners. These last studies emphasise a negative relation between intake level and disease resistance during early infection, possibly by reducing the available nutrients for the invading micro-organism, and/or by production of specific substances that slow down development of the infectious agent. This was also concluded by Isoun (1972), who found evidence that malnutrition leads to lower parasitaemias and increased survival times in rats, infected with *T. brucei*, and fed a diet deficient in protein, thiamine or vitamin A, by comparison with controls adequately fed (Isoun, 1972). In the present experiment, no such effect of growth retardation treatment on parasite counts of infected goats was observed. The shorter duration of the first peak of fever in animals with growth retardation, however, may indicate a quicker clearance from the blood of the first parasite peak (Stephen, 1986).

Effect of growth retardation on intake and energy balance

The differences in feeding regimen in the restriction period led to large differences in body weight at the moment of infection, but apparently the applied feed restriction did not induce substantial lipolysis, because 2 weeks before infection liver TAG was not increased in restricted animals compared with animals fed *ad libitum* (Table 5).

Although DMI during the infection period was not significantly affected by growth retardation, body weight gain over the infection period was higher in these retarded animals than in animals with normal growth (Table 2). According to Hogg (1992) this

may be caused by compensatory mechanisms taking place in animals, just after a period of feed restriction, *i.e.*, a higher feed intake, an increase of gut fill, together with initially lower maintenance requirements. In infected animals, only slightly higher DMI was observed in animals with retarded growth. The observed decrease of ME_m of IR animals compared with IN animals was not seen in control animals with retarded growth, but they showed a larger increase of DMI. It is also possible that gut fill gradually increased in animals with retarded growth in the course of the experiment, due to adaptation from restricted to *ad libitum* feed intake.

Although indications were found for some compensatory intake and body weight gain in animals with retarded growth, these may have been only minor effects, compared with the effect of trypanosome infection.

Conclusions

The course of a *Trypanosoma vivax* infection in West African Dwarf goats with a retarded growth pattern, generally was not different from that in WAD goats with a normal growth pattern. Feed intake and energy retention were reduced. Blood biochemical variables of infected goats were consistent with the reduced energy balance; blood glucose, however, was increased in infected animals. Heat production and ME requirements for maintenance were increased. However, the increase of ME requirements for maintenance tended to be lower in animals with retarded growth; this would imply a lower metabolic cost of infection of animals with retarded growth.

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Chapter 9

General Discussion

General Discussion

Introduction

This thesis describes the results of a series of experiments which were designed to study the effect of experimental trypanosome infection on feed intake, and energy and nitrogen metabolism of trypanotolerant West African Dwarf goats. The following will first report on the general course of infection with respect to anaemia during trypanosome infection. Then possible mechanisms behind the variation in feed intake reduction will be discussed. Subsequently, the effect of trypanosome infection on energy and nitrogen metabolism will be adressed, and the effect of nutrition on the course of infection. Finally, the major conclusions and implications drawn from our studies are formulated.

Anaemia due to trypanosome infection

Infection with *Trypanosoma vivax* caused a progressive anaemia in WAD goats in all reported experiments. The lowest mean packed cell volume (PCV) level reached ranged from 24 % after 3 weeks *T. congolense* infection (chapter 3) down to 17 % after 4 weeks *T. vivax* infection (chapter 8). The decrease is in accordance with studies of Adah *et al.* (1993) and Osaer *et al.* (1994) on WAD goats, and puts serious questions either on the degree of trypanotolerance of the WAD goat breed or on universality of the PCV variable as the ultimate indicator of trypanotolerance of livestock (Murray, 1988; Trail *et al.*, 1991).

The development of anaemia during the acute phase of infection is mainly induced by the immune system (Murray and Dexter, 1988). It is generally accepted that anaemia, certainly in the acute phase, is created by a sharp increase of erythrophagocytosis by macrophages (Murray and Dexter, 1988; Anosa *et al.*, 1992; Sileghem *et al.*, 1993), possibly triggered by cytokines like TNF-α (Lucas *et al.*, 1993; Sileghem *et al.*, 1994). Also erythropoiesis in the bone marrow may be disturbed (ILRAD, 1994).

From our studies no apparent relation between PCV and feed intake and/or feed quality could be found; this means that the development of anaemia during the acute phase of infection is not affected by nutritional status of the animal. This is in agreement with the conclusions of Agyemang *et al.* (1990). They found no effect of feed supplementation of N'Dama cattle on the severity of anaemia during the acute phase of *T. congolense* infection. Also Katunguka-Rwakishaya *et al.* (1993) found no effect of dietary protein level of the diet on the degree of anaemia due to *T. congolense* infection in sheep. The type of anaemia differed between animals fed different protein levels.

The variation in feed intake reduction due to infection

In all experiments, a significant feed intake reduction was observed in infected WAD goats, which is one of the typical signs of trypanosome infection, besides fever and anaemia (Stephen, 1986). This anorexia is probably triggered by cytokines, like TNF-α (Bielefeldt Ohmann *et al.*, 1989), interleukin-1 (McCarthy *et al.*, 1986), prostaglandins and interferon-τ (IFN-τ; Van Miert, 1995). Fever which occurs during infection does not affect feed intake in itself, but is merely another manifestation of cytokine action on the hypothalamus (McCarthy *et al.*, 1986; Van Miert *et al.*, 1986) and coincides with reduced forestomach motility in ruminants (Van Miert *et al.*, 1992). Therefore, bouts of fever often coincide with periods of anorexia. Wassink *et al.* (1993) found a ranking (Pearson) correlation of -0.60 between infection DMI and rectal temperature of WAD goats. The observed anorexia during infection may be benificial to survival of the host (Murray and Murray, 1979), or might be an undesirable side-effect of the excess production of cytokines (Lucas *et al.*, 1993).

The mean feed intake level in our studies differed largely per experiment. In Table 1, the dry matter intake of infected and control animals is presented, and the mean ratio [DMI infected animals / DMI control animals]. Infection DMI was calculated as the mean DMI from day 5 to the end of the infection period, thus taking into account a prepatent period of 4 days.

Table 1. *Ad libitum* dry matter intake (DMI, in $g \cdot kg^{-0.75} \cdot d^{-1}$) during trypanosome infection in the experiments, reported in this thesis.

Experiment, reported in:	Tryps Species	Duration weeks	Age months	Infected n	DMI	sem	Control n	DMI	sem	Ratio Inf/Cont
chapter 3	*T. cong.*	5	18	25	36.3[a]	2.9	25	68.2[1,b]	2.6	0.54
chapter 4/5	*T. vivax*	6	29	12	13.5[a]	2.1	4	35.7[b]	2.3	0.38
chapter 6/7	*T. vivax*	6	14	19	38.1[a]	2.0	10	61.6[b]	3.0	0.62
chapter 8	*T. vivax*	4	7	12	49.4[a]	3.1	12	62.1[b]	2.5	0.80

[1]: DMI before infection served as control DMI level
[a,b]: different superscripts indicate a significant difference between treatments.

In the *T. congolense* study (chapter 3), all animals were infected, so the control DMI was estimated as the pre-infection DMI of the same animals. The animals showed a mean DMI decrease of 46 %. In the respiration study with individually housed WAD goats (chapter 4), a very low feed intake level was observed, which led to negative energy balances, even in control goats. The overall low feed intake in this experiment was ascribed to both the stress due to social isolation in the respiration

chamber (Carbonaro *et al.*, 1992), and the luxurious body condition of the animals. Evidence for a negative relation between feed intake and fat depth was found by Lee *et al.* (1995) in mature ewes. The respiration study with pair-housed animals (chapter 8) resulted in intake levels above maintenance for most infected and all control goats. Finally, the nitrogen balance study (chapter 6/7) showed an intermediate reduction of 38 %, compared with control goats; half of the animals received a chopped grass straw diet, but this did not have significant influence on the relative DMI decrease. The results from Table 1 cannot be compared directly with other studies, due to differences in experimental design, like restricted feeding of control animals (Zwart *et al.*, 1991; Akinbamijo *et al.*, 1992) or because of adding supplement to the basal diet, which encourages diet selection (Akinbamijo *et al.*, 1994a,b). Wassink *et al.*, (1993) studied DMI reduction of WAD goats after infection with successively *T. congolense* and *T. vivax*. The ratio [DMI during infection / DMI before infection] was 0.65 (± 0.036) after *T. congolense* infection and 0.67 (± 0.047) after *T. vivax* infection; the mean age of the animals was 18 months.

The presented data in Table 1 suggest that there is a negative relation between age and DMI ratio, *i.e.*, older animals would suffer more from anorexia during infection. The larger fat deposits in older animals may be a reason for this (Lee *et al.*, 1995). It is also possible that the pathological signs during trypanosomiasis are more pronounced in adult animals, compared to young animals. Likewise is reported that young calves are less susceptible to *T. congolense* infection than adults (Fiennes, 1970; Morrison *et al.*, 1985). Foster *et al.* (1992) reported a higher TNF production after lipopolysaccharide injection in aged rats, compared to young rats, which provides a possible mechanism behind this observation. A more severe DMI reduction in older animals would imply an even larger effect of infection on animal production, because the normal voluntary intake level per kg metabolic weight decreases with age, as was observed in a study with WAD goats (Ketelaars and Tolkamp, 1991). A complicating factor in the comparison of DMI reduction, however, is that the housing and the experimental treatments in our studies were not always the same.

Besides differences in average DMI during infection in the different studies, also variation between animals within studies was observed, and day-to-day variation of individual infected animals. Table 1 indicates that the standard deviation between animals in infected groups (derived from the presented sem), did not increase, compared with healthy controls. This is in conflict, however, with findings of Zwart *et al.* (1991); with respect to DMI response to infection they identified low, medium and high responders, with the standard deviation of the infected group at least doubled,

compared with the control group. Possibly the variation in DMI response in their study was related to variation in the age of the animals (18 - 32 months).

Studies of Wassink *et al.* (1993) and Clausen *et al.* (1993) indicated a high repeatability of individual response to successive infections; this may imply an innate characteristic of genetic origin; in addition Clausen *et al.* (1993) found indications for acquired resistance. The results from the study reported in chapter 3 also provide some evidence that DMI response to infection is affected by genetic factors. As suggested there, this correlation may originate from functional relationships between polymorphism of genes in the CLA region (the TNF-α gene being the most promising candidate) and the degree of anorexia.

In the study described in chapter 2, however, the repeatability of individual DMI response to successive infections was very low. Because the environmental conditions were different during infection period 1 and 2, it is possible that environmental factors played a major role, or possibly that genotype × environment interaction occurred.

Finally, the day-to-day variation of individual DMI was increased by infection; in the N balance experiment (chapter 6/7), the standard deviation of repeated daily DMI measurements increased from on average 5.5 $g \cdot kg^{-0.75} \cdot d^{-1}$ to 9.6 $g \cdot kg^{-0.75} \cdot d^{-1}$; this was also observed in the other trials. It is likely that the consecutive emergence of trypanosome sub-populations in the blood during infection, and the resulting immune responses with its cascade of cytokines (Van Miert, 1995) have created the higher day-to-day variation in feed intake.

It can be concluded that the DMI reduction of WAD goats during acute trypanosome infection is considerable. Average reduction ranges between 20 and 62 %, depending on the severity of disease, the experimental design and the physiological status of the animal. Although environmental conditions like housing and age of the animals seem to play a role, possibly also genetic factors play a role as was suggested by the observed association between polymorphism in the CLA region of the genome and DMI response to infection.

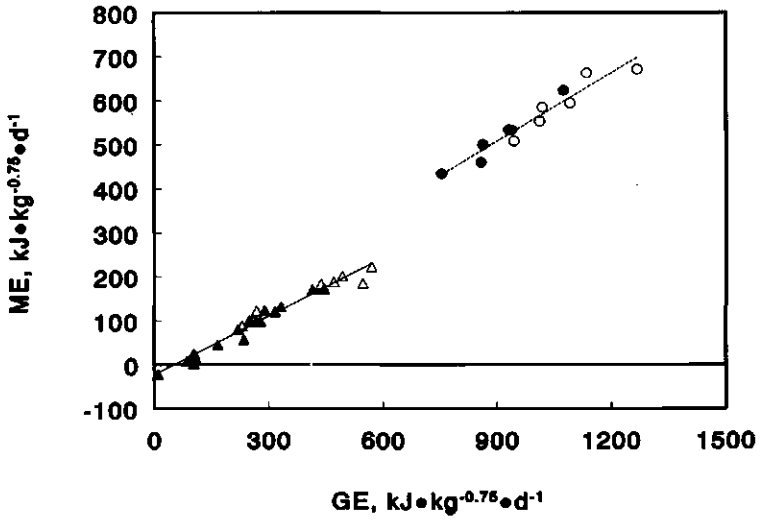
The effect of infection on energy and nitrogen metabolism

As discussed in the previous section of this chapter, daily intake was reduced in infected animals. This implies that both energy and nitrogen intake were reduced to the same extent. The large variation in energy and N intake between experiments and within experiments between animals produced profound differences in energy retention (ER). In chapters 4 and 8, energy and N balances of infected and control

animals are given, whereas in chapter 6 a N balance is presented. In the experiment described in chapter 4, infected animals showed a highly negative ER and N retention (NR). Nearly all infected animals in the experiment in chapter 8, however, showed positive ER and NR. The infected animals in the experiment reported in chapter 6 in general showed negative N retention if fed grass straw and an NR of around zero if fed pelleted lucerne.

In order to investigate the metabolic processes associated with infection, it was studied whether infection leads to increased energy and N losses. Hence the apparent digestibility of energy and nitrogen was estimated, as well as the metabolizability of energy. Apparent digestibility of energy and N, expressed as the proportion digested nutrients relative to intake, was not changed by infection. However, a few animals with very low intake showed somewhat lower apparent digestibilities. These may have been very susceptible animals whose DMI gradually decreased during infection. At very low intake levels, the endogenous fraction in the faeces will be larger; true digestibility of energy and N may not have been changed by infection.

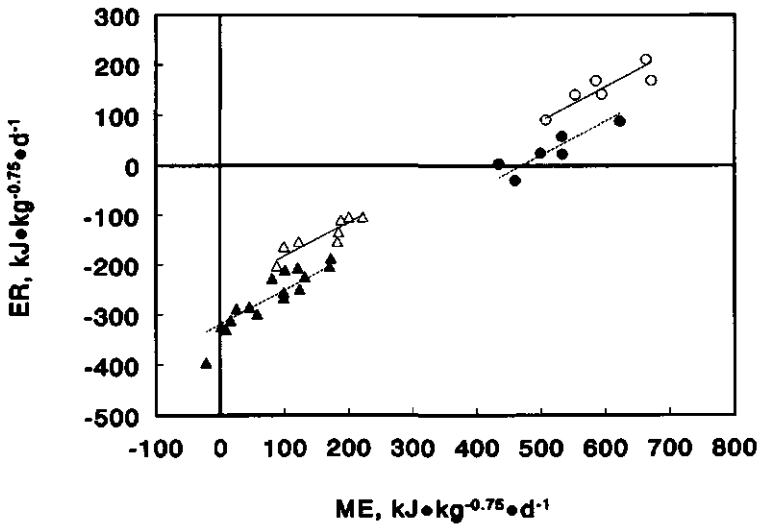
Figure 1. Effect of gross energy (GE) on metabolizable energy (ME) intake in infected and control WAD goats; datapoints consist of pooled observations per animal, and were derived from two separate energy balance trials. The symbol ▲ represent infected goats in trial 1; △ control goats in trial 1; ● infected goats in trial 2, and ○ control goats in trial 2. The line —— represents the regression equation through datapoints from trial 1; - - - the regression equation through datapoints from trial 2.



In Figure 1, results on the relation between metabolizable energy (ME) and gross energy (GE) are presented, showing data from the two energy balance experiments (energy balance trial 1, described in chapter 4, and energy balance trial 2 in chapter 8). Linear regression analysis showed that the slope of the relation was different ($P < 0.001$) for the two experiments, *i.e.*, 0.444 for trial 1 and 0.517 for trial 2. However, no effect of infection on the slope or intercept of the equation was found. This implies that energy losses in faeces or urine, relative to energy intake level, were not increased in infected animals. *Post mortem* marco- and microscopic examination of intestines and kidneys also showed no lesions or improper function in the different experiments.

Figure 2 shows the relation between energy retention (ER) and ME, consisting of datasets from energy balance trial 1 and 2. The two energy balance trials covered a wide range of intake levels, from zero intake up to about 1.8 times maintenance. Regression analysis showed an effect of infection on the intercept ($P < 0.001$), but no effect on the slope of the line.

Figure 2. Effect of metabolizable energy (ME) intake on energy retention (ER) in infected and control WAD goats; datapoints consist of pooled observations per animal, and were derived from two separate energy balance trials. The symbol ▲ represent infected goats in trial 1; △ control goats in trial 1; ● infected goats in trial 2, and ○ control goats in trial 2. The line —— represents the regression equation through datapoints of control goats, and - - - the regression equation through datapoints of infected goats.



This resulted in 2 equations (in $kJ \cdot kg^{-0.75} \cdot d^{-1}$; sem between brackets; combined $r^2 = 0.98$):

Infected: $ER = -319 (+ 6) + 0.678 (\pm 0.017) \times ME$ $(n = 22)$; [1]
Control: $ER = -250 (+ 8) + 0.678 (\pm 0.017) \times ME$ $(n = 14)$; [2]

This means that the decrease of ER due to infection (the difference between the intercepts of equation [1] and [2]), was not affected by ME intake level. It is remarkable that the slope of the equation below maintenance (efficiency k_m) is not different from that above maintenance (efficiency k_f); ARC (1980) reported for pelleted feeds with a metabolizability of 0.50, a k_m of 0.67 and a k_f of 0.48; our k_f is much higher.

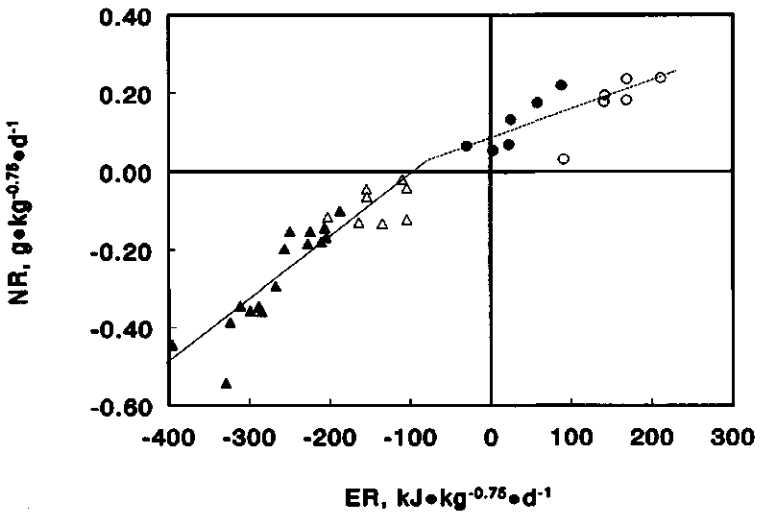
Infection caused a reduction in ER of 125 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in both energy balance trials (chapters 4 and 8). This decrease consisted of about 70 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, attributable to increased heat production mainly due to the fever (difference between intercepts [1] and [2]), and the remainder (55 $kJ \cdot kg^{-0.75} \cdot d^{-1}$) attributable to the intake reduction due to infection (ME intake reduction of 81 $kJ \cdot kg^{-0.75} \cdot d^{-1}$). If results on digestible organic matter intake (DOMI) from the N balance study described in chapter 6, are converted to ME (conversion factor 15.8 kJ ME·g^{-1} DOM, NRC, 1981), the resultant average decrease of ME due to infection was 205 $kJ \cdot kg^{-0.75} \cdot d^{-1}$, much larger than the 81 kJ in the two studies included in Figure 2.

From equations [1] and [2], ME maintenance requirements (ME_m) of 368 and 471 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ in healthy and infected WAD goats can be derived, showing an increase of more than 100 $kJ \cdot kg^{-0.75} \cdot d^{-1}$ (28 %). The increase of ME_m was explained by the increased basal metabolic rate due to the observed fever and possibly other metabolic costs of infection, like the rise of the immune response, and several repair mechanisms like increased erythropoiesis (chapter 5). Apparently, this increase was independent from intake level. Baracos *et al.* (1987) reported on a series of clinical studies in which the increase of basal metabolic rate due to fever ('Q_{10} effect'), resulting from infectious disease or from lipopolysaccharide stimulation, ranged between 13 and 45 % per degree °C. However, this increase included shivering periods, in which heat production may be doubled.

It is noteworthy, that infected animals showed some degree of compensatory behaviour with respect to the increased ME_m, by reducing the standing related energy costs (chapter 5). This reduction of standing time was effected in spite of a higher frequency of postural change than in healthy controls, the latter probably caused by thermal discomfort which occurs more frequently during flucutating fever. The change in behavioral mode of infected animals fits well in the general pattern observed during infection and is closely associated with thermoregulation (Hart, 1988). Also

results on activity related heat production (chapter 7) indicated a decrease of energy spent on physical activity (standing, moving).

Figure 3. Effect of energy retention (ER) on nitrogen retention (NR) in infected and control WAD goats; datapoints consist of pooled observations per animal, and were derived from two separate energy balance trials. The symbol ▲ represent infected goats in trial 1; △ control goats in trial 1; ● infected goats in trial 2, and ○ control goats in trial 2. The line —— represents the regression equation through datapoints from trial 1; - - - the regression equation through datapoints from trial 2.



In Figure 3, the relation between N retention (NR) and ER is depicted for energy balance trials 1 and 2. No effect of infection on the regression equation was detected. A piecewise linear function, pivoting at ER = -85 kJ·kg$^{-0.75}$·d^{-1}, was found to fit the data better than simple linear regression (NR in g·kg$^{-0.75}$·d^{-1}; ER in kJ·kg$^{-0.75}$·d^{-1}; sem between brackets; combined r^2 = 0.93):

for ER < -85 kJ·kg$^{-0.75}$·d^{-1}:

NR = 0.0173 (± 0.0828) + 0.00162 (± 0.00015) × (ER + 85.2); (n = 24); [3]

for ER > -85 kJ·kg$^{-0.75}$·d^{-1}:

NR = 0.0173 (± 0.0828) + 0.00075 (± 0.00023) × (ER + 85.2); (n = 12); [4]

This means, that the relationship between energy and N retained (below maintenance: energy and N depleted) was not changed by infection. These results should be interpreted with care, however, because each of the two linear parts of the line describe observations from only one of the two experiments. Thus an effect of experiment may play a role here. No effect of infection was found on the slopes or intercept of equation [3-4]. This implies that infection did not induce an increase of N losses at a comparable ER as healthy controls. Thus infection caused animals to move down along equation [3-4], rather than moving from the equation.

Therefore the suggestion of Oosting *et al.* (1995) that a balance exists between energy and N available to the tissues, was confirmed. Moreover it was shown that this also holds for infected animals. This was also concluded in our N balance study, reported in chapter 6, in which the relation between NR and DOMI was not different for infected and control animals. However, in this study a linear relationship between NR and DOMI was found, rather than a piecewise linear function. Simple linearity was also reported by Elliott and Topps (1964), Ketelaars and Tolkamp (1991), and Oosting *et al.* (1995). However, the intake level in their studies did not reach below 60 % of maintenance (approximately 14 $g \cdot kg^{-0.75} \cdot d^{-1}$ DOMI), whereas in our energy balance trial 1, much lower ME intake levels were reported. Nevertheless, our observations at very low intake level consisted mainly of infected animals. A few of these observations were made during a moribund state of the animal; it is well possible that in these animals, the energy and N metabolism may have been disrupted due to moribund conditions, as was also suggested for some animals in the N balance trial, reported in chapter 6.

Blood and liver biochemical variables

In the infected goats, generally serum concentrations of non esterified fatty acids (NEFA), ß-hydroxy butyrate (BHB), glucose and insulin appeared to reflect the energy status of the animal. NEFA and insulin concentrations were found to be most sensitive to a negative energy balance (chapter 4, 7 and 8), whereas glucose remained normal and BHB remained low during light to moderate energy shortage. Only in an extremely negative energy balance, glucose concentration decreased and consequently BHB increased, in order to secure the supply of energy substrates to the nervous system (Payne, 1989). However, in energy balance trial 2 (chapter 8), glucose concentration was found to increase in infected animals, despite a reduced intake level. γ-Globulines showed a large increase during infection, and albumin showed a reduction. This led to an overall increase of serum protein.

The decrease of T3 and T4 in infected animals was consistent throughout the reported studies (chapter 4, 7 and 8). Possible mechanisms included a reduced T4 production by the thyroid gland (Mutayoba and Gombe, 1989), increased T3/T4 uptake and degradation by peripheral tissues and increased clearance by the liver (Beisel, 1985), probably triggered by systemically active blood levels of cytokines like TNF-α (Sweep *et al.*, 1992).

Liver triacylglycerol (TAG) was increased in both infected and control animals with a negative energy balance. Therefore, no apparent indications for a direct effect of infection on liver TAG concentration was found. This is in contrast with Grunfeld *et al.* (1989), who found that TNF-α administration induced an increase of hepatic triglyceride production in rats. *Post mortem* examination of our goats showed a non-specific reactive hepatitis in most infected animals. The reduced T3/T4 serum levels may have led to impaired mitochondrial respiratory activity (Lomo *et al.*, 1993). However, the major cause of TAG accumulation in the liver probably was the very high plasma NEFA supply to the liver during a negative energy balance of the animals, and an inadequate capacity of the liver to convert the absorbed NEFA into ketone bodies for use as an energy substrate (Van den Top, 1995).

In Table 2, measurements on DMI, PCV, rectal temperature (T_{rect}), and blood clinical and biochemical variables are presented for animals that died before the end of the experiment, as compared with infected congeners that survived throughout the infection period. Some of the reported animals died spontaneously. Some were euthanized, when spontaneous decease within 24 hours was expected. The DMI was calculated over 7 days preceding decease, whereas mean T_{rect} is calculated over 3 days preceding death. For the values of the blood clinical and biochemical variables, the last observation in the week preceding death is given.

Moribund animals all showed very low DMI. Rectal temperature often was normal, despite high parasitaemia. Apparently the animals did not show a fever response any more to a new subpopulation of parasites emerging in the blood. This may indicate a dysfunction of the immune system. Total protein was usually rather low; however, it cannot be deduced if this indicated a deficient immune response. PCV was low in moribund infected animals, although not different from other infected animals. In a few cases plasma glucose concentration was very low, with NEFA and BHB increased. However, no consistent picture was observed. The in some moribund cases very high serum level of BHB indicates that only in the terminal phase production of ketone bodies was significantly increased, which is in agreement with studies of Wolkers (1993). Urea was increased in some cases, probably due to increased protein catabolism. Serum insulin concentration was often very low, reflecting the intake level of the animal.

Table 2. Clinical parameters of individual moribund WAD goats, infected with *T. vivax*, measured within 3 days from death (spontaneously or by euthanasia); mean values of week 6 p.i. of the remaining infected animals is included.

Goat	Decease day p.i.	Nat./ eut.	DMI[1] $g \cdot kg^{-0.75}$	T_{rect}[2] °C	Parasit.[3] ^{10}log	PCV %	glucose	NEFA	BHB	Urea	insulin	TP
							mmol·L^{-1}				$\mu IU \cdot mL^{-1}$	$g \cdot L^{-1}$
Experiment 1												
1	39	nat.	3.6	38.2	5.7	28	2.5	0.73	0.65	6.0	nd[4]	64
2	35	nat.	12.3	40.3	7.8	20	3.2	0.58	0.10	6.9	11	80
Other infected animals in experiment 1 (not moribund)												
avg	42	eut.	16.4	39.5	4.8	22	2.9	0.65	0.87	7.1	9.2	93
std			10.5	0.8	2.2	4	0.6	0.43	1.99	2.8	5.2	13
Experiment 2												
3	32	eut.	4.9	38.5	6.6	18	1.2	0.73	1.16	6.5	nd	70
4	40	eut.	12.9	38.9	6.8	13	3.7	1.58	1.95	7.6	1.7	58
5	33	eut.	4.7	38.7	8.2	18	1.2	0.16	5.20	8.5	1.7	64
6	30	nat.	12.9	39.9	6.3	17	2.4	0.54	0.26	6.0	1.4	66
Other infected animals in experiment 2 (not moribund)												
avg	42	eut.	42.7	39.3	5.1	18	3.0	0.23	0.15	5.8	9.0	88
std			12.5	0.8	1.1	3	0.4	0.10	0.05	1.6	8.8	12

[1]: mean dry matter intake over the week preceding decease;
[2]: mean rectal temperature over the 3 days preceding decease;
[3]: Parasitaemia, packed cell volume, glucose, non esterified fatty acids, ß-Hydroxy butyrate, urea, insulin and total protein measured from blood, taken in the week preceding decease; from goat numbers 2-2 and 3-2, blood was taken just before euthanization;
[4]: non detectable levels (< 1 $\mu IU \cdot mL^{-1}$).

The data on the death cases therefore roughly provide a picture of a severely malnourished animal, with corresponding blood and liver metabolic profile. High parasitaemia without apparent host response indicate a dysfunction of the defense mechanisms.

The interaction between nutrition and course of infection

In the N balance experiment, described in chapter 6 and 7, two fibrous feeds with a different quality were fed to infected animals. In addition to the usual experimental feed in other studies (pelleted lucerne), chopped grass straw was chosen, which had a low protein content (68 $g \cdot kg^{-1}$ DM). It was aimed to study the interaction between diet quality and the course of infection with respect to feed intake and N metabolism.

Infected animals fed chopped grass straw showed a lower intake than infected animals fed pelleted lucerne. However, the relative decrease of DMI in each feed group, due to infection, was the same (35 - 36 %). Also the relation between NR and

DOMI was not changed by diet type, and agreed with literature reports (Elliott and Topps, 1964; Ketelaars and Tolkamp, 1991; Akinbamijo *et al.*, 1992). Noteworthy is the different γ-globulin fractions of infected animals on either good or poor quality diet. Because a large proportion of the circulating γ-globulins is not specific to the prevalent subpopulation of trypanosomes (Stephen, 1986), this finding does not necessarily indicate differences in immunity.

On basis of these results it was concluded that no interaction between feed quality and course of infection with respect to nutrient metabolism had played a role. Several literature reports, however, indicate that there is interaction between diet quality and production parameters during trypanosome infection, *i.e.*, Katunguka-Rwakishaya et *al.* (1993) found that weight gain of sheep, fed a diet with a high dietary protein content was not reduced in infected animals compared with controls, whereas sheep fed a low protein diet showed a reduced weight gain due to infection. Also Hecker (1994) observed in Djallonké sheep a smaller decrease of weight gain of due to infection, if they were supplemented with concentrate. Also our reported studies, however, do not exclude an interaction of nutrition and infection on animal productivity. This interaction may be twofold. In the first place, in a field setting in which poor feed quality prevents for a high animal productivity (often the case in tropical small ruminant production systems; Ademosun *et al.*, 1988), an extra stress factor like trypanosome infection may bring productivity to zero; in animals fed a better quality feed, however, still some production is possible. Supplementation could therefore increase animal productivity by acting as a lever (due to the fact that a large part of the ingested feed is used for maintenance requirements). In the second place, interaction may be detected with respect to the number of cases of severe undernutrition, which was more often observed in the low quality diet group than in the high quality diet group in our studies; this would probably have led to increased mortality after week 6 post infection, and consequently to a negative flock productivity.

In practical goat keeping, therefore, it is recommendable to pay attention to the quality of the diet, especially during detected trypanosome infection. The applicability of supplements or other high quality feeds, however, is dependent on many factors, including production goals (physical production vs. insurance/financing motives for small ruminant keeping), costs of supplements and of labour, and other management constraints (Bosman, 1995).

In the experiment, reported in chapter 8, the effect of nutritional history on the course of infection with respect to energy and N metabolism was studied. Our considerations for studying nutritional history included the notion that under practical husbandry conditions, feed quality may be poor during part of the year (Ademosun *et al.*, 1988); animals may lose weight during this period, which may leave them more

susceptible to trypanosomiasis (Ferguson, 1988). However, we have found minor interactions with respect to energy and N metabolism during the acute phase (first 4 weeks) of infection. Noteworthy is that infected animals with retarded growth showed a tendency toward lower ME requirements for maintenance, compared with infected congeners with a normal growth pattern; however, this tendency disappeared in the combined data analysis (Figure 2). It was concluded that the course of infection was far more affected by the actual energy and N intake during the infection period, than by nutritional history of the infected animal. It is possible, however, that the applied feed restriction in our reported study was not severe enough to create differences in the response to infection. The compensatory feed intake often observed at realimentation after a period of feed restriction, however (Hogg, 1992), may invert the proposed negative effects on the course of infection.

Conclusions

1. The anaemia due to trypanosome infection was severe, indicating that the suggested tolerance of West African Dwarf goats to trypanosomiasis is different from that in N'Dama cattle.

2. Induced trypanosome infection leads to a reduction in feed intake in West African Dwarf goats, during the acute phase of infection (first 6 weeks of infection). A large variation between animals exists; possible mechanisms behind this variation include:
   - age of the infected animal;
   - genotype of the animal, *viz.* polymorphism in the MHC region, most probably the gene encoding for TNF-α production;
   - environmental conditions, like housing in social isolation.

3. *Trypanosoma vivax* infection increases metabolizable energy requirements for maintenance (average from our studies 28 %). This increase is independent from intake level, and is caused by increased basal heat production, due to fever, and possibly other metabolic costs of infection, like the induction of an immune response.

4. Energy retention is decreased in infected animals due to reduced energy intake and increased energy requirements for maintenance.

5. Infected animals compensate for increased maintenance requirements by reducing activity related energy expenditure (*viz.* standing and moving).

6. Digestibility of dietary N and gross energy are not affected by *T. vivax* infection; metabolizability of the diet is not affected either. The relation between energy retention and nitrogen retention is not changed in *T. vivax* infected animals; this means that infection does not induce an increase of N losses, compared with healthy animals at the same feed intake level.

7. Blood and liver biochemical variables reflect the anorectic state of infected animals. Infection leads to reduced serum concentration of thyroid hormone (T3 and T4), this being independent from feed intake level or quality of the diet.

8. Under the applied experimental conditions, no interaction between diet quality and infection with respect to feed intake and N balance was observed. Effects of diet quality and of trypanosome infection on metabolism are therefore additive. Nevertheless, animal production may be boosted by improving diet quality.

9. During the acute phase of infection, under the applied experimental conditions, no detrimental effects of a retarded growth pattern of young dwarf goats on the energy and N metabolism occur; the actual energy and N intake during infection play a more important role.

10. More knowledge is needed on the mechanisms behind the regulation of feed intake during trypanosome infection, since in our studies variation in feed intake response to infection was proven to affect animal production most.

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Summary

This thesis describes a series of experiments, which were designed to study the effect of trypanosome infection on the energy- and nitrogen (N) metabolism of West African Dwarf (WAD) goats. The possible interaction with nutrition and nutritional status were investigated. Special attention was given to the feed intake response to infection of individual animals, and possible mechanisms behind the variation between animals.

Therefore, castrated WAD bucks were infected experimentally with *Trypanosoma vivax* parasites and were followed for a maximum of 6 weeks afterwards (acute phase of infection). During this period, various traits were measured: individual feed intake, body weight, body temperature, packed cell volume, parasitaemia, energy- and N balance parameters. Moreover blood and liver biochemical variables, signifying the metabolic profile were measured, *i.e.*, serum (or plasma) concentrations of glucose, non esterified fatty acids, ß-hydroxy butyrate, urea, insulin, thyroxine (T4) and triiodothyronine (T3) and hepatic triacylglycerol (TAG). After termination of the experiment, the animals were euthanized and *post mortem* micro- and macroscopic examination was done.

In general infection caused severe anaemia, fluctuating fever and parasitaemia. *Post mortem* analysis showed the signs of *T. vivax* infection, with hyperplasia of the lymphoid tissues and mononuclear infiltrates in several tissues. Also oedema of lungs and other tissue was observed, and frequently a mononuclear myocarditis.

Variation in dry matter intake (DMI) reduction due to infection differed widely between animals and experiments. The mean ratio (DMI during infection / DMI before infection) for the different experiments ranged between 0.38 and 0.80, whereas variation in individual response was even higher. In the infection trial reported in chapter 2, it was studied if the individual DMI responses to successive trypanosome infections had a high repeatability if environmental conditions were changed during the second infection period. This would give insight in the sources of variation that caused the differences in DMI response among animals. Little correlation was found between the individual DMI response to successive trypanosome infections. It is concluded that environmental factors, like a different housing system in the subsequent infection periods, has played a large role in the DMI response. In the study described in chapter 3, the variation in clinical traits among infected goats was related to polymorphism of the major histocompatibility complex (MHC) region on the genome. Some evidence was found that differences in DMI response among animals was related to MHC class I and II polymorphism. It is suggested that this correlation may be attributed to genetic differences among animals

in the production capacity of tumor necrosis factor-α (TNF-α), a cytokine which negatively affects feed intake during infection.

In chapters 4 and 8 results from two experiments are reported, which were designed to study the effect of trypanosome infection on energy- and N metabolism. The results demonstrate that infection led to reduced dietary energy- and N uptake, with no changes in diet digestibility and metabolizability. Metabolizability was found to be different for the two experiments (general discussion). The metabolizable energy (ME) requirements for maintenance were increased by infection (average increase over different studies equalled 28 %). This increase appeared to be independent from feed intake level (general discussion), and was mainly caused by an increase of heat production (HP) in infected animals. Individual heat production, body temperature and body posture were measured continuously to study the effect of infection on these traits and the relationships between these traits (chapter 5). It could be concluded that the short term variation in HP increase was related to the fluctuant fever during infection. Infected animals spent less time standing, which reduced energy costs. Indications were found that postural behaviour was thermoregulatory induced. Possibly also other metabolic costs like the induction of an immune response played a role in the increase of ME maintenance requirements.

The increase of N retention with energy retention was not different in infected and control animals. Therefore infection did not induce an increase of N losses at iso-nutritional comparison. A piecewise linear function was found to describe the combined dataset best (chapter 9), indicating a different relationship below and above zero maintenance, and/or a difference between the two energy balance experiments (chapters 4, 8).

The blood and metabolic biochemical parameters generally reflected the nutritive state of the experimental animals, with insulin and non esterified fatty acids being most sensitive to undernutrition. Fatty livers were observed in both infected and control animals with a negative energy balance. T3 and T4 were largely decreased by infection, irrespective of feed intake level (chapters 4, 7, 8).

It was concluded from an experiment with a good and a poor quality fibrous feed (chapters 6, 7), that diet quality did not interact with the course of infection. This means that the effects of diet quality and of infection were additive. A few moribund animals with very low feed intake tended to show increased N losses. Therefore, under practical husbandry conditions, strategic supplementation during infection may offset the wasting effects of infection, and prevent for high mortality.

The nutritional history of young dwarf goats , *viz.* a retarded growth pattern, did not interact with the course of trypanosome infection. However, a tendency toward lower ME maintenance requirements in infected animals with retarded growth, compared with

infected animals with a normal growth pattern was observed. It was concluded that the dietary provisions during infection were far more important for the outcome of disease than the nutritional history of the infected animal.

The results of these studies make clear that future research should concentrate on the variation in feed intake response to infection in (trypanotolerant) breeds, as the major determinant of biological production.

Résumé

Cette thèse décrit un ensemble d'essais conduits afin d'étudier l'effet de l'infestation par le trypanosome sur le métabolisme énergétique et azoté de la chèvre naine d'Afrique de l'ouest (West African Dwarf goats, WAD). Les interactions pouvant exister avec l'alimentation et le statut nutritionnel ont été recherchées. L'effet sur l'ingestion de l'infestation des animaux, et les mécanismes pouvant expliquer les variations entre individus ont fait l'objet d'une attention particulière.

Ainsi des boucs WAD infestés expérimentalement avec des parasites Trypanosoma vivax ont fait l'objet d'un suivi sur une période maximale de 6 semaines suivant l'infestation expérimentale. Durant cette période correspondant à la phase aiguë de l'infestation, plusieurs critères ont été mesurés : quantités ingérées, poids vif, température corporelle, hématocrite, parasitémie, paramètres décrivant les bilans énergétique et azoté, paramètres métaboliques sanguins et hépatiques - glucose, acides gras non estérifiés, β-hydroxy-butyrate, urée, insuline, thyroxine (T4), triiodothyronine (T3) et triacylglycérol (TAG) -. A la fin de l'expérimentation, les animaux ont été enthanasiés et ont fait l'objet d'examens macro- et microscopiques post-mortem.

Dans le cas général, l'infestation provoque une anémie sévère, une fièvre oscillante and une parasitémie. L'autopsie permet de mettre en évidence des signes liés à l'infestation par T. vivax, en particulier une hyperplasie des tissus lymphoides et des infiltrats mononucléaires dans plusieurs tissus. Cet examen a également permis d'observer un oedème des poumons et de plusieurs autres tissus, ainsi que fréquemment une myocardite mononucléaire.

La réduction de quantités de matière sèche ingérée (MSI) avec l'infestation varie fortement entre animaux et essais. Les valeurs du rapport moyen entre quantité de MSI au cours de l'infestation et quantité de MSI avant infestation s'étendent de 0,38 à 0,80 d'un essai à l'autre, tandis que ce rapport présente de plus larges variations entre animaux.

Dans l'essai décrit au chapitre 2, la répétabilité des quantités de MSI en réponse à des infestations successives par T. vivax a été étudiée dans le cas où les facteurs environnementaux étaient modifiés pendant la seconde période d'infestation. Cette étude avait pour but d'évaluer les facteurs de variation des quantités de MSI entre individus. Les quantités de MSI en réponse à des infestations successives sont faiblement corrélées. Ainsi, les facteurs environnementaux, tels que les différents types de logement dans les périodes successives d'infestation, jouent un rôle important dans les variations de quantité de MSI.

Dans le chapitre 3, les relations entre les différents signes cliniques observés chez les chèvres infestées et le polymorphisme du Complexe Majeur d'Histocompatibilité ont été étudiées. Il apparaît que des différences de quantité de MSI par les animaux peuvent être liées au polymorphisme des classes I et II du Complexe Majeur d'Histocompatibilité. Cette corrélation pourrait être attribuée à des différences génétiques dans l'aptitude à produire le facteur a de nécrose des tumeurs (tumor necrosis factor-α, TNF-α), ce facteur étant une cytokine qui réduit l'ingestion au cours de l'infestation.

Les essais décrits dans les chapitres 4 et 8 avaient pour objectif d'étudier l'effet de l'infestation par le trypanosome sur le métabolisme énergétique et azoté. Les résultats montrent que l'infestation conduit à une diminution de l'absorption d'énergie et d'azote des aliments, alors que la digestibilité et la valeur métabolisable de la ration ne varient pas. La valeur métabolisable des rations était différente d'un essai à l'autre (cf. discussion générale). Les besoins d'entretien exprimés en énergie métabolisable augmentent avec l'infestation (augmentation moyenne de 28 % sur la base de différents essais). Cette augmentation apparaît indépendante du niveau d'ingestion (cf. discussion générale), et semble principalement due à une augmentation de la production de chaleur chez les animaux infestés.

Dans le chapitre 5, la production de chaleur, la température corporelle et la posture des animaux ont été mesurées en continu afin d'étudier l'effet de l'infestation sur ces critères et les relations entre ces critères. Les variations à court terme de production de chaleur sont liées à une fièvre oscillante pendant l'infestation. Les animaux infectés passent moins de temps debout, ce qui réduit les dépenses énergétiques. Il apparaît que le comportement postural est thermorégulé. D'autres éventuelles dépenses métaboliques, telles que l'induction d'une réponse immunitaire jouent un rôle dans l'augmentation des besoins d'entretien exprimés en énergie métabolisable. L'augmentation de la rétention azotée avec la rétention d'énergie ne diffère pas entre animaux infectés et témoins. Ainsi, l'infestation n'induit pas d'augmentation de pertes azotées pour des régimes iso-nutritionnels.

Dans le chapitre 9, une fonction linéaire d'ajustement aux données a été décrite, permettant de montrer des relations différentes en fonction du niveau de couverture des besoins d'entretien, et des différences entre les bilans énergétiques des deux essais (chapitres 4 et 8).

Les paramètres métaboliques et sanguins reflètent en général l'état nutritionnel des animaux. Les concentrations en insuline et acides gras non estérifiés apparaissent plus sensibles à la sous-nutrition. Des foies stéatosés ont été observés chez les animaux infectés comme chez les témoins avec des bilans énergétiques négatifs. Les concentrations en T3 et T4 sont fortement diminuées chez les animaux infectés, indépendemment du niveau d'ingestion (chapitres 4, 7 et 8).

Un essai conduit avec deux qualités de ration fibreuse (bonne/mauvaise) a permis de montrer que la qualité de la ration n'interagit pas avec l'évolution de l'infestation (chapitres 6 et 7). Les effets de la qualité de la ration et de l'infestation sont donc additifs. Quelques animaux moribonds avec une faible ingestion ont montré des pertes accrues en azote. Ainsi, en pratique, une supplémentation pendant l'infestation pourrait contrecarrer les effets négatifs de l'infestation, et empêcher la mortalité élevée.

Les antécédents nutritionnels de jeunes chèvres naines, mesurés au travers de leur retard de croissance, n'intéragit pas avec l'évolution de l'infestation par le trypanosome (chapitre 8). Toutefois, les besoins d'entretien exprimés en énergie métabolisable tendent à être plus faibles chez les animaux infestés avec un retard de croissance que chez les animaux infestés avec une croissance normale. Ainsi, il apparaît que l'effet du niveau des apports alimentaires pendant l'infestation sur les conséquences de la maladie est bien plus important que celui des antécédents nutritionnels des animaux infestés.

Les résultats de ces essais montrent que les futurs travaux de recherche devraient plus particulièrement porter sur l'étude des variations de quantités ingérées en réponse à l'infestation dans les races trypanotolérantes, en raison de son rôle majeur sur la production.

Samenvatting

Het proefschrift dat voor u ligt, is het resultaat van viereneenhalf jaar promotieonderzoek naar de invloed van trypanosomiasis (slaapziekte) op een aantal aspecten van de stofwisseling van Westafrikaanse dwerggeiten. Eerst zal op de achtergrond van dit onderzoek worden ingegaan, waarna de belangrijkste resultaten zullen worden besproken.

Introductie

Slaapziekte is een protozoaire ziekte, en komt voor in een groot aantal Afrikaanse landen ten zuiden van de Sahara; de ziekte bedreigt zowel mens als dier. De herkauwers onder de landbouwhuisdieren (koeien, schapen, geiten) worden relatief het vaakst besmet met *Trypanosoma vivax* en *Trypanosoma congolense*, twee van de species die slaapziekte veroorzaken. De ziekte wordt overgebracht via de bloedzuigende tseetseevlieg. Naar het ziektebeeld is veel onderzoek verricht; men vond dat infectie in het algemeen leidt tot sterke bloedarmoede (anaemie), koorts en verminderde eetlust, en dat de afloop vaak dodelijk was. Het afweersysteem heeft enige tijd nodig voor de specifieke herkenning van de indringer en het ontwikkelen van een afweerreaktie ('immuun respons') hiertegen; als deze immuun respons in werking treedt, blijken ondertussen echter enige protozoën zich te hebben 'vermomd' voor het immuunsysteem, zodat zij niet gevoelig zijn voor deze immuun respons. Deze protozoën kunnen zich daardoor enige tijd ongehinderd vermeerderen, waarna de volgende wèl tegen hen gerichte immuun respons in werking treedt. Ondertussen hebben sommige parasieten echter een wéér ander 'jasje' aangetrokken, en het proces herhaalt zich. Zo kan dit lange tijd doorgaan, terwijl de patient langzaam wegkwijnt.

Reeds lang geleden werd door onderzoekers de aandacht gevestigd op een aantal runder- geiten- en schapenrassen, afkomstig uit de landen waar slaapziekte voorkomt, die tolerant bleken te zijn voor slaapziekte. Dit betekent dat dieren wel geïnfecteerd kunnen worden, maar dat de ziekte een veel milder verloop heeft en vaak weer spontaan geneest. Uitgebreid onderzoek aan zo'n 'trypanotolerant' runderras, de N'Dama, wees uit dat deze dieren tijdens een infectie met trypanosomen weinig anaemie vertonen, en zelfs kunnen blijven doorgroeien, zodat de boer toch nog enige productie van zijn kudde heeft. De mate van deze tolerantie voor slaapziekte lijkt echter afhankelijk van

omgevingsfactoren, zoals de kwaliteit van de voeding van het dier, het productieniveau en eventueel tegelijkertijd optredende andere infecties.

De laatste jaren is meer aandacht gekomen voor de mogelijkheden van kleine herkauwers (schapen en geiten). Zij zouden beter passen in kleinschalige en in gemengde landbouwsystemen dan runderen, en daarnaast is hun produktiviteit relatief hoog. Het algemene voordeel van herkauwers daarbij is, dat ze laagwaardige en voor menselijke consumptie ongeschikte voedingsbronnen kunnen omzetten in voor de mens nuttige produkten zoals melk, vlees, huiden, trekkracht en mest. In de gebieden waar slaapziekte voorkomt, zouden daarom trypanotolerante kleine herkauwers een bijdrage kunnen leveren aan de ontwikkeling van een duurzame(re) landbouw.

De mate van tolerantie van kleine herkauwers (geiten, schapen) is echter minder uitgebreid onderzocht dan die van runderrassen. Een serie veldstudies onder wisselende omstandigheden heeft inzicht gegeven in de produktiviteit van verschillende rassen geiten en schapen, maar naar het effect van trypanosomiasis op de stofwisseling (energie- en eiwithuishouding) en andere fysiologische processen en de eventuele wisselwerking met de aangeboden voeding is nog weinig onderzoek verricht. Een vergroting van het inzicht in deze onderliggende processen kan indirect helpen om de produktiviteit van de dierlijke component van kleinschalige landbouwsystemen te vergroten. Daarnaast kan het bijdragen aan het vergroten van het inzicht in andere, vergelijkbare ziekten.

Doel en methoden van het onderzoek

In dit onderzoek bestudeerden we de mate van trypanotolerantie van Westafrikaanse dwerggeiten, één van de belangrijkste geitenrassen in door slaapziekte geteisterde gebieden. Hiertoe infecteerden we groepen gecastreerde bokken kunstmatig door een dosis van 100.000 tot 1.000.000 trypanosomen per dier in de halsader te spuiten. Vervolgens bestudeerden we van individuele dieren de voedselopname (het voedsel bestond in de meeste proeven uit gepelletteerde luzerne). Verder waren we geïnteresseerd in de variatie tussen dieren, en zochten we naar verklaringen voor het verschil in reactie tussen dieren. Daarnaast bestudeerden we de invloed van infectie op de energie- en eiwithuishouding van het dier, en onderzochten we of de kwaliteit van de geboden voeding invloed had op het verloop van de infectie.

De energie- en eiwit-huishouding werd bestudeerd in respiratiecellen; dat zijn afgesloten ruimten waarin het zuurstofgebruik en de kooldioxide/methaan productie van de dieren bepaald kunnen worden. Met deze gegevens kan de warmteproductie berekend worden. Als van de opgenomen hoeveelheid energie ('bruto energie') de energie in faeces en urine, en de energie in de uitgeademde hoeveelheid methaan wordt

afgetrokken resteert de zg. metaboliseerbare energie. Als van deze metaboliseerbare energie de warmteproductie wordt afgetrokken, blijft de zg. energie retentie over, hetgeen de hoeveelheid energie is, die door het dier gebruikt kan worden voor enige vorm van productie (melk, vleesgroei, trekkracht). Deze energie balans is daarom een methode om te bepalen hoeveel energie een dier produceert (of inteert) in relatie tot de opgenomen hoeveelheid opgenomen energie, en ook kan de invloed van omgevingsfactoren, zoals kwaliteit van voeding en het voorkomen van infecties, op de energiebalans worden beoordeeld.

De eiwithuishouding werd bestudeerd met behulp van de stikstofbalans (stikstof is een kenmerkende bouwsteen van eiwitten). De stikstofbalans is evenals de energiebalans opgebouwd uit enerzijds opname (van in dit geval stikstof dat in het voedsel zit) en anderszijds uit verliezen (stikstof in faeces en urine en in vervluchtigde ammoniak), en kan zowel in respiratiecellen als in balanskooien gemeten worden. Het verschil tussen stikstof opname en stikstof verliezen is de stikstof retentie. De verschillende onderdelen van de energie- en stikstofbalans worden gecorrigeerd voor verschillen in lichaamsgewicht tussen dieren, door te delen door het lichaamsgewicht tot de macht ¾; hierdoor worden gegevens van lichte en zware dieren met elkaar vergelijkbaar.

Naast de hiervoor beschreven metingen, werd de concentratie van een aantal stoffen in het bloed gemeten, die informatie geven over de energiebalans; dat zijn de concentraties in het bloed van glucose, insuline, vrije vetzuren, ß-hydroxyboterzuur, ureum, thyroxine (T4) en triiodothyronine (T3). Omdat een van de gevolgen van een negatieve energiebalans is dat lichaamsvet wordt gemobiliseerd en dat daardoor in extreme gevallen leververvetting kan optreden is aan het einde van de infectie ook het vetgehalte van de lever gemeten. Om een indruk te krijgen van de ernst van de opgewekte infectie werd de mate van de door de infectie veroorzaakte bloedarmoede (anaemie) gevolgd. Hiertoe werd regelmatig het celvolume (haematocriet) van het bloed gemeten. Verder werden het aantal parasieten per ml bloed, en de lichaamstemperatuur gemeten.

Uitkomsten van het onderzoek

De opgewekte infectie leidde tot onregelmatige koorts met pieken en dalen, en tot sterke anaemie. Het lymfesysteem (lymfeknopen, thymus) was sterk geactiveerd; de longen en sommige andere organen vertoonden oedeem. Vaak werd een ontsteking van de hartspier geconstateerd.

In de hoofdstukken 2 en 3 wordt de invloed van infectie op de voedselopname (uitgedrukt als de drogestofopname) beschreven. De drogestofopname bleek bij alle

geïnfecteerde dieren verlaagd te zijn tijdens de infectie. Bij de verschillende experimenten die we hebben gedaan, werden verlagingen tussen 20 en 62 % gevonden. De mate waarin de drogestofopname was verlaagd, uitgedrukt als de ratio (drogestofopname tijdens infectie / drogestofopname voor infectie), verschilde echter sterk van dier tot dier. Het bleek, dat als dieren tweemaal achter elkaar worden geïnfecteerd met verschillende soorten trypanosomen (respectievelijk *T. congolense* en *T. vivax*), de individuele voedselopnamedaling beide keren niet even groot was (Hfdst. 2). Dit werd met name veroorzaakt door de verschillende omstandigheden waaronder de twee infectie-experimenten plaatsvonden. Bij de tweede infectie waren de dieren individueel gehuisvest, waarbij de dieren geen soortgenoten konden zien of horen. Dit heeft waarschijnlijk stress veroorzaakt, hetgeen weer de voedselopname negatief beïnvloedde. Bovendien waren de dieren zwaarder (en vetter) tijdens de tweede infectie; in het algemeen zorgt dit voor een verlaging van de voedselopname.

In een ander experiment werd een aanwijzing gevonden voor een verband tussen de variatie in de ratio drogestofopname tussen dieren enerzijds, en bepaalde genetische eigenschappen van deze dieren anderzijds. Van alle dieren in deze proef was namelijk het zg. Major Histocompatibility Complex (MHC) klasse I en II bepaald. Dit complex van genen is nauw betrokken bij de immuun respons tegen infecties. We vonden dat dieren met een bepaald MHC klasse I en II genotype een hogere ratio drogestofopname vertoonden dan dieren met een ander MHC genotype. Twee verklaringen zijn hier mogelijk. Het is mogelijk dat de MHC genen zelf betrokken zijn bij de voedselopname-regulatie tijdens infectie, maar het is ook mogelijk dat andere genen op hetzelfde chromosoom de voedselopname tijdens infectie reguleren. Dit laatste ligt meer voor de hand, omdat het gen dat de productie van tumor necrosis factor-α (TNF-α) reguleert vlakbij de klassen I en II van het MHC gelegen is. En van dit TNF-α is weer bekend dat het tijdens slaapziekte infectie geproduceerd wordt, en een negatieve invloed op de voedselopname heeft. Als dit verband tussen voedselopname tijdens infectie en TNF-α genotype werkelijk bestaat, kan dit een handvat vormen om de dieren met de kleinste voeropnamedaling in een populatie te selecteren.

In de hoofdstukken 4 en 5 wordt het onderzoek beschreven naar de energie- en stikstofbalans van geiten, die geïnfecteerd zijn met *T. vivax*. De energie- en stikstofopname daalde onder invloed van de infectie. De energie- en stikstofverliezen in de faeces en urine bleken echter steeds een vast percentage van de opgenomen hoeveelheden aan energie en stikstof uit te maken, onafhankelijk van het feit of dieren geïnfecteerd waren of niet. Dit betekent dat de schijnbare verteerbaarheid van energie en stikstof (d.i. de verteerde hoeveelheid gedeeld door de totale opgenomen hoeveelheid) en de metaboliseerbaarheid van energie (d.i. de metaboliseerbare energie als

proportie van de bruto energie) niet werden beïnvloed door infectie. Het verteringsproces wordt dus waarschijnlijk niet beïnvloed door trypanosomiasis.

De infectie bleek echter wel aanleiding te geven tot een sterke verhoging van de warmteproductie. Deze verhoogde warmteproductie, samen met de verlaagde bruto energie-opname, leidde tot een veel lagere en zelfs negatieve energie retentie bij geïnfecteerde dieren, met als gevolg dat geïnfecteerde dieren gewicht verloren. Verder was ook de stikstof retentie negatief; het bleek dat de energie retentie en de stikstof retentie nauw met elkaar verbonden waren. Deze relatie werd niet verstoord door de infectie. In een volgende proef, die beschreven is in Hfdst. 8, lag het voedselopname-niveau van de proefdieren hoger. Hierdoor was de energie- en stikstof retentie van geïnfecteerde dieren nog positief en namen de dieren nog licht in gewicht toe. Op basis van de resultaten van deze twee experimenten werd geschat dat in de acute fase van de infectie de onderhoudsbehoefte aan energie 28 % verhoogd is. Dit wil zeggen dat geïnfecteerde dieren ten opzichte van gezonde dieren gemiddeld 28 % meer energie moeten opnemen om alle onderhoudsprocessen in het lichaam te bekostigen. Uiteraard betekent dit een sterke verlaging van de productie ten opzichte van gezonde dieren.

Vervolgens werd de variatie van uur tot uur van de individuele warmteproductie bestudeerd in relatie tot de variatie in lichaamstemperatuur en tot het gedrag van deze dieren (Hfdst. 5). We vonden dat de verhoging van de warmteproductie bij geïnfecteerde dieren duidelijk in verband staat met de verhoogde lichaamstemperatuur. Ook zagen we dat zieke dieren energie besparen door meer te blijven liggen, omdat dit minder energie kost dan staan. Daarnaast werden aanwijzingen gevonden dat het moment waarop zieke dieren opstaan of juist weer gaan liggen, te maken heeft met gevoelens van onderkoeling of oververhitting.

In een tweetal proeven, beschreven in hfdst. 6 - 8, werd onderzocht of de kwaliteit en/of de aard van de voeding invloed kan hebben op het verloop van de infectie. Daartoe kregen de dieren onbeperkt voer aangeboden (ca. 40 % meer dan ze opkunnen). De ene groep kreeg een laagwaardig ruwvoeder (gehakseld grasstro) aangeboden, de andere een goede kwaliteit ruwvoeder (gepelletteerde luzerne). Het bleek dat het laagwaardige voeder in vergelijking met het hoogwaardige voeder tot een lagere voedselopname leidde en tot een lagere lichaamsgroei. In de groep geïnfecteerde dieren was de voedselopname en lichaamsgroei lager dan in de groep gezonde dieren. We vonden echter een even grote procentuele verlaging van de voedselopname door de infectie in beide voedingsgroepen (ca. 35 % verlaging ten opzichte van gezonde dieren). Hieruit valt af te leiden dat er geen sprake was van een interactie tussen de infectie en de voerkwaliteit voor wat betreft de voedselopname, hetgeen toch de belangrijkste bepalende factor in de dierlijke productie is. Op grond hiervan zou geconcludeerd kunnen worden dat het niet uitmaakt of je hoogwaardig of laagwaardig ruwvoeder

verstrekt aan geïnfecteerde dieren. Toch kan het raadzaam wezen een hoogwaardig ruwvoeder te verstrekken aan geïnfecteerde dieren, omdat de dieren hiermee beter in conditie blijven en wellicht op de langere termijn beter de infectie de baas kunnen.

In de proef, beschreven in Hfdst. 8, werd onderzocht welke invloed een periode van ondervoeding heeft op een daaropvolgende slaapziekte infectie. Het is immers bekend, dat in de tropen regelmatig perioden van ondervoeding voorkomen, bijvoorbeeld door verslechtering van de voederkwaliteit in de droge tijd. Deze proef werd als volgt uitgevoerd. Aan jonge, groeiende bokken werd gedurende een periode van 17 weken een beperkte hoeveelheid luzerne gevoerd ('vertraagde groei'), terwijl een groep leeftijdsgenoten volop luzerne konden eten ('normale groei'). Dit leidde tot een verschil in gewicht van 6 kg tussen deze twee groepen dieren aan het begin van de infectie. Om de mogelijke verschillen in het verloop van de infectie uitsluitend te kunnen toeschrijven aan de in de voorperiode opgelegde verschillen in het rantsoen, werd tijdens de infectie periode aan alle dieren volop voeder verstrekt.

Ook in deze proef werden er weinig aanwijzingen gevonden voor een interactie tussen infectie en de voederrantsoenen. Het bleek dat de nutriëntenopname niet wezenlijk verschilde tussen de geïnfecteerde dieren met normale groei en met groeiachterstand. Wel werden aanwijzingen gevonden, dat bij dieren met een groeiachterstand de verhoging van de onderhoudsbehoefte als gevolg van infectie, kleiner was. Dit zou kunnen wijzen op een efficiëntere stofwisseling van deze dieren. Op grond hiervan kwamen we tot de conclusie dat het belangrijker is kwalitatief hoogwaardig voeder beschikbaar te stellen tijdens de infectie, dan in de periode voor de infectie.

Curriculum Vitae

Johannes Teunis Pieter van Dam werd geboren op 16 april 1967 in Bodegraven. Hij kreeg de liefde voor de veeteelt met de paplepel ingegoten op het melkveehouderij-bedrijf van zijn ouders. Daarom koos hij in 1985, na een succesvol verlopen VWO opleiding op de Rijksscholengemeenschap F.A. Minkema, te Woerden, voor de studie Zoötechniek aan de Landbouwuniversiteit te Wageningen, oriëntatie Veehouderij. In juni 1991 studeerde hij af met afstudeervakken in de Veehouderij, Veevoeding en Graslandkunde. In september 1991 werd hij vervolgens aangesteld als assistent in opleiding bij de vakgroep Veehouderij op het onderzoek waarvan de resultaten in dit proefschrift zijn beschreven.