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Evaluation of the pathogenicity in goats of *Trypanosoma congolense* from Matutuine, Mozambique

By

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Abbreviations

DINAP- National Directorate of Livestock

PCV- Packed Cell Volume

UEM - Universidade Eduardo Mondlane

RTTCP- Regional Tsetse & Trypanosomosis Control Programme

UPBRC - Biological Research Center of the University of Pretoria

CH - control on high-energy diet

CL - control on low energy diet

IH - Infected on high energy diet

IL- Infected on low energy diet

MEm - Metabolizable energy for maintenance

EDTA - Ethylenediamine tetra-acetic acid

PBS - Phosphate buffered saline

PCV - Packed Cell Volume

RBC- Red Blood Cell

MCV- Mean corpuscular volume

MCH-Mean corpuscular hemoglobin

MCHC- Mean corpuscular hemoglobin concentration

TSP- Total serum protein level

BIL-T - Total bilirubin level

AST- Serum aspartate aminotransferase level

SIP- Inorganic phosphate level

GGT - Serum gama glutamyltransferase

CK - Creatin kinase level

Declaration

I hereby declare that this dissertation, submitted by me to the University of Pretoria for the degree of Master of Science has not previously been submitted for a degree at any University.

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Cesaltina da Conceição Lopes Menete Tchamo

Abstract

A study was conducted to determine the impact of infections with *Trypanosoma congolense* on various variables (including hematological and biochemical parameters) of South African Boer goats kept on various planes of nutrition.

Sixteen goats with eight months old divided into four groups and weighing on average 12 kg were used in the experiments. Two groups (of four goats each) were infected with a *T. congolense* isolated in the Matutuine District of Mozambique. One group (of four goats) was kept on a low energy budget (9.72 MJ of metabolizable energy per kg and 0.16 kg of digestible protein per kg) whereas another group (of four goats) was kept on a high-energy budget (9.72 MJ of metabolizable energy per kg and 0.09 kg of digestible protein per kg). Two uninfected control groups kept on a low or high-energy budget were included in the experiment.

In each of the experimental groups, the parasitemia, packed cell volume (PCV), the hemoglobin level, the red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), the total serum protein level (TSP), the total bilirubin level, the serum aspartate aminotransferase level (AST), the inorganic phosphate level (SIP), the serum gama glutamyltransferase level (GGT), the serum urea level, the creatinin and the creatin kinase (CK) levels, the body temperature and weighs were monitored and compared during the pre-patent from day -13 to 7 and during the chronic one from day 22 to 56.

The first peak of parasitemia occurred earlier in infected goats kept on a low energy diet. Throughout the observation period, infection with *T. congolense* and diet had no significant effects on body weight. On the other hand, PCV, RBC counts reduced in both groups of infected goats. The pre-infection phase urea levels were higher in the animals (infected and control) kept on a high-energy diet. No significant changes were observed in the plasma of Albumin, AST, Creatinin, GGT, Globulin, SIP and TSP levels as a result of diet and/or infection with *T. congolense.*

These results suggest that the infection with the Mozambican isolate of *T. congolense,* which was used in this study, induces a mild infection with no or minimal effect on the health goats even when kept on a low energy plane of nutrition.

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CHAPTER 1: INTRODUCTION

Background

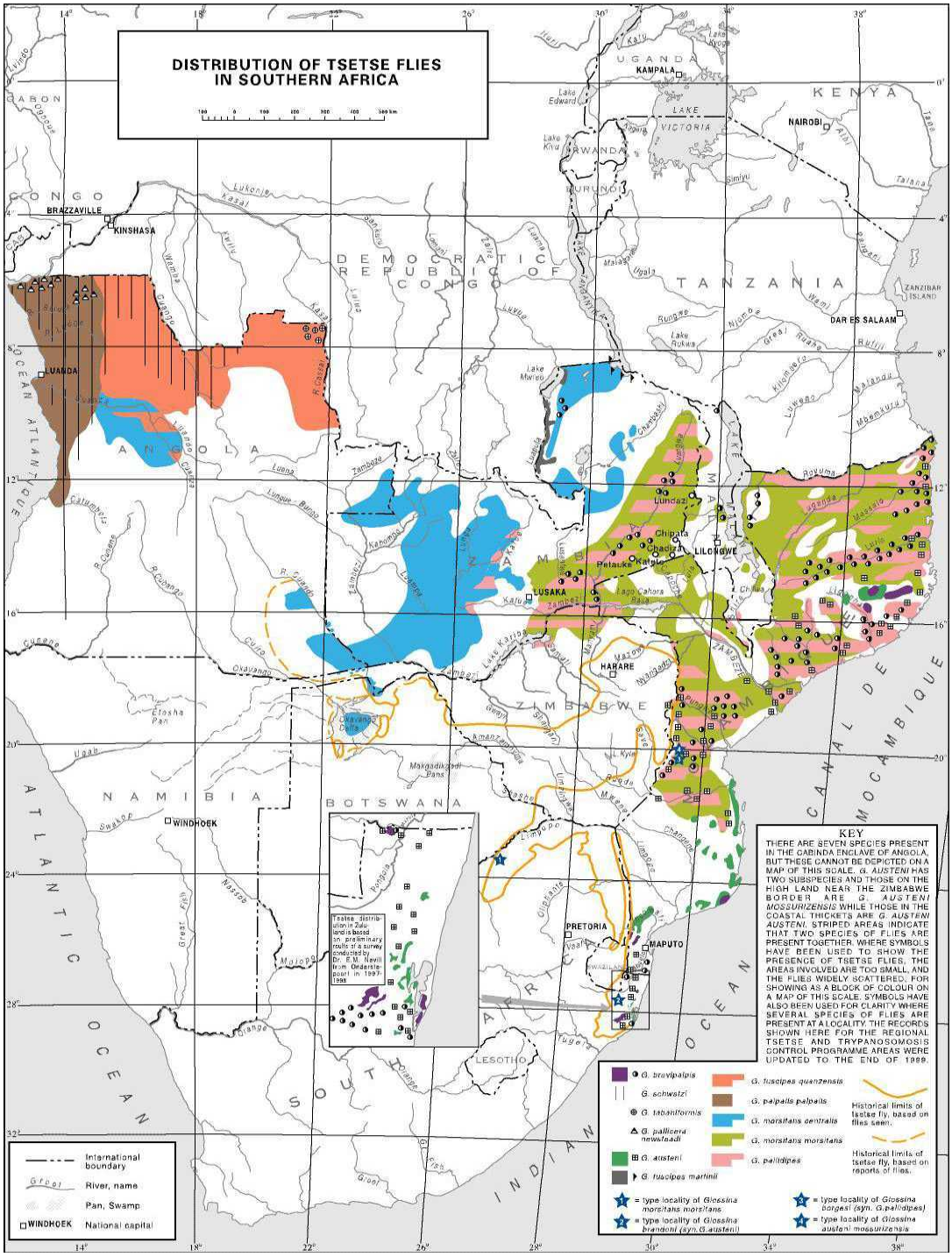
Tsetse-transmitted trypanosomosis is a disease caused by several species of protozoan parasites of the genus *Trypanosoma* (Masiga *et al.,* 2002). It is an important disease that limits animal production in Sub-Saharan Africa causing great economic losses that cannot be prevented because the methods to control trypanosomosis are often difficult to implement and to sustain. This aggravates the situation.

Approximately 10 million square kilometers of the African continent are infested by tsetse flies, the vectors of trypanosomes, and a total of 61 million bovines in 38 African countries are at risk of contracting the disease (Murray & Gray, 1984). The annual losses in livestock production due to trypanosomosis are estimated at USD 5 billion (Murray & Gray, 1984; D'Ieteren *et al*., 1998).

In Mozambique trypanosomosis is also considered an important constraint to livestock and rural development. Between 1949 and 1974, the Missão de Combate a Tripanosomíases or Mozambican Trypanosomosis Control Department was active. Its main objectives were to determine the distribution of the tsetse fly and conduct tsetse and trypanosomosis control. Its activities resulted in the identification of areas where trypanosomosis was present and the production of maps of the distribution of tsetse and *Trypanosoma* species in Mozambique.

The main tsetse species present in Mozambique are *Glossina morsitans morsitans, G. brevipalpis, G. austeni* and *G. pallidipes.* Historical data indicate that 70% of the country is suitable for tsetse: with the exception of some highlands in Niassa, Cabo Delgado, Tete and Zambézia Provinces, most of the areas north of the Save river are supposed to be tsetse-infested. A study carried out under the auspices of the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) in the year 2000 confirmed that *G. morsitans* and *G. pallidipes* have the widest distribution, while *G. austeni* is found mainly in dry coastal thickets.

Figure 1: Map showing the distribution of tsetse flies in Southern Africa (from RTTCP).



At independence in 1975, the number of cattle and small ruminants in Mozambique numbered 1,422,100 and 661,000 animals, respectively. Since then this number has declined mainly due to

the lack of technical expertise following independence. This situation was aggravated by the civil war which made it impossible to increase or even maintain the number of animals. By 1995, cattle and small ruminants had decreased by about 80 and 34%, respectively, compared to their numbers in 1975 (Figures 2 and 3).

Figure 2: Changes in the number of cattle in Mozambique between 1975 and 2003 (Source: National Directorate of Livestock, DINAP).

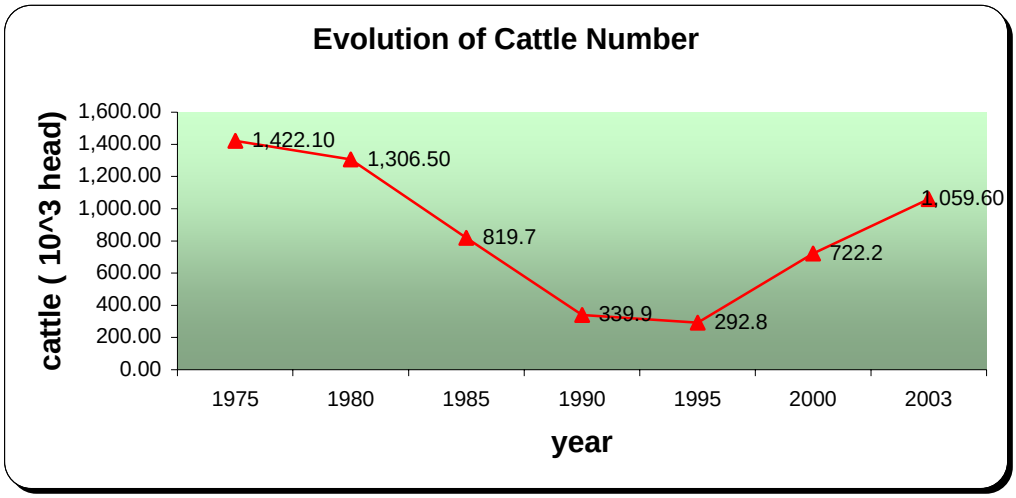
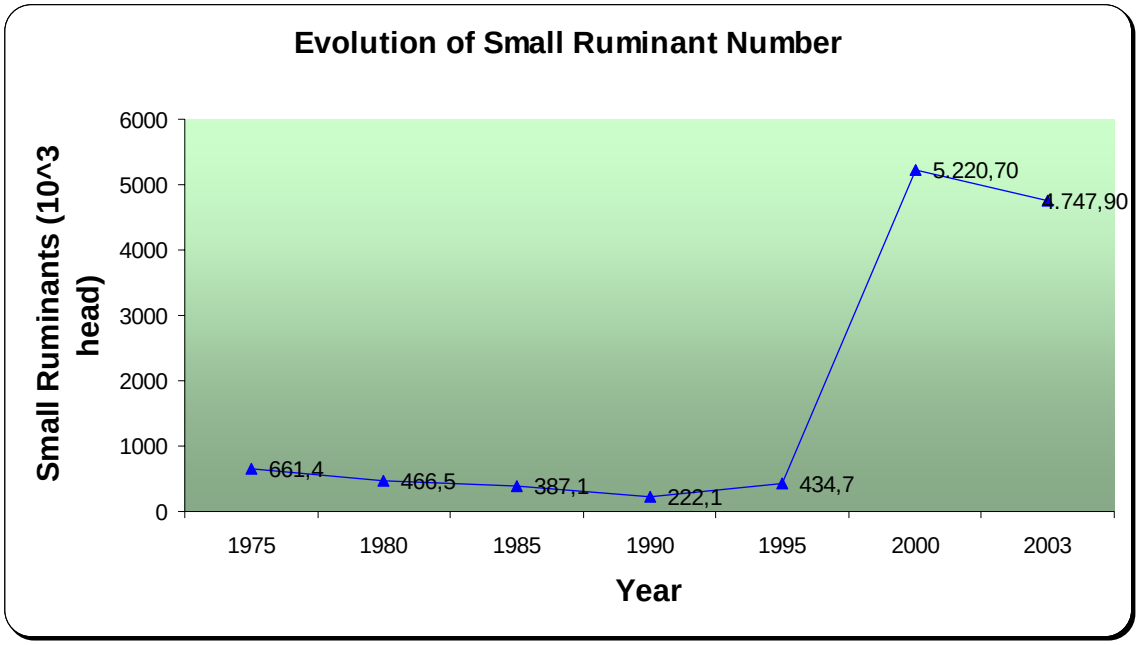


Figure 3: Changes in the number of small ruminants in Mozambique between 1975 and 2003 (Source: National Directorate of Livestock, DINAP).



In an effort to increase the national herd after the civil war, restocking programmes were initiated in 1992. They aim at reintroducing improved breeds in areas where smallholder farmers traditionally kept livestock. Animals from South Africa, Swaziland and Zimbabwe have been imported into Mozambique for the restocking purposes. Improved goat breeds, imported from South Africa and Zimbabwe, were the most preferred by potential livestock keepers. This is due mainly to the ease with which they adapt to the national livestock management system, their superior heat stress and drought tolerance, their tick-borne disease resistance and their potential meat-producing qualities.

Nevertheless, due to a variety of reasons, including erratic rainfall, the goat restocking program has faced many difficulties. One potential animal-health constraint is trypanosomosis and the impact of trypanosomal infections on goat health. Extensive areas in which goats are being restocked are infested with tsetse flies. The impact of this challenge on imported goat breeds is not known. Furthermore, the effect of poor nutrition on the impact of a trypanosomal infection in imported goats needs to be further assessed.

1.2.Objectives of the Study

The objectives of the present study were to investigate (i) the development of a *T. congolense* strain isolated in Matutuine District (Maputo Province, Mozambique) in South African goats and (ii) to determine the effect of nutrition on the development and pathogenicity of the parasite in goats by measuring specific haematological and biochemical parameters.

CHAPTER 2: LITERATURE REVIEW

2.1. The parasite (*Trypanosoma*)

Trypanosomes are protozoan parasites of the genus *Trypanosoma*, order Kinetoplastida.. They have as characteristic organelles, a kinetoplast and a flagellum. Typically, trypanosomes require a host and a vector to complete their life cycle. They multiply in blood, tissues or body fluids of a vertebrate host and, with the exception of *T. equiperdum* which is sexually transmitted, are transmitted by a haematophagous invertebrate vector (Connor, 1994).

Pathogenic trypanosomes of the genus *Trypanosoma* are divided into two sections: Salivaria, when their development in the vector and transmission is by saliva and, Stercoraria, when their development in the vector and transmission is by faecal contamination of the wound caused by a bite of the vector. Each section is subdivided into subgenera and species and, according to Hoare (1972), they can be classified as follows:

Salivaria

Subgenus *Duttonella*

Species *Trypanosoma vivax*

Subgenus *Nannomonas*

Species *Trypanosoma congolense* and *T. simiae*

Subgenus *Trypanozoon*

Species *Trypanosoma brucei brucei, T. b. rhodesiense, T. b. gambiense, T. evansi* and *T. equiperdum*

Subgenus *Pycnomonas*

Species *Trypanosoma suis*

Stercoraria

Subgenus *Schizotrypanum*

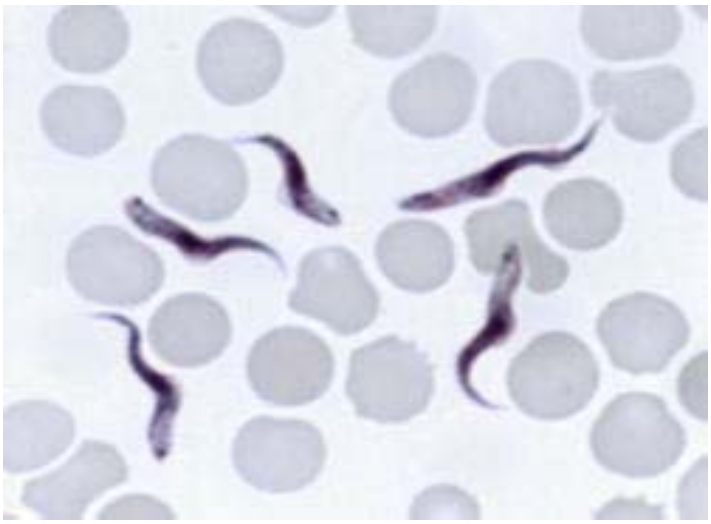
Species *Trypanosoma cruzi*

There are evidences that subgenus Nannomonas contains one additional species, *Trypanosoma godfreyi.* Among the three species of subgenus Nannomonas the *T. congolense* is subdivided into four subgroups: Savannah, Forest, Kilifi and Tsavo (Asbeck, *et al. ,*2000). Species and subgroups can be identified by isoenzyme analysis or DNA probes specific for the major repetitive DNA element of each group It is currently accepeted that *T. congolense* is subdivided into three types or groups: savannah, riverine/forest and Kenya coast. (Stevens and Brisse, 2004)

Trypanosoma brucei, *T. congolense*, *T. simiae* and *T. vivax* are the pathogenic species for domestic animals that generally are transmitted by tsetse flies (Hoare, 1972).

All species of salivarian trypanosomes have a wide range of domestic and wild mammalian hosts. In domestic animals, *T. brucei, T. evansi* and *T. congolense* infect cattle, sheep, goats, horses, donkeys, camels, pigs, dogs and cats. In addition to these hosts, *T. evansi* also infects water buffalo. *Trypanosoma vivax* does not infect dogs, cats, or pigs. *Trypanosoma equiperdum* is restricted to horses and donkeys. *Trypanosoma simiae* infects pigs, camels, sheep, and goats, but in the last two species the infectivity is variable. It is not infective for cattle (Losos, 1986).

Figure 4: Picture showing *Trypanosoma congolense*



Trypanosoma congolense (Figure 4*)* is a major cause of disease in livestock in Africa. It is currently regarded as a strict plasma parasite on the basis of its distribution in the mammalian host and characteristic pathological changes which develop in the tissues during infection (Luckins & Gray, 1979). *Trypanosoma congolense* is the smallest species and is between 8 and

20 μm in length. It has no free flagellum and the kinetoplast is usually subterminal and marginal Connor (1994).

Trypanosoma vivax is a member of the subgenus *Duttonella*. This species has a large terminal kinetoplast, a distinct free flagellum and an inconspicuous undulating membrane. *Trypanosoma vivax* is a large (18-26 μm long) monomorphic organism that is very active in wet blood smears. Cattle, sheep and goats are primarily affected. Although this organism is considered to be less pathogenic for cattle than *T. congolense*, it is nevertheless an important cause of African Animal Trypanosomosis in West Africa (www.vet.uga.edu/vpp/gray_book/FAD/aat.htm). According to Losos (1986), primary lesions due to *T. vivax* occur in the blood, blood vessels, lymphoid system and, to a limited degree, in some of the solid tissues, particularly the heart.

Trypanosoma brucei belongs to the subgenus *Trypanozoon*. The blood forms of *T. brucei* measure from 11 to 42 μm. *Trypanosoma brucei* is an extremely polymorphic trypanosome occurring as short, stumpy organisms without a flagellum, long slender organisms with a distinct flagellum, and intermediate forms that are usually flagellated. Horses, dogs, cats, camels and pigs are very susceptible to *T. brucei* infections. Infections in cattle, sheep, goats and sometimes pigs often result in mild or chronic infections (www.vet.uga.edu/vpp/gray_book/FAD/aat.htm).

2.2. The Vector

Trypanosomes are transmitted primarily by tsetse flies, belonging to the genus *Glossina,* in which they undergo a cyclical transmission. Tsetse flies are virtually restricted to Africa and some offshore islands such as Zanzibar (Phelps *et al*., 1990)

They infest about 10 million square kilometers and affect 38 countries, which makes African animal trypanosomosis a problem of truly continental magnitude (Connor & Van den Bossche, 2004)

The distribution of tsetse-transmitted bovine trypanosomosis in southern Africa is largely determined by the distribution of tsetse which consists of three major fly belts. The first belt, with *G. morsitans morsitans* and *G. pallidipes* as the main tsetse species, links tsetse foci in Malawi with the large fly belt common to eastern Zambia, Zimbabwe and Mozambique. The *G. morsitans centralis* fly belt covers western Zambia, parts of Angola, the Kwando River drainage in Namibia′s Eastern Caprivi district and the Okavango Delta in Botswana. Finally, a third fly

belt with the species *G. austeni* and *G. brevipalpis* covers part of Mozambique′s southernmost Matutuine district and north-eastern KwaZulu-Natal in South Africa **(**Connor & Van den Bossche, 2004**).**

2.3. Importance of trypanosomosis

Most governments in southern Africa recognize bovine trypanosomosis as a serious constraint to development and a serious threat to the agricultural sector. In Zambia, for example, bovine trypanosomosis is listed as a disease of national importance. In Mozambique, trypanosomosis is considered a serious threat to the cattle-restocking programme. In Zimbabwe, the financial implications for the communal and commercial farming sector of tsetse reinvading cleared areas are enormous and substantial efforts are made to maintain artificial barriers to tsetse reinvasion.

Recent surveys have been conducted to determine the impact of trypanosomosis on animal productivity. The results suggested that the largest and most consistent impact of trypanosomosis is on birth and mortality rates of both adult and young animals. The general implications are that the trypanosomosis (i) reduces calving rates by 1-12% in tolerant breeds of cattle and by 11-20% in susceptible breeds and (ii) increases calf mortality by 0-10% in tolerant breeds of cattle and by 10-20% in susceptible breeds. Two studies from The Gambia indicated that trypanosomosis reduces milk off-take from trypanotolerant cattle by 10-26%. Studies on trypanotolerant sheep and goats also indicated that the main impacts of trypanosomosis are on lambing (reduced by 4-38%) and kidding rates (reduced by 37%) (Swallow, 1999). Nevertheless, studies carried out in various African countries showed that the effect of trypanosomosis in small ruminants can differ from breed to breed (Adah *et al.*, 1993; Katunguka-Rwakishawa *et al.,* 1999; Faye *et al.,* 2005).

Trypanosomosis is also responsible indirectly for impaired productivity because of the failure of infected stock to utilize available food as efficiently as healthy animals, loss of carcasses quality and weight, loss of milk yield and, not infrequently, loss of productivity through abortion.

2.4. Trypanosomosis in Goats

Small ruminants play a crucial role in providing protein (meat and milk) whilst they also serve as a cash reserve and protection against agricultural crop failure. In terms of reproductive performance, small ruminants have an early puberty, short lambing intervals and non-seasonal breeding and less watering requirements.

For many years, trypanosomosis research focused mainly on the effects of the disease on cattle, with minimal concern for sheep and goats. It was then believed that sheep and goats were little affected by trypanosome infections (Stephen, 1970). However, it has now been demonstrated that great economic losses occur in small ruminants due to trypanosome infections (Kalu *et al*., 2001).

Recent studies had shown that tsetse flies preference to a host may differ depending on the prevailing ecological conditions of a region. For example, surveys carried out on the plateau of eastern Zambia where livestock is a potential host of tsetse, indicate that cattle are the preferred host and undergo the highest level of challenges than goats (Simukoko *et al*, 2007). Van den Bossche and de Deken (2002) have identified two factors that may explain why cattle are the most preferred by tsetse over the goats. One is the fact that cattle are spread more evenly in the area while goats distribution is restricted to the vicinity of Villages and, the other, is that individual cattle or groups of cattle herds produce odour plumes that makes them more attractive to tsetse flies. Similar observation of low level preference to goats in other tsetse infected areas was described by Ahmadu *et al*, (2002).

Susceptibility to an infection with trypanosomes can also differ between breeds and it is reflected in the development of anaemia, increased mortality rates, weight loss and reduced growth rates. In general, susceptibility is high in goats not been exposed previously to an infection with trypanosomes (Whitelaw *et al*., 1985).

2.5. Pathophysiology of *T. congolense*

In southern Africa, *T. congolense* is responsible for the most important form of African trypanosomosis in domestic mammals, including goats. The manifestation of an infection with *T. congolense* in goats varies according to the trypanosome strain and the breed, but is characterized generally by anaemia, fever, weakness, lameness, anorexia, weight loss, swollen lymph nodes and oedema in different regions of the body.

The pathological changes caused by the different trypanosomes depend further on whether the parasite is located intra or extravascularly. *Trypanosoma congolense* and to a lesser extent *T. vivax* are considered as strictly bloodstream parasites causing no direct tissue lesions. However,

splenomegaly subcutaneous or intramuscular haemorrhages (*T. vivax)* and serous fluid in pericardial, pleural and peritoneal cavities has been observed (Losos *et al.,* 1972*)*.

2.5.1 Anaemia

The course of the disease is characterized by a rapidly developing anaemia due to high levels of parasitaemia. In sheep and goats, animals that have a PCV below 15% as the result of an infection with trypanosomes rarely recover (Masiga *et al.,* 2002). Anaemia is considered a significant pathological feature with a multifactoral origin (Murray and Dexter, 1988; Katunguka *et al*. (1992). A dysfunctional immunological system, the secretion of toxins by trypanosomes and ineffective haematopoiesis contribute to the development of anaemia (Adrianarivo *et al*., 1994).

2.5.2. Metabolical and Biochemical changes

Infection with *T. congolense* in sheep is associated with a drop in plasma cholesterol and phospholipids concentration (Katunguka-Rwakishawa *et al.*, 1992). This decline commences when trypanosomes appear in the circulation. This implies that it may be a result of products released by trypanosomes as they undergo lysis or as the result of an uptake by trypanosomes. Other changes in biochemical parameters include changes in plasma proteins (Holmes, 1976) and serum iron (Tartour & Idris, 1973). A significant decline in plasma total protein and albumin was also reported by Katunguka-Rwakishaya *et al*. (1999). Akinbamijo *et al.* (1992) reported an increase in total protein during the last period of infection in West African Dwarf goats. This was attributed to a rise in the gamma globulin concentration. It has been suggested that the degree of changes in these parameters may be an indication of the severity of the infection.

2.5.3. Imunosuppression

The antibody responses of *T. congolense* or *T. vivax*-infected animals to non-trypanosomal antigen are often depressed. The exact mechanism involved in this trypanosome-induced immunosuppression is not clear (Holmes *et al*, 1974).

2.5.4. Tissue damage

Infections by all tsetse-transmitted trypanosome species begin with the injection or penetration of trypanosomes into the subcutaneous or submucosal connective tissue. At this site, multiplication may occur which may be associated with a local inflammatory reaction (Losos *et al*., 1986).

Conventionally, *T. vivax* and *T. congolense* are considered strictly plasma parasites confined to the circulatory system, whereas *T. brucei* has been considered a parasite of intercellular fluids, connective tissue, parenchymatous tissues and the fluids of the body cavities. However, *T. congolense* undergoes an early extravascular phase of development at the site of the tsetse bite and in the prefemoral lymph nodes (Abebe *et al.,* 1993).

Central nervous system (CNS) involvement has been reported in cattle with concurrent experimental infections with *T. congolense* and *T. vivax.* The pituitary gland, which is connected to the CNS through the hypothalamus, is one of the endocrine organs that is affected during a trypanosome infection (Abebe *et al*., 1993).

It has been described that *T. congolense* shows a preference for specific locations within the microvascular system; more parasites are found in skeletal muscle, brain and myocardium than in spleen and liver. The microvasculature of the pituitary gland may be another site of preferential localization of *T. congolense* (Abebe *et al.,* 1993).

The heart is an organ that is consistently damaged by all three pathogenic trypanosome species. Cattle deaths from trypanosomosis are frequently the result of congestive heart failure brought about by a combination of anaemia, myocarditis and circulatory disturbances. Myositis of the skeletal muscle is partly caused by emaciation, characteristic, of the disease (Urquhart, 1980).

2.5.5. The effect of Diet

The course of the disease may be aggravated by poor nutrition because it undermines the resistance of the host against infection. Conversely, sick animals will lose appetite due to certain physiological changes and voluntary food intake will reduce.

A range of stress factors such as pregnancy, parturition, lactation, poor nutrition, overwork and intercurrent disease increases susceptibility to trypanosomosis. Diet plays an important role in determining the severity of the pathological effects of an infection with *T. congolense*.

Ruminants show considerable variations in their susceptibility to parasitic infections and the nutritional status of the host has been suggested as one of the possible causes of this variation (Murray and Dexter, 1988 cited by Katunguka-Rwakishaya *et al.,* 1993). It has been observed in the field that the impact of trypanosomal infections becomes more severe during the dry season

when feed is in short supply (Agyemang *et al*., 1992 cited by Katunguka-Rwakishaya *et al.,* 1993).

Katunguka-Rwakishaya *et al*. (1999) also reported that adequate nutrition enhanced the ability of infected sheep to withstand the adverse effects of the infection by promoting body weight gains and moderating the severity of pathophysiological changes associated the disease. Faye *et al*. (2005), on the other hand, observed that the *T. congolense* parasitaemia tended to be high in West African Dwarf goats receiving supplemental feed, compared to infected goats kept on a basal diet. Furthermore, they found positive effects of the plane of nutrition on some biochemical parameters such as total plasma protein, plasma albumin, and plasma cholesterol and plasma urea.

CHAPTER 3 - MATERIALS AND METHODS

3.1. Experimental animals

Sixteen South African Boer goats (eight males and eight females) with an average weight of about 12 kg and about 8 months old were purchased from the University of Pretoria and transported to the Biological Research Center of the University of Pretoria (UPBRC).

3.2. Pre-experimental procedures

Upon arrival, all animals were tagged and weighed. A jugular blood sample was collected from each animal for haematological and biochemical analyses to establish base-line data.

Animals were dewormed 15 days before infection with trypanosomes using a combination of a broad spectrum anthelmintic, Albendazole 1.9 % (Valbazen^{R}), Pfizer AH, at a dose of 0.2 ml/kg body weight and a coccidiostatic Diclazuril 2.5% (Vexocan^{R}), Bayer AH, at 2 ml / 5 kg body weight.

3.3. Feeding and housing

The animals were housed individually in an isolation unit at the UPBRC for 75 days. They were divided into 4 groups of 4 goats each (two males and two females) as follows:

- Group 1: Uninfected on high energy (HE) diet (control group)
- Group 2: Uninfected on low energy (LE) diet (control group)
- Group 3: Infected on high energy (HE) diet
- Group 4: Infected on low energy (LE) diet

Feeding of specific diets commenced 2 weeks before infection. The animals in the high-energy (HE) group were given 1.2 kg of fresh matter (FM) per day and those in the low-energy (LE) group were given 0.71 Kg FM each.

The low-energy ration consisted of the following (per 100 kg):

Maize meal	25.2
Sunflower oil cake	39.4
Eragrostis	17.9
Molasses	10.7
Limestone	3.2
MonoCaP	1.4
Salt	1.1
Commercial premix	1.1
	100

This provided 9.72 MJ of metabolizable energy per kg and 0.16 kg of digestible protein per kg.

The high-energy ration consisted of the following (per 100 kg):

Maize meal	42.8
Sunflower oil cake	16.5
Eragrostis	18.9
Molasses	14.3
Limestone	2,9
MonoCaP	1.8
Salt	1.4
Commercial premix	1.4
	100

This provided 9.7 MJ of metabolizable energy per kg and 0.09 kg of digestible protein per kg.

Metabolizable energy for maintenance (MEm) for growing indigenous goats was taken as 489 kJ/kg. This was determined by regressing metabolizable energy intake against average daily gain (Luo *et al*., 2004). Goats on the high-energy diet were given 120% of their calculated MEm requirement, while goats on the low-energy diet were given 80% of their calculated MEm requirement. If a goat on the latter diet lost weight, the daily ration was increased by 10%. The percentage of digestible protein in the low-energy diet was relatively high, so as not to cause

catabolic breakdown of muscle and other tissue. The feed consisted of a milled mixture (not pelleted).

3.4. Trypanosomes and preparation of the inoculum

3.4.1. Study area

An isolate of *T. congolense* from local cattle breed in Matutuine district. (Maputo Province, Mozambique), stored in liquid nitrogen, was used in the present study. Matutuíne is one of the seven districts that comprise the Maputo Province. It is bordered by Republic of South Africa in the South, the Indian Ocean in the East, the Kingdom of Swaziland and Boane District in west and the city of Maputo in north (Sigauque *et a*l. 2000).

Climate – the climate of Matutuíne is subtropical with two main seasons: a hot and rainy season which covers the month of November up to March with temperatures varying from 18˚ C to 39˚C and a cool dry season from April to October with temperatures ranging from 12˚ C to 26˚C. The average annual rainfall is about 750 mm.

Vegetation – the vegetation is of the savannah type. The predominant species are of the type *Acacia* spp, *Terminalia sericia*, *Dichartachys cinera*, *Eulia natalensis*, *Diopyrus mespilliformes* and *Albizia* spp.

3.4.2. Isolation of the trypanosomes

An isolate of *T. congolense* from Matutuine (Maputo Province, Mozambique), stored in liquid nitrogen, was used. The thawed stabilate (0.3 ml) was injected intraperitoneally into 10 immunosuppressed mice. Mice to be inoculated with trypanosomes were immunosuppressed 24h beforehand by intraperitioneal injection with 300mg/kg cyclophosphamide (Endoxan®, Asta Medica, Cambridge, UK). The mice were housed in standard cages at the research facilities of the Faculty of Veterinary Medicine, Eduardo Mondlane University, and Maputo. Afterwards they were transported to the UPBRC and held in isolation. Blood from the tail of each infected mouse was examined to determine the parasitaemia. In the present experiment the *T. congolense* subgroup typing acoording to Stevens and Brisse (2004) was not performed.

3.4.3. Infection of goats

Parasitaemic mice were anaesthetised by isofluroane inhalation and were exsanguinated into tubes containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. An estimate of the

parasitemia (for scale see Table 1) was made on the pooled blood sample, which was then diluted with phosphate-buffered saline (PBS) pH 7.4 to give 10^4 trypanosomes in 2 ml of the PBS/blood mixture.

Eight goats were inoculated subcutaneously (at one site on the left flank) with 0.5 ml of infected blood.

3.5. Clinical observations and collection of blood samples

3.5.1. Clinical examination

The infected animals were monitored daily. Rectal temperature was taken, colour of mucous membranes was evaluated by means of the FAMACHA chart and habitus and appetite were recorded. The control groups were monitored twice a week.
All goats were weighed weekly, using an electronic scale.

3.5.2. Haematology

A total of 0.5 milliliters of jugular blood was collected into paediatric tubes containing EDTA as anticoagulant three times a week (Monday, Wednesday and Friday) from each animal of the infected groups and on Monday and Friday from each animal of the control groups.

The packed cell volume (PCV), haemoglobin level, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined through standard procedures in the Clinical Pathology Laboratory, Department of Companion Animal Clinical Studies

3.5.3. Goat parasitaemia

From all infected animals, ear vein blood was collected daily into microhematocrit tubes. The blood was centrifuged, the PCV measured and the parasitaemia quantified by examination of the buffy coat as described by Murray *et al.* (1983).

Table 1: Estimation of the parasitaemia according to the method of Murray *et al.* (1983)

Number of trypanosomes per field (magnification x 250)	Score	Estimated parasitaemia (Tryps/ml blood)
>100	6+	> $5x10^6$
>10	5+	> $5x10^5$
1-10	4+	10^4-$5x10^5$
1 per 2 fields –1 per 10 fields	3+	$5x10^3$-$5x10^4$
1-10 per slide	2+	10^3-10^4
1 per slide	1+	10^2-10^3

Animals with a PCV ≤ 14 % were euthanized by administration of an overdose of pentobarbitone sodium into the jugular vein. The carcasses were submitted for *post mortem* examination at the Pathology Section. Similarly, animals that died during the experimental procedures were also subjected to a *post mortem* examination.

3.5.4. Biochemical profile

To determine the effect of the trypanosome infection on the liver, kidney, heart and skeletal muscles (Table 2) of the various experimental animals, 5 millilitres of jugular blood was collected into a plain pediatric tube on Mondays. This blood sample was used to determine the total serum protein level (TSP), the total bilirubin level (BIL-T), the serum aspartate aminotransferase level (AST), the inorganic phosphate level (SIP), the serum gama-glutamyltransferase level (GGT), serum urea, creatinin levels and the creatin kinase level (CK). Moreover, one milliliter of blood was collected, by jugular venipuncture, into tubes containing fluoride as anticoagulant for the determination of glucose levels.

Table 2: Various biochemical parameters measured and their biological interpretation.

Parameter	Indicator of:
TSP	Dehydration, malnutrition and malabsorption, loss of protein.
BIL-T	Haemolysis, liver damage
AST	Liver, skeletal and heart muscle affected
SIP	Kidney affections
GGT	Liver damage
Urea	Kidney function when (↑), hepatic function when (↓)
Creatinin	Kidney function
CK	Muscle damage
Glucose	Hyperglycaemia, hypoglycaemia, malabsorption and severe malnutrition

Determination of the biochemical profile was conducted using standard procedures of the Clinical Pathology Laboratory, Department of Companion Animal Clinical Studies (University of Pretoria) using an automated system (Next Clinical Chemistry System – Alfa Wasserman Bayer SA*).*

Table 3: Weekly schedule of specimen collection.

	Infected			Control		
	EDTA	Plain	Fluoride	EDTA	Plain	Fluoride
Day 1	0,5 ml	0,5 ml	1 ml	0,5 ml	0,5 ml	1 ml
Day 2		-	-	-	-	-
Day 3	0,5 ml	-	-	-	-	-
Day 4		-	-	-	-	-
Day 5	0,5 ml	-	-	0,5	-	-
Day 6		-	-	-	-	-
Day 7		-	-	-	-	-

At the end of the experiment (a maximum of two months after infection), the infected animals were euthanized as described above. The carcasses were submitted for *post mortem* examination at the Pathology Section, Department of Paraclinical Sciences. The control animals were slaughtered.

3.6. Statistical Analyses

For analytical purposes, the observation period for each of the experimental groups was divided into two periods *i.e.* the pre-infection period from day –13 to 7 (day 0 being the day of infection) and the chronic infection period from day 22 to 56. The parametric variables measured during the two periods of study were compared using MANOVA statistical design with repeated measurements groups. The statistical analyses were carried out using SPSS 13.0

CHAPTER 4 – RESULTS

4.1. Rectal temperature

During the pre-patemt period only the effect of the infection with *T. congolense* was borderline significant (p=0.043) (Figure 5 and Table 4). During the chronic phase of infection, on the other hand, there was no significant effect of neither the nutritional status nor infection on the rectal temperature.

Figure 5: Rectal temperature of animals belonging to the four experimental groups.

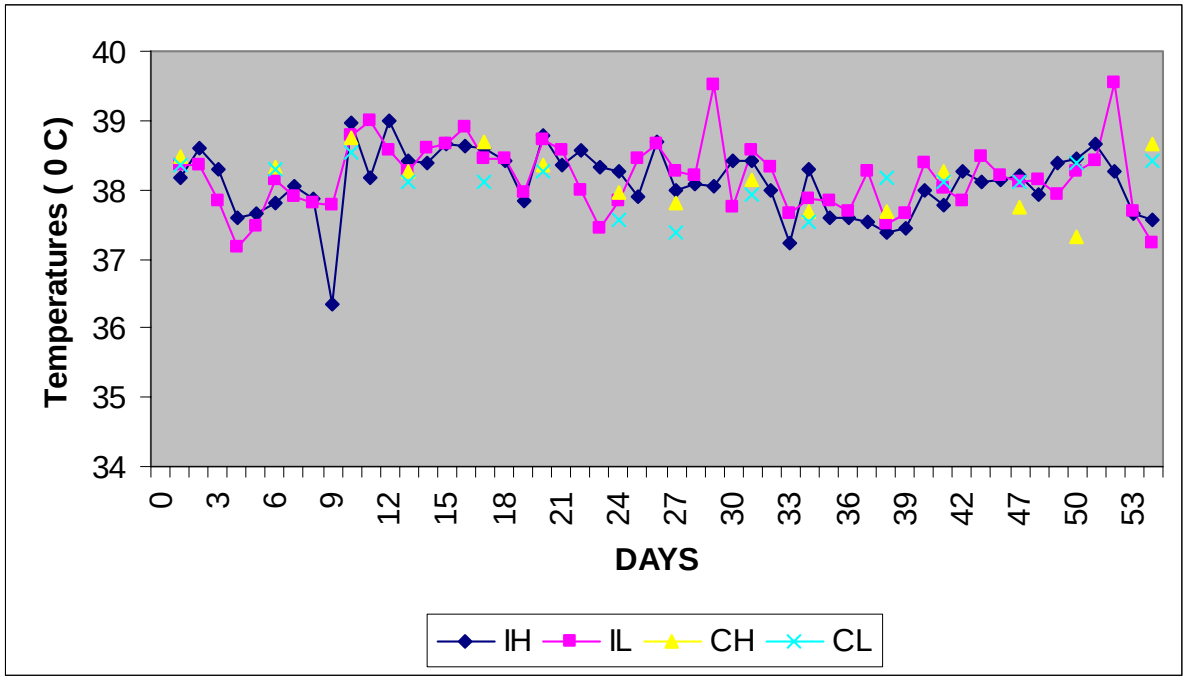


Table 4: Average Temperature (in °C ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) or low (IL) energy diet and non-infected control animals also subjected to a high (CH) or low (CL) energy diet during the pre-patent (day –13 -7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	38 ± 0.5	38 ± 0.5	38 ± 0.3	38 ± 0.3
Chronic	38 ± 0.8	38 ± 0.7	38 ± 0.7	38 ± 0.6

4.2. Parasitaemia

The first trypanosomes in the peripheral circulation were detected on day 7 post-infection by buffy- coat examination (Table 5). One animal belonging to the IL group had not developed a parasitaemia by day 19 and was reinfected. Parasitaemia was detected three days later. After becoming positive, the levels of parasitaemia fluctuated considerably. The parasitaemia increased in both infected groups to reach the first peak by day 11 in the IH and by day 9 in the IL group (Table 5). The mean prepatent period in the high energy infected (IH) and low energy infected (IL) groups was 7.5 days.

Table 5: Variations in the parasitaemia of each individual animal (estimated using the Murray scale) during infection.

Parasitemia of group									
Date	**days**	**IH-1**	**IH-2**	**IH-3**	**IH-4**	**IL-1**	**IL-2**	**IL-3**	**IL-4**
27-Jan	0	neg	neg	neg	neg	neg	neg	neg	neg
28-Jan	1	neg	neg	neg	neg	neg	neg	neg	neg
29-Jan	2	neg	neg	neg	neg	neg	neg	neg	neg
30-Jan	3	neg	neg	neg	neg	neg	neg	neg	neg
31-Jan	4	neg	neg	neg	neg	neg	neg	neg	neg
1-Feb	5	neg	neg	d/neg	neg	neg	neg	neg	neg
2-Feb	6	neg	neg	neg	neg	neg	neg	neg	neg
3-Feb	7	d+1	neg	neg	2+	1+	neg	neg	neg
4-Feb	8	4+	2+	2+	5+	5+	4+	1+	neg
5-Feb	9	5+	5+	2+	5+	5+	6+	4+	neg
6-Feb	10	6+	6+	5+	5+	5+	6+	5+	neg
7-Feb	11	5+	5+	5+	5+	5+	5+	5+	neg
8-Feb	12	2+	1+	2+	2+	2+	2+	1+	neg
9-Feb	13	4+	4+	4+	5+	4+	4+	1+	neg
10-Feb	14	5+	2+	2+	3+	5+	5+	4+	neg
11-Feb	15	5+	4+	6+	4+	6+	5+	6+	neg
12-Feb	16	4+	4+	4+	6+	6+	4+	4+	neg
13-Feb	17	4+	4+	2+	6+	4+	3+	4+	neg
14-Feb	18	4+	4+	6+	1+	4+	1+	2+	reinfected
15-Feb	19	4+	4+	4+	1+	2+	1+	4+	neg
16-Feb	20	4+	4+	4+	2+	3+	1+	2+	neg
17-Feb	21	5+	5+	5+	4+	4+	4+	1+	2+
18-Feb	22	5+	1+	4+	5+	4+	4+	1+	2+
19-Feb	23	5+	2+	5+	4+	5+	1+	2+	4+
20-Feb	24	3+	3+	4+	5+	4+	1+	2+	4+
21-Feb	25	4+	3+	4+	4+	4+	4+	4+	1+
22-Feb	26	2+	4+	5+	4+	5+	5+	4+	4+
23-Feb	27	4+	4+	1+	4+	4+	3+	3+	5+
23-Feb	28	4+	1+	1+	4+	4+	3+	3+	5+
24-Feb	29	4+	1+	4+	4+	3+	2+	4+	3+
25-Feb	30	2+	2+	2+	1+	3+	1+	2+	***2+***
26-Feb	31	5+	4+	3+	3+	5+	2+	4+	2+
27-Feb	32	2+	2+	3+	4+	1+	1+	3+	5+
28-Feb	33	4+	5+	1+	4+	1+	3+	4+	5+
1-Mar	34	3+	1+	2+	5+	1+	4+	5+	5+
2-Mar	35	3+	4+	1+	2+	***2+***	2+	3+	4+
3-Mar	36	2+	4+	3+	3+	5+	5+	5+	6+
4-Mar	37	4+	2+	3+	5+	5+	4+	1+	5+
5-Mar	38	2+	1+	4+	6+	4+	6+	3+	3+
6-Mar	39	2+	2+	4+	4+	4+	1+	2+	2+
7-Mar	40	3+	2+	4+	1+	5+	4+	4+	3+
8-Mar	41	3+	2+	5+	4+	2+	5+	3+	3+
9-Mar	42	5+	2+	5+	4+	1+	5+	2+	5+
10-Mar	43	1+	4+	4+	5+	3+	5+	3+	6+
11-Mar	44	4+	4+	5+	4+	3+	2+	1+	4+
12-Mar	45	4+	6+	4+	4+	5+	5+	3+	6+
13-Mar	46	6+	3+	5+	4+	5+	5+	5+	5+
14-Mar	47	3+	1+	4+	2+	5+	5+	5+	5+
15-Mar	48	6+	2+	5+	4+	5+	3+	4+	4+
16-Mar	49	5+	4+	4+	4+	5+	4+	4+	3+
17-Mar	50	Died	4+	5+	3+	5+	4+	4+	3+
18-Mar	51	Died	6+	3+	5+	5+	4+	4+	2+
19-Mar	52	Died	6+	6+	4+	1+	4+	3+	4+
20-Mar	53	Died	2+	4+	4+	1+	3+	4+	4+
21-Mar	54	Died	1+	3+	4+	3+	4+	5+	5+
22-Mar	55	Died	4+	3+	2+	3+	3+	3+	4+
23-Mar	56	Died	3+	3+	2+	5+	5+	4+	1+
24-Mar	57	Died	2+	2+	1+	4+	3+	3+	2+

4.3 Body weights

Throughout the observation period, infection and/or diet had no significant effects on body weight ($p>0.05$) (Table 6).

Table 6: Average body weights (in kg ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and not infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-infection (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	11 ± 1.5	9 ± 1.7	11 ±2.4	11 ± 2.7
Chronic	11 ± 1.5	9 ± 1.8	11 ± 2.1	11 ± 2.1

4.4. Haematological parameters

4.4.1. Packed-cell volume

During the pre-patent phase, the PCV values of both groups (control and infected) did not differ significantly ($p>0.05$). However, during the chronic phase of infection differences became significant ($p<0.01$). Moreover, during chronic phase, the PCV values of the CH group were significantly higher compared to the PCV values of both infected groups (IH ($p=0.02$) and IL ($p=0.001$)) (Figure 6 and Table 7).

Figure 6: Mean PCV of animals belonging to the four experimental groups.

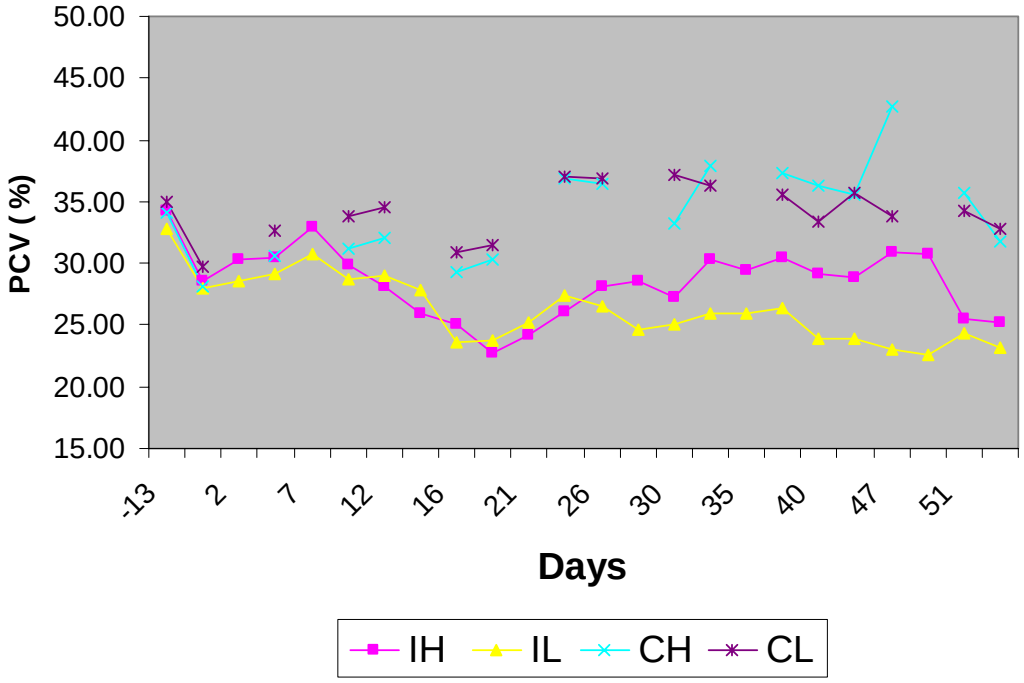


Table 7: Mean PCV (in % ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	31.1 ± 2.94	29.9 ± 1.92	30.2 ±4.04	32.0 ± 0.64
Chronic	29.5^{ad} ± 1.9	24.8^{c} ± +2.2	38.3^{b} ± 5.2	35.0^{bd} ± 2.7

Note: a-c Means within a row with no common superscripts are significantly different ($p<0.05$)

4.4.2. Haemoglobin

The effects of infection and/or diet on the haemoglobin level were not significant ($p>0.05$) (Table 8).

Table 8: Mean haemoglobin (in g/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	100.5 ± 10.7	95.5 ± 4.4	97.6 ± 10.9	99.8 ± 5.9
Chronic	90.2 ± 6.4	125.1 ± 96.1	115 .8 ± 14.4	104.5 ± 5.06

4.4.3. Red blood cell counts

During the pre-patent phase, the RBC counts of control and infected groups were not significantly different ($p>0.05$). During the chronic phase the RBC counts of the CH and IH groups and of the CL and IL groups were significantly different ($p=0.03$ and $p=0.001$, respectively) (Figure 7 and Table 9).

Figure 7: Mean red blood cell counts of animals belonging to the four experimental groups.

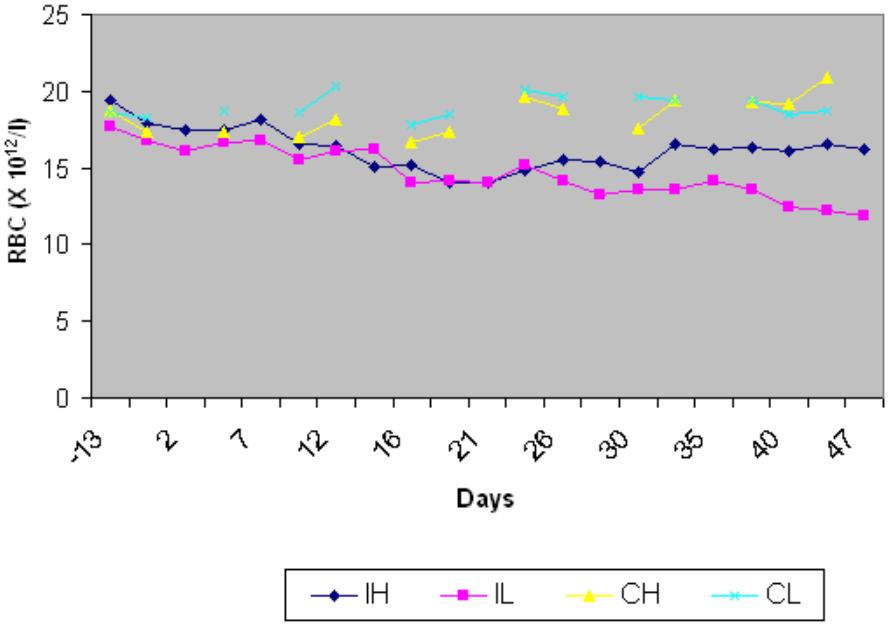


Table 9: Mean red blood cell count (x10^{12} /L ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	18.2 ± 1.5	17 ± 1.0	17.4 ± 2.1	18.4 ± 1.2
Chronic	16^{ac} ± 1.1	13.2^{a} ± 0.7	19.5^{b} ± 2.1	19^{bc} ± 1.2

Note: a-b Means within a row with no common superscripts are significantly different ($p<0.05$)

4.4.4. Mean Corpuscular Volume (MCV)

During both observation periods, the MCV did not differ significantly ($p<0.05$) between experimental groups (Table 10).

Table 10: Average Mean Corpuscular Volume (in fl ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	17.0 ± 0.7	17.5 ± 0.2	17.2 ± 0.2	17.2 ± 0.3
Chronic	18.4 ± 0.6	18.7 ± 0.9	19.5 ± 1.0	18 ± 3.6

4.4.5. Mean Corpuscular Haemoglobin (MCH)

Throughout the observation period the effects of infection and diet on the mean corpuscular haemoglobin level were not significant ($p>0.05$) (Table 11).

Table 11: Average Mean Corpuscular Haemoglobin concentration (in pc ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	5.4 ± 0.2	5.6 ± 0.1	5.5 ± 0.7	5.4 ± 0.1
Chronic	5.6 ± 0.1	5.8 ± 0.1	5.9 ± 0.2	5.4 ± 0.1

4.4.6. Mean Corpuscular Haemoglobin Concentration (MCHC)

During the pre-patent phase the MCHC showed no significant difference between groups ($p>0.05$). However, during chronic phase, the difference between control and infected groups was significant ($p<0.05$). The MCHC of the CL group was significantly lower compared to the MCHC of the IL group ($p=0.02$) (Figure 8 and Table 12).

Figure 8: Mean MCHC of animals belonging to the four experimental groups.

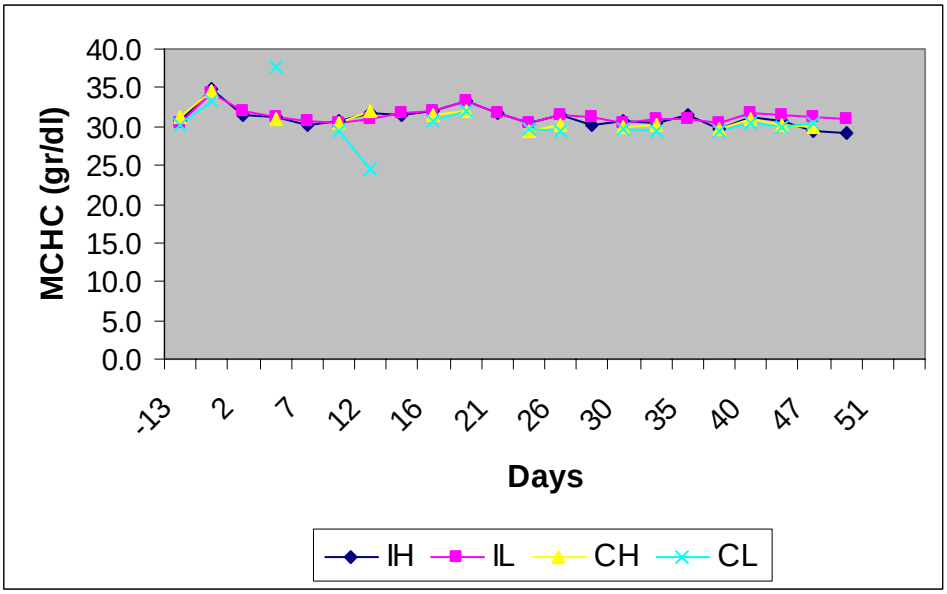


Table 12: Average Mean Corpuscular Haemoglobin Concentration (in g/dl ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	32.3 ± 0.5	32.0 ± 0.8	32.4 ± 0.7	33.7 ± 4.5
Chronic	30.5 ± 0.3	31.3[a] ± 0.4	30.2 ± 0.3	29.8[b] ± 0.9

Note: a-b Means within a row with no common superscripts are significantly different (p<0,05)

4.6. Biochemical parameters

4.6.1. Albumin

Throughout the observation period the effects of infection and diet on the albumin level were not significant (p>0.05) (Table 13).

Table 13: Average albumin level (in g/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and not infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	25.3 ± 3.2	25.8 ± 1.9	25 ± 3.6	24 ± 2.2
Chronic	23 ± 2.3	23.6 ± 1.6	24.8 ± 0.4	22.7 ± 1.72

4.6.2. Aspartate aminotransferase

Throughout the observation period the effects infection and diet on the aspartate aminotransferase level were not significant ($p>0.05$) (Table 14).

Table 14: Average aspartate aminotransferase level (in U/liter ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	97.3 ± 43.5	87.1 ± 24.7	84.1 ± 15.7	72 ± 8.3
Chronic	71.4 ± 6.7	76.1 ± 14.1	76.2 ± 17.1	61.3 ± 5.1

4.6.3. Total Bilirubin

During the pre-patent phase, the effects of infection and diet on the total bilirubin level were not significant ($p>0.05$) but during the chronic phase differences became significant between the infected and the control groups ($p<0.05$) (Figure 9 and Table 15).

Figure 9: Mean total bilirubin of animals belonging to the four experimental groups.

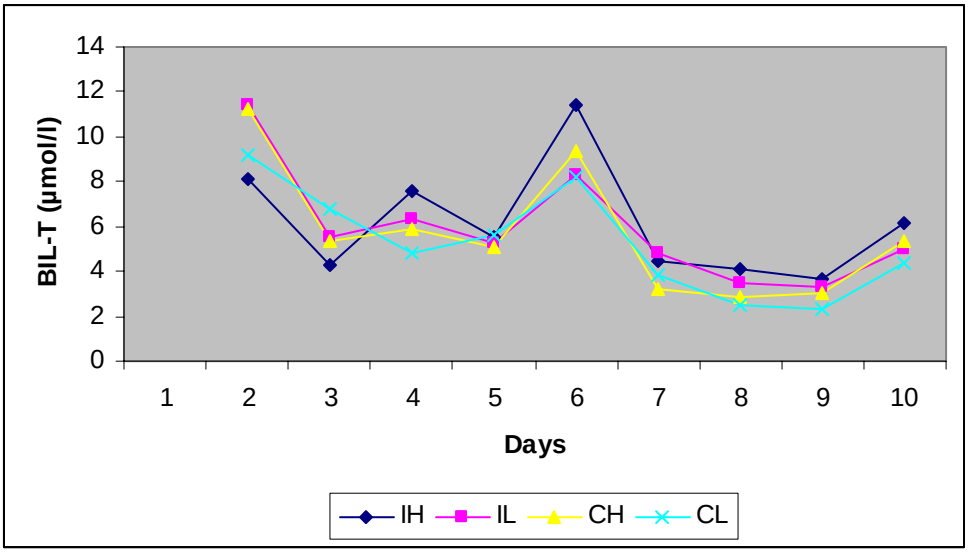


Table 15: Mean total bilirubin level (in µmol/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	6.6 ± 0.8	7.7 ± 1.2	6.9 ± 2.0	6.9 ± 0.8
Chronic	4.3 ± 0.8	4.0 ± 0.3	3.6 ± 0.4	3.2 ± 0.8

4.6.4. Creatine Kinase

The creatine kinase values, during the pre-patent and the chronic phase did not differ significantly as a result of infection and/or diet ($p>0.05$) (Table 16).

Table 16: Average CK (in U/liter ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	169.5 ± 42.6	167.7 ± 34.6	172 ± 11.5	159.6 ± 48.5
Chronic	107 ± 39.2	123.2 ± 40.2	175.1 ± 57.8	134.9 ± 48.7

4.6.5. **Urea**

During the pre patent phase the urea value differed significantly among groups ($p<0.05$). The IH group differs significantly from the IL group ($p=0.02$) and the CH group differed significantly from IL group ($p=0.01$). However, during the chronic phase, those differences disappeared (Figure 10 and Table 17).

Figure 10: Mean urea levels of animals belonging to the four experimental groups.

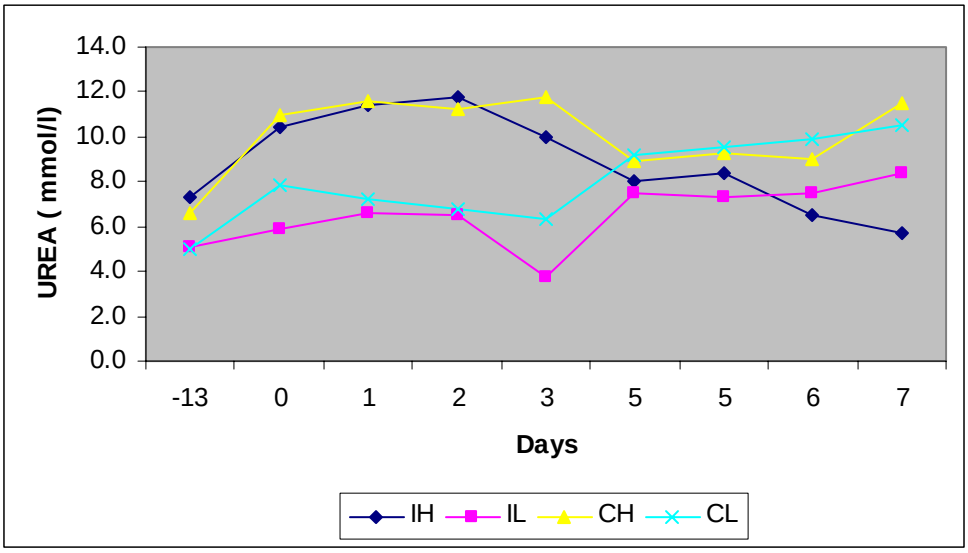


Table 17: Mean urea (in mmol/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	9.7^{a} ± 2.4	5.6^{b} ± 0.4	9.9^{ac} ± 1.5	6.7^{a} ± 0.7
Chronic	7.2 ± 1.7	7.9 ± 1.0	9.6 ± 1.2	9.7 ± 0.5

Note: a-b Means within a row with no comon superscrips are significantly different ($p<0.05$)

4.6.6. Creatinin

Throughout the observation period the effects of infection and diet on the creatinin level were not significant ($p>0.05$) (Table 18).

Table 18: Mean creatinin (in µmol/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	60.3 ± 10.7	64.1 ± 8.9	58.4 ± 4.3	64.8 ± 5.1
Chronic	70.4 ± 16.6	64.7 ± 9.2	68.6 ± 3.7	66.8 ± 5.3

4.6.7. Gama-glutamyltransferase

Throughout the observation period the effects of infection and diet on the gama-glutamyltransferase level were not significant ($p>0.05$) (Table 19).

Table 19: Mean GGT (in U/liter ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	32.2 ± 1.0	30.8 ± 6.1	34.2 ± 1.6	31.4 ± 2.1
Chronic	32.8 ± 2.8	36.3 ± 4.0	35.8 ± 1.2	37.8 ± 3.9

4.6.8. Globulin

Throughout the observation period the effects of infection and diet on the globulin level were not significant ($p>0.05$) (Table 20).

Table 20: Mean globulin level (in g/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	42.8 ± 6.0	40.3 ± 4.9	44.7 ± 9.5	42.8 ± 5.3
Chronic	35.2 ± 6.6	34.7 ± 2.6	34.0 ± 2.9	34.7 ± 4.8

4.6.9. Glucose

Throughout the observation period the effects of infection and diet on the glucose level were not significant ($p>0.05$) (Table 21).

Table 21: Mean glucose level (in mmol/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	3.0 ± 0.19	3.0 ± 0.17	2.9 ± 0.04	3.0 ± 0.26
Chronic	2.7 ± 0.27	3.0 ± 0.13	2.9 ± 0.41	2.9 ± 0.23

4.6.10. Inorganic Phosphate

Throughout the observation period the effects of infection and diet on the inorganic phosphate level were not significant ($p>0.05$) (Table 22).

Table 22: Mean inorganic phosphate level (in mmol/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phase of the observation period.

	IH	IL	CH	CL
Pre-patent	3.6 ± 0.2	3.1 ± 0.4	3.6 ± 0.4	3.3 ± 0.5
Chronic	2.7 ± 0.59	2.6 ± 0.34	2.8 ± 0.19	2.8 ± 0.56

4.6.11. Total Serum Protein

Throughout the observation period the effects of infection and diet on the total serum protein level were not significant ($p>0.05$) (Table 23).

Table 23: Average total serum protein level (in g/l ± s.d.) of animals (n=4 in each group) infected with *T. congolense* subjected to a high (IH) and low (IL) energy diet and non-infected control animals also subjected to a high (CH) and low (CL) energy diet during the pre-patent (day -13-7) and chronic (day 22-56) phases of the observation period.

	IH	IL	CH	CL
Pre-patent	61.8 ± 9.9	62.4 ± 4.7	61.7 ± 3.9	61.4 ± 7.0
Chronic	61 ± 8.1	62 ± 6.3	63 ± 5.8	62 ± 6.8

CHAPTER 5 – DISCUSSION

The study presented in this thesis was conducted to evaluate the effect of different nutritional levels on the development and pathogenicity of a *T. congolense* infection in goats by monitoring haematological and biochemical parameters.

For analytical purposes, the observation period was divided into two periods *i.e.* the pre-patent phase from day -13 to 7 and the phase when the infection was well established (or chronic phase) from day 22 to 56.

5.1. Temperature, Parasitemia and Body Weight

As could be expected, infection with *T. congolense* resulted in fever in both experimental groups. However, once the infection was established, no fever was observed in the infected groups, with rectal temperatures being similar to the rectal temperatures of uninfected animals.

This observation suggests a low impact of the trypanosome infection in all experimental animals independent of the level of nutrition. Although the parasitaemia showed a fluctuating pattern throughout the infection, again no significant differences were observed between the infected groups (IH and IL) in the chronic phase of the infection.

This suggests that the nutritional plane did not influence the level of parasitemia. This observation is in agreement with the findings of Osaer *et al*. (1998) studying the effect of *T. congolense* infections and nutritional supplements on the establishment and outcome of pregnancy in trypanotolerant Djallonké ewes, and Osaer *et al*. (1999) investigating effects of an infection with *T. congolense* and diet on puberty in Djallonké ewes.

In contrast, Faye *et al*. (2005) observed that the *Trypanosoma* parasitaemia tended to be higher in animals receiving supplemental feed than in animals kept on a basal diet. Although the parasitaemia differed little between the experimental groups, the first peak of parasitaemia occurred earlier in infected goats kept on a low-energy diet. This observation is in accordance with the observation of Hecker *et al.* (1991) who found that feed supplementation of sheep

exposed to natural fly challenge delayed the onset of parasitaemia, but did not affect the prevalence rates. On the other hand,

Katunguka-Rwakishaya *et al.* (1995) found that the time to reach the first peak of parasitaemia was affected by the nutritional level of Scottish Blackface Sheep infected with *T. congolense.* Indeed, according to their observations, the parasitaemia increased in both experimental groups to reach the first peak by day 23 after infection in the high-energy group and by day 34 after infection in the low-energy group.

The mean prepatent period in both infected groups was 7.5 days. A similar duration has been reported in the small East African goat breed infected with *T. congolense* (Mutayoba *et al.*, 1989), where infected goats became parasitaemia within 5-8 days after infection. In contrast, Katunguka-Rwakishaya *et al.* (1995) observed a prepatent period of 14.8 days (range 12-16 days) and 17.8 days (range 12-26) in Scottish Blackface sheep infected with *T. congolense* and kept on high-energy and low-energy diets, respectively.

Throughout the observation period, infection with *T. congolense* and diet had no significant effects on body weight. In contrast, Katugunka-Rwakishaya *et al.* (1999) observed that infection and energy intake had significant effects on body-weight gain. Infected animals on a low-energy intake showed greater retardation in growth than animals on a high-energy intake. The reason for the absence of significant differences in our study probably is due to low pathogenicity of the local *T. congolense* strain having minimal effect on the health of the infected animal under the experimental conditions.

Specht (1982) studied the effect of simultaneous infections with trypanosomes (*T. vivax* and *T. congolense*) and gastrointestinal nematodes on productivity of local breeds of goats and sheep in Mozambique. She was able to show that animals receiving treatment against worm parasites gained more weight than animals that only received treatment against trypanosomes. Thus the double infections with trypanosome impaired the weight gain of goat and sheep. Up to now there no others studies in Mozambique showing the impact of trypanosomes on goat production and productivity.

There is another important parameter apart from nutritional factor, which may influence the pathogenicity of trypanosome infection that should be considered. That is the subgroups of *T.*

congolense (Stevens and Brisse, 2004). However, in the present study subgroups were not dealt with. Only *T. congolense* species were considered. Data from recent survey in Matutuine area indicated that 95% of *T. congolense* isolates belongs to *savannah* subgroup, which may strongly suggest a high probability that the subgroup of this study could be *savannah* (Neves, 2007 personal comunication).

5.2. Haematological parameters

The results obtained during the observation period suggest that there was interaction between dietary plane and *Trypanosoma* infection resulting in a decrease in RBC and PCV in both infected group (IH and IL). This decrease was more severe in the IL group. In the chronic phase, on the other hand, there was a significant difference between the control and infected groups with regard to the PCV, RBC and MCHC as a result of infection. This suggests that infection affects PCV, RBC and MCHC and the effect of infection was minimized by the high dietary supplementation

Similar observations were made by Osaer *et al.* (1999) investigating the effects of an infection with *T. congolense* and diet on puberty, age at first lambing and haematological changes in Djallonké ewes. Here also diet supplementation resulted temporarily in a better haematopoietic response following trypanosome infection.

Results from our study suggest that adequate nutrition minimizes the effect of infection. Similarly, a better control of the anaemia caused by natural trypanosome infection was observed in N'Dama cattle kept on a high plane of nutrition (Agyemang *et al*., 1990).

Throughout the observation period the mean MCHC level remained within normal values. According to Coles (1986**),** normal physiological values for MCHC are 28-34 (32) g/dl. Hence, this suggests that in both infected groups there was a normochromic anaemia.

The absence of change of MCV suggests that the anaemia is normocytic. There seems to be a relation between the diet and infection, because the anaemia was lower in animals kept on a low-energy diet.

Different studies came up with different conclusions related to effect of the of nutrition plane on the development of anaemia. In the present study, anaemia was normocytic and normochromic and was more explicit in the groups fed a low-energy diet. Similar observations were made by Katunguka-Rwakishaya *el al.* (1995) who observed that infected sheep fed on high-energy food, developed a less severe anaemia than the animals on low-energy intake.

In contrast, Osaer *et al.* (1998) observed that a high level of dietary supplementation did not ameliorate the degree of anaemia following trypanosome infection and there was also evidence of a better erythropoietic response.

Haemolysis is by far the most important mechanism causing anaemia in *Trypanosoma*-infected susceptible animals (Anosa, 1988). This is attributed to insufficient compensation through erytropoiesis of the destruction of RBC, especially in the early stage of infection. Once the infection persists, the destruction and replacement of RBC becomes more balanced.

In contrast, Osaer *et al.* (1999) observed a reduction in RBC in the beginning of the infection. In response to the loss of red cells, increased numbers of immature cells were released from the bone marrow, resulting in a macrocytic anaemia.

A macrocytic, hypochromic anaemia with reduced PCV levels in sheep fed roughage with lower energy and protein was also found in *T. congolense*-infected sheep (Wassink *et al*., 1997).

5.3. Biochemical parameters

No significant changes were observed in plasma levels of albumin, AST creatinin, CK, GGT, globulin, glucose, SIP and TSP, as a result of diet and/or infection with *T. congolense.* All values were within the normal range (Tennant, 1997; Kaneko *et al*., 1997; Braun *et al.* cited by Doxey ,1983). Those normal values suggest that a function of the various organs was little affected by the infection and nutritional level. The pathological changes caused by trypanosomes depend on whether the parasite is located intra- or extravascularly. *Trypanosoma congolense* is an intravascular parasite and is considered to cause few direct tissue lesions (Losos & Ikede, 1972). However, it can induce changes in the endothelium of capillaries, and so indirectly damage adjacent tissues. One vital organ that is consistently damaged by this trypanosome species is the

heart. Cattle deaths from trypanosomosis are frequently the result of congestive heart failure brought about by a combination of anaemia, myocarditis and circulatory disturbances. Myositis of skeletal muscle is partly caused of the emaciation characteristic of the disease (Urquhart, 1980).

The differences found in the urea level in the present study could not be explained. Under normal circumstances, a high plasma urea concentration is associated with excessive breakdown of protein. However, Blowey *et al.* (1973) and Al-Rabat *et al.* (1971) (cited by Katunguka-Rwakashaya *et al.*, 1999) suggested that high-energy intake facilitates microbial protein synthesis in the rumen and thus reduces ammonia and blood urea concentration levels. However, low-energy intake tends to increase protein degradation in the rumen, resulting in production of large quantities of ammonia, which is converted into urea by liver and excreted.

In conclusion, the results of this study suggest that the *T. congolense* strain used in this experiment is mild, with minimal effect on the health of the infected goats. The outcomes of our study also suggest that the effect of plane of nutrition in such infections with low pathogenicity has minimal impact on haematological and biochemical parameters.

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