THE INFLUENCE OF GRADED DIETARY ENERGY ON DYNAMICS OF *Trypanosoma congolense* INFECTION IN WEST AFRICAN DWARF GOATS

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A THESIS IN THE DEPARTMENT OF VETERINARY MEDICINE SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF
DOCTOR OF PHIOSOPHY

OF THE

UINIVERSITY OF IBADAN

NIGERIA

2011

ABSTRACT

Malnutrition increases goats' susceptibility to diseases thereby hindering their profitable production. Low calorie diet and infection have impact on health and survival of livestock. The influence of Graded Dietary Energy (GDE) on dynamics of *Trypanosoma congolense* (*Tc*) infection in goats is not fully elucidated. The influence of GDE levels on *Tc* infection in West African Dwarf (WAD) goats was therefore investigated.

A preliminary survey for trypanosomes involving 675 goats was conducted during the dry and rainy seasons in Ogbomoso, Oyo State using standard techniques. Fifty four goats were randomly divided into three dietary groups A, B and C of 18 animals each and fed with 2426.7 or 2548.6 or 2670.4 kcal/kg isonitrogenous diet. After four weeks on the respective diet, 1.0 x 10^6/ml of *Tc* were administered intraperitoneally to 14 goats from each group AI, BI and CI , while four goats from each group served as non-infected controls AC, BC and CC. The animals were monitored for body weight changes post-infection, and after treatment with diminazene aceturate on week 5. Blood and serum samples collected weekly for 10 weeks, were assessed for changes in parasitaemia, haematology, serum biochemistry, complement levels and lymphocyte proliferation using standard methods. Data from the survey were analysed using descriptive statistics and Students't-test, experimental data by ANOVA while parasite counts were correlated with haematology, serum biochemistry, and complement levels at $p < 0.05$.

Dry season prevalence rate (4.5%) was significantly higher than that of rainy season (2.3%).While mean parasite count of group AI at weeks 3-5 was significantly higher than those of BI and CI, growth was retarded significantly in AI than BI and CI. Post-treatment growth acceleration was significantly higher in CI than BI and AI from

weeks 7-10 but, CC grew at a significantly higher rate than BC and AC throughout the experimental period. Mean erythrocytic values fell with highest decline in

AI than BI and CI from weeks 3-5 with normocytic normochromic anaemia. Mean serum Na^{+} , Ca^{2+}, PO_4^{2-} , globulin and transaminases increased from weeks 2-5 while mean serum K^{+}, HCO_3^{-}, total protein, albumin, complement C3, total and alternative haemolytic complement levels decreased, being significant only in group AI. In all infected groups increased parasitaemia correlated negatively with erythrocytic values, serum K^{+}, HCO_3^{-}, total and alternative haemolytic complement levels and positively with serum Na^{+}, PO_4^{2-}, total protein, globulin and transaminases. Mean lymphocyte count per minute within stimulated infected groups from weeks 1-5 were significantly increased in the order of CI > BI > AI, while unstimulated control cells maintained uniform response over a 72–hour period. Following treatment with berenil®, mean erythrocytic values and other parameters returned to pre-infective values by week 10, but earliest in group CI at Week 8.

The prevalence of caprine trypanosomosis was higher in dry than rainy season. Increased dietary energy intake in goats increased their tolerance to *Tc* infection. It is recommended that dietary energy along with other nutrients be adequately provided for goats to reduce clinical effects of trypanosomosis.

Key words**:** West African Dwarf goats, Dietary energy levels, *Trypanosoma congolense* infection and Trypanotolerance

Word counts**:** 492

CERTIFICATION

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DEDICATION

This project is dedicated to the following people who have contributed immensely to my progress in life:

Dr. A. A. Owoade of the Department of Veterinary Medicine, University of Ibadan, who provided most of the reagents used in this work, courtesy of Prof. C.P. Muller of the Institute of Immunology, Luxemburg.

Dr. Anumudu of the Department of Zoology, University of Ibadan, Ibadan .

To my late brother, Mr. Mufutau Ameen who passed away in 2005, May his soul rest in peace.

To my Father-in-Law and former Chief Imam of University of Ibadan mosque, Dr. Dawood Ahmad Adekilekun Tijani, May his soul rest in peace.

ACKNOWLEDGEMENTS

My sincere thanks and gratitude go to Almighty God, who out of infinite mercy and grace has helped me to complete this course of study.

I am sincerely grateful to my supervisors Professor R.A .Joshua and Dr.E.A. Okewole, both of whom are fathers, teachers, guides and mentors and despite their tight work schedule found time to supervise this work.

My appreciation goes to Head, Department of Veterinary Medicine, Dr. A.O. Ayoade and other former H.O.Ds including Professor (Mrs) H.O. Notthidge for their advice. I would like to express my appreciation to all my lecturers: Professors B . E. Olufemi, L.A. Oladosu, C. A. O. Adeyefa, and Drs. A. A. Owoade, Mrs. Oladele, T.O.Lasisi. and all others in the Department of Veterinary Medicine.

I am indebted to Professor (Dr) Claude Muller of the Institute of Immunology ,Luxemburg, who provided some of the reagents used in running the complement system and lymphocyte proliferation studies , courtesy of Dr. A. A. Owoade.

I am grateful to the Laboratory staff of the Department of Veterinary Medicine:Mrs Iyabo Adetiba and Mr .Lekan Lateef for their technical assistance in these studies.

To my colleagues, Drs. A.K. Raheem, F.M. Lawal, O.O. leigh, O.O. Aina and a host of others, I say a big thank you. Also, to all my colleagues in the Department of Animal Production and Health, LAUTECH, Ogbomoso. I say a big thank you. A special thank you is extended to Dr. T.A.Adedeji, LAUTECH for the Computer analysis of the experimental data.

Finally, I wish to thank very sincerely my wife Mrs Iyabo Ajarat Adekilekun Ameen and our children, I love you all.

May Almighty God bless and reward you all (Amen).

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CHAPTER ONE

INTRODUCTION AND PROJECT JUSTIFICATION

The biochemical mechanisms by which the chemical energy contained in foodstuffs are made available to the animals encompass the biochemical events that occur from the moment of ingestion to its final breakdown and excretion. Classically, these biochemical events have been divided into the metabolism of the three major constituents of food; carbohydrates, proteins, and lipids (Kaneko, 2008).The major function of ingested carbohydrates is to serve as energy sources, and their storage function is relatively minor. Carbohydrates are also precursors of essential intermediates for use in synthetic processes. When the metabolic machinery of an animal is disrupted, a disease state prevails (Kaneko, 2008). It has long been generalized that famine and disease are closely associated, while malnutrition increases susceptibility to infection. Malnutrition can include not only deficiencies but also excesses or imbalances of specific nutrients (Joshua *et al*., 1993). In general, severe nutritional deficiencies reduce T-cell functions impairing cell mediated responses but sparing B-cell function and humoral immunity (Sheffy and William, 1982; Joshua *et al*., 1993).

West African Dwarf (WAD) sheep and goats are the small ruminants which are ubiquitous in villages throughout the Nigerian rainforest and the derived savannah (ILCA, 1987).The animals have great economic potential due to high fertility, fecundity, prolificacy,early maturity and their adaptability to the humid environment (Ademosun, 1988).However, the economic benefits obtained from these animals are far below expectation due to low productivity.This low productivity is due to numerous factors of which the major one is disease (Schilhorn van Veen,1973).Diseases constitute great impediments to small ruminant production, a mortality rate of 34.12% and 36.20% have been reported respectively for sheep and goats in the old Bendel State (ILCA,1987).Of the great importance are vector-borne diseases which are difficult to control are tsetse-transmitted trypanosomosis and tick-borne or tick associated diseases like babesiosis and cowdriosis (Uilenberg *et al.*,1993).

Ruminant animals show considerable variation 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) . Small ruminants are fully susceptible to trypanosomosis (Adah *et al.*,1993;Ogunsanmi *et al.*,1994) and the economic impact of the disease on these animals has been shown to be substantial (Luckins, 1992). Disruption

of erythrocyte membrane integrity has been reported to be caused directly by the trypanosomes (Banks, 1979, 1980; Anosa and Kaneko, 1983) or indirectly by secreted products of trypanosomes (Huan *et al.*,1975;Tizard *et al.*,1978;Esievo, 1981; Pereira, 1983;Knowles *et al.*,1989). Significant reduction in erythrocyte membrane sialoglycoproteins, due to increased activity of circulating neuraminidases (sialidases) has also been reported to play a significant role in the development of anaemia in African animal trypanosomosis (Esievo *et al.*,1982;Aminoff,1988;Olaniyi *et al.*,2001). These phenomena have been reported to be responsible for early sequestration and destruction of erythrocytes by cells of the mononuclear phagocytic series and subsequent anaemia during trypanosomosis (Murray and Dexter, 1988). Erythrocyte peroxidation has been observed to be one of the factors, which play an important role in the pathogenesis of anaemia in acute trypamosomosis in mice infected with *T. brucei* (Igbokwe *et al.*,1994). Trypanosomes and activated phagocytes (macrophages and neutrophils) are known to elaborate sialidases (Esievo *et al.*,1982; Lambre *et al.*,1990), proteases (Khaukha and Rasamany, 1981; Knowles *et al.*,1989), reactive oxygen radicals such as O^{2-}, OH^{-} on erythrocyte membranes leading to their rapid destruction during infection (Aminoff,1988;Olaniyi *et al.*,2001). Studies in cattle experimentally infected with *T. congolense* have demonstrated that the rate of development of anaemia in cattle given ground nut cake supplementation was slower than in those that were not supplemented (Little *et al*., 1990). Similar observations were made by Hecker *et al.*,(1991) in Djallonke sheep exposed to high tsetse challenge. However, these results are at variance with those of Agyemang *et al*., (1990) on N'Dama cattle infected with trypanosomes. The blood-stream forms of some trypanosomes scavenge blood glucose as a source of energy (Chaudhuri *et al*., 2006). This may partly contribute to the development of hypoglycemia observed in some trypanosome-infected animals. Indeed, Faye *et al.*, (2005) reported that the high energy demands of trypanosome infection may lead to severe energy shortage and this might be reflected in the changes to energy and protein metabolism. Whitlock, (1949); Gibson, (1963) reported that nutritional states of the host can influence the pathogenicity of parasitic infection and that it is generally accepted that well-nourished animals withstand parasites better than those that are poorly-fed. The present study investigated the influence of graded dietary energy on parasitaemia, live weight, haematology, serum biochemistry, complement levels and lymphocyte proliferation on WAD goats experimentally infected with *T.congolense* .

Project Justification:

. The resistance of some trypanosomes to trypanocidal drugs has been reported in many parts of Africa (Jones, 1967; Authie 1984; Peregrine *et al.*, 1988, Joshua *et al.*,1988,1995) and toxicity resulted from administration of these drugs. Malnutrition increases goats' susceptibility to diseases thereby hindering their profitable production.Low calorie diet and infection have impact on health and survival. There is dearth of information on the use of natural means such graded dietary energy in the prevention of trypanosomosis and the influence of Graded Dietary Energy (GDE) on dynamics of *Trypanosoma congolense* (*Tc*) infection is not fully elucidated in goats. The influence of GDE levels on *Tc* infection in West African Dwarf (WAD) goats was therefore investigated vis-a-vis the course of parasitaemia, body weight gain, blood-biochemical changes, complement levels and lymphocyte proliferation .

BROAD OBJECTIVE

To investigate the influence of graded dietary energy on dynamics of *Trypanosoma congolense* infection in West African Dwarf (WAD) goats.

SPECIFIC OBJECTIVES

(1) Preliminary survey on seasonal influence on prevalence of trypanosomosis in West African Dwarf goats in Ogbomoso area of Oyo State, Nigeria

(2) To assess the course of *Trypanosoma congolense* infection in WAD goats fed three different levels of dietary energy.

(3) To assess the influence of three different levels of dietary on body weight changes of *Trypanosoma congolense* infected (WAD) goats .

(4) To assess the influence of three different levels of dietary energy on haematological changes of *Trypanosoma congolense* infected WAD goats.

(5) To assess the influence of three different levels of dietary energy on serum bio-chemical changes of *Trypanosoma congolense* infected WAD goats.

(6) To assess the influence of three different levels of dietary energy on complement levels of *Trypanosoma congolense* infected WAD goats .

(7) To assess the influence of three different levels of dietary energy on lymphocyte proliferation in *Trypanosoma congolense* infected WAD goats .

(8) To assess the influence of three different levels of dietary energy on chemo-therapeutically treated WAD goats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 RUMINANT NUTRITION

Goats are small ruminants that are widely distributed throughout geographical and climatic regions of the world and have been found to adapt quickly to new environment (Huston, 1998).There are 34.5 million goats in Nigeria (FD&PCS,1991) and they supply about 38% of the nations total meat output (Umoh *et al.*,1996).The West African Dwarf (WAD) goats belong to small ruminants with four compartments: the rumen, reticulum, omasum and abomasum. Collectively, these organs occupy almost three quarters of the abdominal cavity, filling virtually all of the left side and extending significantly into the right. The reticulum lies against the diaphragm and is joined to the rumen by a fold of tissue (Phillipson, 1970). The rumen, far and away the largest of the forestomaches, is itself sacculated by muscular pillars into what are called the dorsal, ventral, caudodorsal and caudoventral sacs (McDonald *et al.*, 1979). In many respects, the reticulum can be considered a "cranioventral sac" of the rumen; for example, ingesta flow freely between these two organs. The reticulum is connected to the spherical omasum by a short tunnel. The abomasum is the ruminant's true or glandular stomach which secretes digestive fluids. Histologically, it is very similar to the stomach of monogastrics. The interior of the rumen, reticulum and omasum is covered exclusively with stratified squamous epithelium similar to what is observed in the esophagus. Each of these organs has a very distinctive mucosa structure, although within each organ, some regional variation in morphology is observed. The anatomic features described above are exemplified by cattle, sheep and goats. Certain other animals are also generally called ruminants, but have slightly different forestomach anatomy. Camelids (camels, llamas, alpacas, vicunas) have a reticulum with areas of gland-like cells, and an omasum that is tubular and almost indistinct. These animals are occasionally referred to as pseudoruminants or as having "three stomachs" rather than four. A Stratified, squamous epithelium such as found in the rumen is not usually considered an absorptive type of epithelium. Ruminal papillae are however very richly vascularized and the abundant volatile fatty acids produced by fermentation are readily absorbed across the epithelium. Venous blood from the forestomachs, as well as the abomasum, carries these absorbed nutrients into the portal vein, and hence, straight to the liver. The rumen is a fermentation vat *par excellence*, providing an anaerobic environment, constant temperature and pH, and good mixing.

Well-masticated substrates are delivered through the esophagus on a regular schedule, and fermentation products are either absorbed in the rumen itself or flow out for further digestion and absorption downstream(Phillipson,1970). Ruminants evolved to consume and subsist on roughage - grasses and shrubs built predominantly of cellulose. Despite the fact that some ruminants, feedlot steers for example, are feed large quantities of grain, this section will focus on a ruminant's "natural diet". Feed, water and saliva are delivered to the reticulorumen through the esophageal orifice. Heavy objects (grain, rocks, nails) fall into the reticulum, while lighter material (grass, hay) enters the rumen proper. Added to this mixture are voluminous quantities of gas produced during fermentation. Ruminants produce prodigious quantities of saliva. Published estimates for adult cows are in the range of 100 to 150 liters of saliva per day, aside from its normal lubricating qualities, saliva serves at least two very important functions in the ruminant:

- provision of fluid for the fermentation .
- alkaline buffering - saliva is rich in bicarbonate, which buffers the large quanitity of acid produced in the rumen and is probably critical for maintainance of rumen pH.

All these materials within the rumen partition into three primary zones based on their specific gravity. Gas rises to fill the upper regions, grain and fluid-saturated roughage ("yesterday's hay") sink to the bottom, and newly arrived roughage floats in a middle layer. The rate of flow of solid material through the rumen is quite slow and dependent on its size and density. Water flows through the rumen rapidly and appears to be critical in flushing particulate matter downstream. As fermentation proceeds, feedstuffs are reduced to smaller and smaller sizes and microbes constantly proliferate. Ruminal contractions constantly flush lighter solids back into the rumen. The smaller and more dense material tends to be pushed into the reticulum and cranial sac of the rumen, from which it is ejected with microbe-laden liquid through the reticulo-omasal orifice into the omasum. The function of the omasum is rather poorly understood. It may function to absorb residual volatile fatty acids and bicarbonate (Radostitis *et al.*,.2007). The tendency is for fluid to pass rapidly through the omasal canal, but for particulate matter to be retained between omasal leaves. Periodic contractions of the omasum knocks flakes of material out of the leaves for passage into the abomasum. The abomasum is a true, glandular stomach which secretes acid and otherwise functions very similarly to the stomach of a

monogastric. One fascinating specialization of this organ relates to its need to process large masses of bacteria. In contrast to the stomach of non-ruminants, the abomasun secretes lyzozyme, an enzyme that efficiently breaks down bacterial cell walls. The processes described above apply to adult ruminants. For the first month or so of life, the ruminant is functionally a monogastric. The forestomachs are formed, but are not yet fully developed. If milk is introduced into such a rumen, it basically rots rather than being fermented. To avoid this problem in such young ruminants, suckling causes a reflex closure of muscular folds that form a channel from the esophageal orifice toward the omasum (the esophageal groove), shunting milk away from the rumen and straight toward the stomach where it can be curdled by rennin and eventually digested enzymatically. An orderly pattern of ruminal motility is initiated early in life and, except for temporary periods of disruption, persists for the lifetime of the animal. These movements serve to mix the ingesta, aid in eructation of gas, and propel fluid and fermented foodstuffs into the omasum. If motility is suppressed for a significant length of time, ruminal impaction may result. A cycle of contractions occurs 1 to 3 times per minute. The highest frequency is seen during feeding, and the lowest when the animal is resting. Two types of contractions are identified:

Primary contractions originate in the reticulum and pass caudally around the rumen. This process involves a wave of contraction followed by a wave of relaxation, so as parts of the rumen are contracting, other sacs are dilating.

Secondary contractions occur in only parts of the rumen and are usually associated with eructation.

The forestomachs possess a rich enteric nervous system, but coordinated contractions require central input. Motility centers in the brainstem control both the rate and strength of contraction via vagal efferents. Cutting the vagus nerve in a ruminant abolishes coordinated reticuloruminal motility. There are also vagal afferents from the rumen to the motility centers which allow stretch receptors and chemoreceptors in the rumen to modulate contractility. Conditions inside the rumen can significantly affect motility. If, for example, ruminal contents become very acidic (as occurs in grain engorgement), motility will essentially cease. Also, the type of diet influences motility: animals on a high roughage diet have a higher frequency of contractions than those on a diet rich in concentrates. Ruminants are well known for "cud chewing". Rumination is regurgitation

of ingesta from the reticulum, followed by remastication and reswallowing. It provides for effective mechanical breakdown of roughage and thereby increases substrate surface area to fermentative microbes. Regurgitation is initiated with a reticular contraction distinct from the primary contraction. This contraction, in conjunction with relaxation of the distal esophageal sphincter, allows a bolus of ingesta to enter the esophagus. The bolus is carried into the mouth by reverse peristalsis. The fluid in the bolus is squeezed out with the tongue and reswallowed, and the bolus itself is remasticated, then reswallowed. Rumination occurs predominantly when the animal is resting and not eating, but that is a considerable fraction of the animal's lifespan. Fermentation in the rumen generates enormous quantities of gas were about 30-50 liters per hour in adult cattle and about 5 liters per hour in a sheep or goat. Eructation or belching is how ruminants continually get rid of fermentation gases. As mentioned above, an eructation is associated with almost every secondary ruminal contraction. Eructated gas travels up the esophagus at 160 to 225 cm per second and, interestingly, a majority is actually first inspired into the lungs, then expired. Anything that interferes with eructation is lifethreatening to the ruminant because the expanding rumen rapidly interferes with breathing. Animals suffering from ruminal tympany (bloat) die from asphyxiation.

2.1.1 Feed utilization

Digestibility trials are used to estimate the degree of utilization of feed as a whole or of special groups of constituents. The differences between the chemical composition of the nutrient intake and faeces voided expressed a as a percentage of intake, without correction for nutrient of metabolic faecal origin, is referred to as “apparent digestibility “coefficient”. Metabolic faecal nitrogen (MFN) has been defined as that protein of faecal nitrogen which is not of dietary origin but originates within the body from a variety of sources, such as epithelial cells, bacteria, mucus, residues from bile and digestive juices (Schneider, 1935; Blaxter and Mitchell, 1948). Faecal output, which is contacted for its execretory material (MFN), expressed as a ratio of nutrient intake, given an idea of true digestibility. Maynard and Loosli, (1969) gave details of the conventional methods of determining MFN.

Digestibility coefficients are used to compute the total digestible nutrient (TDN) from a feed of known nutrient content. Pigden and Bell,(1955) obtained a regression equation relating predicated TDN to actual TDN determined by digestion trials as :

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TDN = $\frac{\%\ OM\ dig.\ in\ vitro \times \%\ OM\ in\ forage}{100}$ +dig. EE g/100g. DM x 1.25

Where OM is = Organic matter and EE is = Ether Extract.

This regression equation relating in vitro estimate is faster than the conventional trail but theses estimated TDN values were lower than the actual TDN values. There is a close correlation (r = 0.7 – 0.8) between DM digestibility in vivo and fatty acid production in vitro (Asphind et al., 1958). Clark and Mott, (1960) also obtained a regression equation for predicating digestibility of forages from their chemical components instead of using the conventional trail.

The total energy liberated when feed is burnt in a bomb calorimeter is called "Gross Energy" or "heat of combustion". The sum of energy value of the components constitute the energy value of the food. The gross energy of a food or feed is not all available to the animal. Correction of gross energy for the heat of combustion of faeces gives the digestible energy. Deduction of the combustible energy of urine for digestible energy gives the apparent metabolizable energy, is the portion of the gross energy which undergoes transformation within the body (Maynard and Loosli, 1969).

There is further loss of energy in the form of combustible gases which is made up almost of methane. When this is taken care of the true metabolizable energy is obtained. Methane is produced from the rumen following rumen fermentation of feed and is eructed by the ruminant. This result in a loss of equivalence of 8 to 10% of the energy intake (Body , 1945; Blaxter, 1967). Heat lost as methane is usually determined in a respiratory chamber which is really expensive. The amount of methane produced is a function of the types of diet and the level of intake. Low quality diets result in larger proportion of methane, and the percentage of gross energy loss as methane declines as feed intake increases. Several formulae exist for calculating gaseous energy loss in ruminants, Blaxter and Clapperton, (1965) stated as follows:-

CH_4 = 1.30 + 0.112 D-L (2.37 – 0.050 D)

Where D = digestible energy at the maintenance level of feeding.

L = the level of feeding as a multiple of maintenance.

Metabolizables energy (ME) for ruminant is often calculated by the formula:

ME = DE x 0.82

Where DE = digestible energy

It has been pointed out, however, by Flat and Moe, (1969) that this is only approximation as the ME/DE ratio may vary considerably by the nature of the diet and the level of feeding.

Methane production is a wasteful outlet of gross energy which could have been used by the animal. By feeding unsaturated fatty acid, methane production was reduced (Blaxter and Czerkawski, 1966). This is found possible because of the fact that double bonds of unsaturated fatty acids compete with carbon dioxide for hydrogen, the hydrogen being required for the reduction of carbon dioxide to form methane (Carrol and Hungate, 1955). It has also been observed by Czerkawski and Breckenridge, (1972) that by variation of feeding regime to increase propionate production at the expenses of acetate and butyrate, methane production was inhibited by 20 to 40%. Trei *et al.*, (1972) and Johnson (1972, 1974) utilized hemiacetal of chloral and starch as an effective methane inhibitor. They therefore concluded that energy normally lost as methane could be retained as production metabolites. Digestibility trials are therefore important for the determination of TDN, digestible energy (DE), metabolizable energy (ME) and starch equivalent (SE), which are feeding standards used in animal nutrition.

2.1.2 Protein Metabolism

The quality of the protein in animal with simple stomach is determined by its ability to supply essential amino acids in available forms and in balanced proportions when digested by the animal (Sotola,1930;Blaxter,1967;Maynard *et al.*,1979). Complex stomach animals present interesting studies in their digestion of food by virtue of the large number of bacterial and protozoal population of their rumen. Protein entering the rumen is hydrolyzed by the micro-organisms to yield peptides, amino acids and ammonia (McDonals, 1948, 1954; Lewis *et al.*, 1957). It has been observed by McDonald, (1948), Belasco, (1964), Lewis *et al.*, (1957) and Annison and Lewis, (1959) that non-protein nitrogenous compounds (Asparagine, Urea, and even Ammonium salts) also give ammonia as an end product of bacterial action. The microbial population utilize ammonia in the presences of adequate energy to synthesize the proper amino acids needed for their own protein requirements. It has been shown that 80 percent of the bacterial species existing in the rumen can utilize ammonia as the sole source of nitrogen for growth while 25 percent require it absolutely an 55 percent could use either ammonia or amino acids (Maynard *et al.*, 1979).

Ingesta from the rumen is subject to a rapid fall in pH in the abomasum. This kills the bacteria and the protozoa, which are easily digested by the host enzymes to release

amino acids (Annison and Lewis, 1959). The nutritive value of protein fed depends on the extent to which the food protein is converted to microbial protein and efficient utilization of ammonia which is of no use to the host animal by the micro organisms in the rumen it has been noted by McDonal (1952); Annison *et al.,* (1954); Lewis *et al.,* (1957); Mba *et al.,* (1971) that efficient utilization of ammonia was greatly enhanced in the presence of readily fermentable carbohydrate, such as starch. Lowering of ruminal pH which delays the absorption of ammonia through the rumen wall is favourable by such energy substrate. The ammonia eventually becomes available for the synthesis of microbial protein. The ruminal amino acids which are always in low concentration are further degraded to volatile fatty acids(VFA), ammonia and carbondioxide ($C0_2$). On high protein diets, protein degradation may contribute a significant proportion of the VFA produced (Hungate, 1966). Unused ammonia for the synthesis of microbial protein is excreted as urea when absorbed through the rumen wall, thus representing a net loss of dietary protein to the animal. McDonald (1969) described the recycling of urea. On low – proteins diets, the kidney reabsorbs a greater quantity of urea and thus a fair proportion is returned to the blood to be recycled into the rumen to provide added nitrogen for microbial fermentation (Schmidt-Nielsen, 1977). Young growing lambs extract more urea than mature wether (Allen and Miller, 1976). Proteins, such as casein, soybean meal, groundnut cake, which are highly soluble in rumen, are easily broken down by bacterial for the production of ammonia (Challmers *et al.,* 1954; Annison *et al.,* 1954; Little *et al.,* 1963). Such high soluble proteins has been associated with decreased nitrogen retention if a large proportions is hydrolyzed to ammonia, and if a significant amount of ammonia is excreted as urea by the animal (Lewis *et al.,* 1957; Preston *et al.,* 1963). Heat treatment has been tried (Sherrod *et al.,* 1967), chemical action of formaldehyde on case in (Reis and Tunks, 1969; 1970)and treatment with tannic acid (Driedger and Hatfield , 1970) to reduce the solubility of protein to allow adequate nitrogen to become available for optimal rumen microbial growth, yet allow a significant amount of the higher quality dietary protein to pass the rumen for postruminal degradation. This enhances the protein utilization of the animal as a whole.

2.1.3 Non – protein Nitrogen utilization by Ruminants

Several studies have been carried out to determine the ability of a large number of non – protein nitrogenous substances to support growth of ruminal bacterial in vitro (Belasco, 1954; Henderikx and Martin, 1963). It has been shown by Loosli *et al.,* (1949) and Ducal *et al.,* (1953) that's all essential amino acids were synthesized by the rumen

microbial population of sheep and goats from urea nitrogen. There is enough evidence to show that efficient utilization of urea nitrogen depends on the availability of a readily fermentable carbohydrate (Mills *et al.*,1942; Pearson and Smith, 1943; and Oltjen and Putnan, 1966). For fear of toxic effects, limited to moderate quantities of non - protein nitrogen (NPN) has been fed to ruminant (Phillipson, 1964; Waldo, 1968; Chalupa, 1968). This fear however was allayed by Oltjen (1969).

Ellis *et al.*, (1956), reported biological values of 54, 57, 73 and 84 for urea, gelatin, casein and soy-protein respectively in metabolism trials using purified diets with lambs.It has been shown by Virttanen,(1966) that milk production could be maintained in cows on purified protein – free feed, using urea and ammonia salts as the main source of nitrogen (N), provided that energy and mineral were adequate. Reports on the results of feeding urea to zebu cattle and buffaloes indicated interested increased voluntary intake of dry matter, and higher dry matter, crude protein and crude fibre digestibility. Ichnoponani and Sidhu, 1966a; Briggs *et al.*,1960) have both reported increase in roughage consumption and reduction in body weight losses by feeding sheep with straw sprayed with molasses and urea. Nitrogen retention of steers fed was 60 percent of steers fed isolated soy- protein (Oltjen and Putnan, 1966) while Clifford and Tillman, (1968) reported nitrogen retention of sheep fed urea diets as 70 percent and as good as with sheep fed isolated soy-protein diet. In comparative metabolism trails involving NPN like urea, biuret, urea phosphate and uric acid with Steers, nitrogen retention values expressed as a percentage of intake were 18.4, 16.9, 12.3 and 23.1% respectively (Oltjen *et al.*, 1968). Even though the nitrogen retention had shown no significant differences, uric acid appeared to be a good source followed by urea. It seems therefore that great potentials exist in increasing ruminant production by making maximal use of the ability of the ruminal microbial population in making efficient use of NPN source as a source of high quality protein for themselves and for the host animal.

2.1.4 Conversion of Dietary protein into microbial protein

For the efficient utilization of the end products of protein fermentation a readily available source of energy is necessary. Such energy sources like pure starch or starch feeds like cereals, cassava and potatoes are usually most satisfactory. Molasses or sugars are less satisfactory because they pass out of the rumen too rapidly. Cellulose on the other hand, is made available too slowly. Rations low in protein but high in readily available carbohydrates are most favourable to protein synthesis in the rumen. It has been observed by Lewis *et al.*,(1957) that at high pH, the ammonia molecules are mostly present in the

unionized form and the presence of glucoses or its derivative lactic acid, lowers the pH and the ammonia molecules are mostly present in the ionized form. They also noted that unionized ammonia molecules pass through the rumen epithelia much more quickly than the ionized forms, thus in the ionized form ammonia molecules pass through the rumen epithelia much more slowly giving time for the rumen micro- organisms to incorporate ammonia for microbial protein. Reis and Reid ,(1959) found that high pH favours ammonia production in the rumen, and that the optimum pH for ammonia production in the rumen varied between 6.0 and 7.0.

The synthesis of amino acids from ammonia by mciro-organisms requires the presence of ammonia, carbon skeleton and energy. Studies have shown that the utilization of carbon from carbohydrate (Hoover *et al.,* 1963) carbon dioxide (Huntanen *et al.*,1954), Isovaleric acid, acetate and other volatile fatty acids (Hoover *et al.*, 1963) indicates that carbon from a wide variety of sources could be used for the synthesis of amino acids. However, other studies have shown that the synthesis of certain amino acids require certain specific carbon skeleton. Microbial proteins constitute the major part of Nitrogen continuing compounds reaching the lower gastrointestinal tract, and so factors which affect the microbial populations also affect the availability of microbial protein to the host animal (Weller *et al.,* 1958).Semi – purified diets and antibiotics have been shown to influence the concentration of protozoas in the rumen and to modify the bacterial population of metabolic activity (Bryant and Small, 1960; Purser *et al.*, 1965). It has been reported, however, that changes in ration did not modify the amino acid composition of the rumen bacteria (Weller, 1957 and Meyer *et al.,* 1967) while the amino acid composition of protozoa have been found to vary (Poley, 1965; Hoeller and Harmyer, 1964). Hogen and Weston (1967a) summarized some of the factors influencing the conversion of dietary – Nitrogen to microbial Nitrogen as:-

a. The time spent by the feeds participles in the rumen, the longer the time, the greater the conversion.
b. The resistance of dietary –Nitrogen source to deaminative degradation, the more resistant, the less the conversion.
c. Availability of nitrogen for microbial protein synthesis.
d. Energy availability for rumens fermentation.
e. Presence of growth factors for instance, minerals such as cobalt, and also vitamins such as B_{12}.
f. The population composition of rumen micro – organisms.

2.1.5 Limiting Amino Acids

The amino acid constituents of the microbial protein determines its nutritive value. The results obtained in estimates made on the amino acid composition of microbial proteins showed no consistent deficiency in the essential amino acids as compared with that of whole egg (Duncal *et al.*, 1953). A technique for the determination of plasma amino acid score (PAAS), which was claimed to be very reliable for apparition in ruminants, was developed by McLaughlan (1964). Bergen *et al.*, (1968a), using this technique fed microbial proteins to rats and found that protozoan protein was extremely low in histamine, and low in valine. The lowest value was recorded by cystine for bacterial protein while arginine, histidine, leucine and lysine were also considered low. The results suggest that these amino acids are potential limiting amino acids in rumen microbial proteins. Halfpenny and Rook, (1968), however observed the defect in relying on plasma amino acid assay for they noted that changes in certain plasma amino acids tend to parallel the physiological state of the animal. It has been pointed out that the amino acids in shortest supply are methionine, cysteine and isoleucine (McDonald, 1969). Studies have shown that methionine, lysine and histidine could be limiting for tissue growth (Schelling *et al.*, 1967; Virtanen, 1966; Bergen *et al.*, 1968a; Smith, 1969). Methionine appears to be the first limiting amino acid for high yielding cows and sheep (Hutton and Annison, 1972). Good results have been obtained when methionine was fed to lambs (Mowat and Deelstra, 1972; Scott *et al.*, 1972). Supplementation of methionine or sulphur- containing amino acids appear to be beneficial to ruminants with regards to N-utilization.

2.1.6 Energy metabolism in the rumen

Radostitis *et al.*,(2007) observed that the break down of carbohydrates and other constituents of the diet by ruminal micro – organisms gives rise to volatile fatty acids; these are formic acid, acetic acid, propionic acid, normal and isolbutyric acid. In addition carbon dioxide, methane, and bacterial cells (Elsden, 1945), acetic acid and traces of other acids such as succinic are also produced. Phillipson and McAnally, (1942) showed also that the volatile fatty acids (VFA) are absorbed, from the rumen directly into the blood stream (Bacroft et al., 1944a, 1944b' Mason and phillipson, 1951). Acetic, propionic acids are utilized immediately or store for subsequent use. It has been shown (Leng *et al.*, 1967) that about half of the glucose, the major energy substrate for metabolic process is formed, from propionate produced in the rumen, and as such propionic acid is glucogenic. Acetic and butyric acids are not glucogenic(Radostitis *et al.*,2007).They

either serve the energy needs of the body through the Kreb's cycle (Armstrong, 1965) or enter the metabolic cycle of fat to form body fat (Armstrong and Blaxter, 1957; Armstrong *et al.*, 1958). Acetic acid has been shown to be used in the synthesis of the short chain fatty acids of milk (Barry, 1964). The gaseous product of fermentation, mainly methane, escapes and is of no energy values to the animal. It has been estimated that 15 to 30 percent of the digestible dry matter escaped ruminal action (Gray, 1947; Nicholson and Sutton, 1969). The digestion in the small intestine is carried out by the intestinal enzymes as obtained in the non –ruminants.

2.1.7 Energy requirement for maintenance, production and haemopoiesis

Lack of growth, body tissue losses, or reduced production are manifestation of energy deficiency rather than any specific signs such as those which characterize the deficiency of some specific nutrients (Radostitis *et al.*,2007; Flatt and Moe, 1969). When an animal is neither lactating nor pregnant and is neither gaining nor losing body tissue, it is said to be at "Maintenace" level. The maintenance requirement is a measure of the amount of feed energy necessary to maintain an animal for long periods of time without gaining or losing body weight. In fasting animals, the quantity of heat produced is equal to the quantity of chemical energy expended for body maintenance, and when measured under specific conditions is known as the animals basal metabolism. Basal metabolism per unit metabolic weight is higher in young animals than in old, being for example 120 kcal/ kg metabolic live weight in a young calf but only 80 kcal/ kg metabolic live weight in a mature cow. It also tends to be higher in males than females (Maynard and Loosli, 1969). Most feeding standards are based on the assumption that maintenance requirements under normal condition are appreciably higher than basal or fasting metabolism rates, and 1.25 to 1.35 times fasting metabolism values are frequently used when calculating maintenance requirements (Church and Pond, 1976). Brody, (1945) claimed that basal metabolism is proportional to the body weigh "w" to the exponent "n", w^n is therefore the metabolic size of animals ranging from mice to elephant. He suggested the power 0.73 for weight as metabolic size. He suggested the power 0.73 for weight as metabolic size. Kleiber, (1947) felt that $W^{3/4}$ provided a better fitting formula rating basal metabolism to body size than does $W^{0.734}$. The National Research Council (NRC) adopted the exponent 0.73 while Armstrong and Mitchell ,(1955) utilized the exponent 0.734.

Maintenance need is usually determined by feeding trials. The method involved the determination of food required to keep animals at constant body weight. Devendra,

(1967b) used this method by adjusting and Neville, (1974) on non-lactating and lactating Hertford cows. The daily intake corrected for energy due to any fluctuation in live weight, become the maintenance requirement. Kost *et al.*, (1935) proposed the following figures;-

Kg gained X7.8 = TND required for gain.

Kg lost X 6.0 = TND equivalent of loss.

These corrections are approximations because of lack of tissue gained or lost. The change in weight might be due to water, which would have no food equivalent. This experimental period (Devendra, 1967b) or eliminated by including a slaughter test (Garrett *et al.*, 1959). Coop, (1962) used the equation:

Daily DOM intake $=aw^{3/4} + bg$.

Where DOM is the digestible organic matter intake,

"$aw^{3/4}$ is the maintenance requirement which is proportional to "w"

"b" is the DOM per kg live weight gain.

Errors due to live weight measurement were minimized by using long experimental periods. Most of the experiments on estimation of maintenance needs have been experiments on estimation of maintenance needs have been done on adult animals. Estimates of requirements for gain (Fattening, 1967b) are made mainly with adult as well (Coop,1962; Devendra, 1967b). The maintenance and growth requirements of adult sheep cannot be the same as those of the lamb. Growth in young animals is principally due to increase in the bone and muscle tissue, in the adult, it is mainly accomplished by addition, in the adult, it is mainly accomplished by addition of fatty tissues (McMeekan, 1940) in pigs and Palsson and Verges,(1951) in sheep. Garrett *et al.*,(1959) showed that TDN, digestible energy (DE) or metabolically energy per unit metabolic size is also equal to a +bg.

Where "b" is the energy required per kg live weight gain and "g" id the average daily gain kg and "a" the intercept on ordinate axis is the energy requirement for maintenance. This relationship has been used by Swanson, (1971) to obtain the requirements for different ages. In effect, energy requirements for maintenance, growth lactation and pregnancy are usually determined using balance technique or feeding trials. Such trials which have been carried out by many workers (Garrett *et al.*, 1959; Coop, 1962; Maynard and Loosli, 1969; Neville and McCullough, 1969; Moe *et al.*, 1970). Swanson,(1971) and Neville, (1974) facilitated the expression of requirements as TDN, ME, DE which are some of the feeding standards commonly used for energy values. Energy balance studies can also be used with animals in respiration chambers, described

by Kleber, (1958). The chamber provides for the measurement of gaseous exchange and thus for calculation of that production. Facilities for the measurement of feed intake, urine and faecal losses, and methane production provided for the determination of metabolizable energy (Maynard and Loosli, 1969). Respiration chambers produced highly reliable date requirements obtained with these chambers agree with values obtained by conventional method as shown from results of Beakley and Findley, (1955); Garrett *et al.,* (1959); Blaxter, (1967) and Sawyer *et al.,* (1971).In the recent refereces a value of 101.38kcal ME/kg$W^{0.75}$ (424) was established as the maintenance energy requirement for goats with no distinction as to age, stage of growth, maturity or biotype(NRC 1981). Recently Luo *et al.*,(2004) estimated the ME maintenance requirement of goats consuming at near or above maintenance to be 431 kj/kg $BW^{0.75}$.The metabolizable energy required for weight gain was estimated from three experimental values and established at 7.25 kcal ME/g(30.33kJ) of gain (NRC 1981) for all rates of gain and with no differentiation as to biotype or growth stage. Luo *et al.,* (2004b) predicted ME requirements for gain for the same classes of goats with values ;13.4,23.1,23.1,19.8 and 28.5 kJ/g for preweaning, growing meat, growing dairy, growing indigenous and mature goats respectively.

2.1.8 Energy and Protein Interaction

Studies have shown that high solubility of dietary proteins may decrease nitrogen retention especially if large proportion is hydrolyzed to ammonia and significant amount excreted as urea (Preston *et al*., 1963; Lewis *et al.,* 1957; and Leng, 1970). There is however enough evidence to show that the end products of readily available carbohydrates lowered the ruminal PH, which in turn delayed the absorption of ammonia through the rumen epithelium enabling ammonia to become available for microbial protein synthesis (Annison, 1956;Lewis, 1957; Phillipson,1964; Mba *et al.,* 1972). Dietary nitrogen favors effective utilization of energy. For example when ruminants are fed low nitrogen diets or on diets containing large amounts of soluble carbohydrates, there is an extremely high efficiency of nitrogen usage in both phases because rumen micro-organisms synthesize protein from recycled urea and more nitrogen reaches the duodenum than is consumed (Hogan and Weston, 1970; Leng, 1970). In the later case, cell growth were facilitated (Leng, 1970). Since nitrogen retention is always dependent on energy intake (Black *et al.,* 1973), it is very requirements. In one nitrogen utilization experiment Black *et al.,* (1973) regressed nitrogen balance (g/kg $w_{kg}^{0.734}$ per day) on the

contribution protein made to the digestible energy (DPE) to DE ratio. DPE is obtained by multiplying the digestible crude protein by 4.0 kcal per g,. DCP intake (Broody, 1945).

2.1.9 Interaction between nutrition and immunity in ruminant

In cattle, sheep, or goats- particularly those reared primarily on home-grown forages and grains, and when there are considerable nutrient losses from leaching during curing or losses from long dry-storage-conditions of marginal nutrition may exist (Alloway and Hodgson, 1964). Varying degrees of malnutrition can be expected and do occur in areas where soil mineral deficiencies are found or in arid regions where forages are cured on the stem or when total feed supplies are marginal during prolonged dry seasons. Such nutritional states must contribute negatively to level of immunocompetence, and decrease general resistance to infection. As is the case with non ruminants, deficiency or marginal nutrition states for selenium and/or vitamin E have routinely been diagnosed in the field for ruminants in the field, for ruminants in the Northeast (Kubota *et al.*, 1967;Perry *et al.*, 1976). For many ruminants under range conditions, protein-calorie malnutrition, if not mineral and vitamin malnutrition as well is common.Starvation often results in negative nutrient balances and losses in body weight of 10%-20% during the dry period, the extent of the effect depending upon the severity of environmental conditions.Neither of these conditions can be expected to contribute positively to optimal immune function.

2.1.10 Nutrition and Immunodeficiency

Immune deficiency may be congenital, genetic, and acquired.The latter can be caused by virus infection (human HIV and cat FIV), ingestion of certain mycotoxins,(T-2 toxin,Fekete *et al.*,1989),peroxides of rancid feed (Fekete *et al.*,2009), long lasting stress and corticosteroid treatment. Immune deficiency increases susceptibility to infection and therefore connected with some types of allergies and autoimmune diseases.Immune deficiency correlates only indirectly with the nutrition, namely if the primary cause of disease leads to chronic diarrhea and vitamin, malabsorption and there are significant faecal blood, protein, mineral losses.During designing the daily ration for these patients, the replacement of the lost subtances should be taken into consideration.

2.1.11 Influence of nutrient deficiency on the Immunity.

The extremely insufficient feed intake (general energy and protein deficiency) and also the ingestion of quantitatively sufficient, but unbalanced rations alter immune functions.However, the protein-energy malnutrition (PEM) is the primary factor in affecting immune response.Interpretation of the related data is difficult,because generally there is an accompanying vitamin and/or mineral deficiency,too(Tanner and

Taylor,1965).Moreover, the commonly occurring secondary infection weakens the immune system and also worsens the general body condition,although most of the knowledge of this field derives from epidemiological studies, and the results of experiments and investigation on the effect of a single nutritional factor on the immunity are difficult to extrapolate to the complex situations of the real life.

2.1.12 Energy and Protein deficiency.

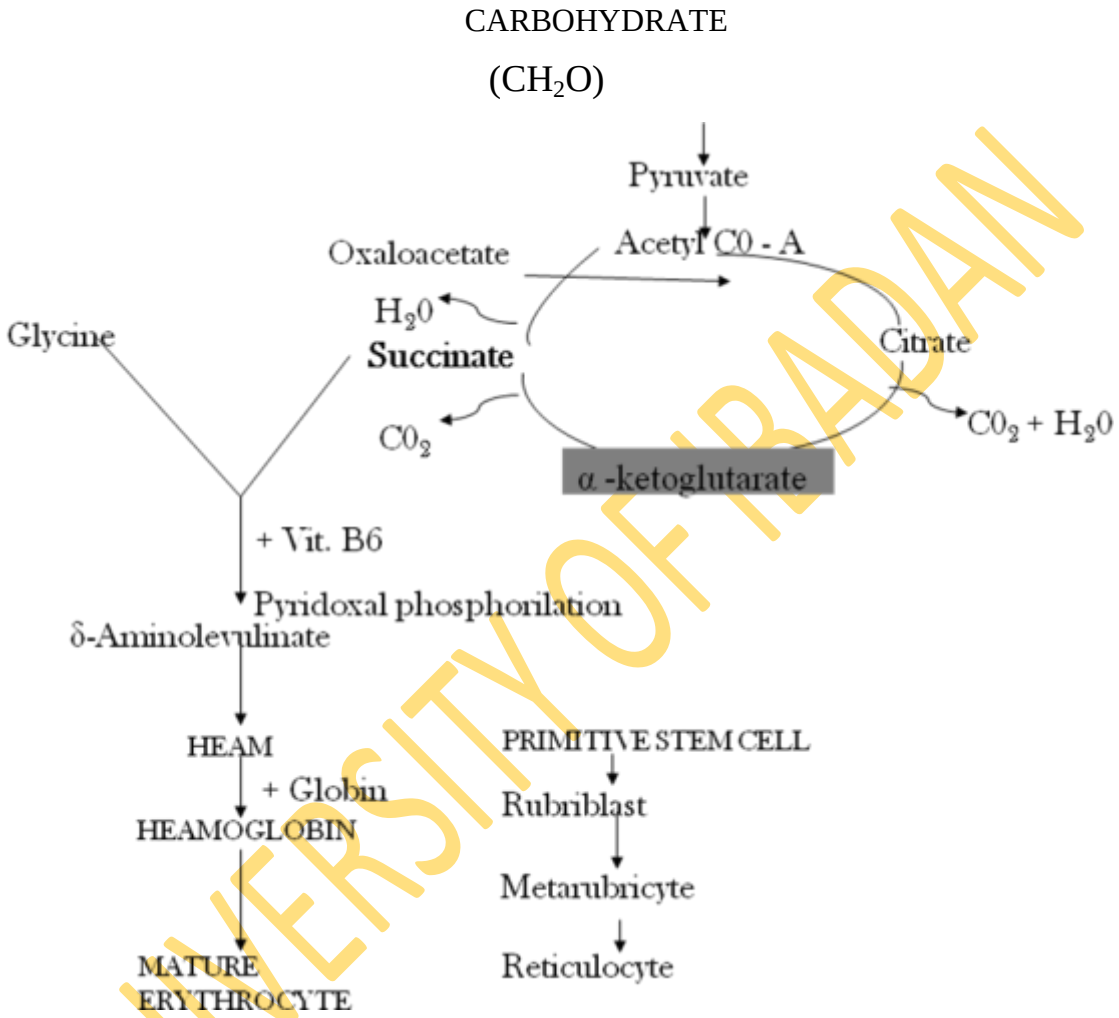
Lack of energy and protein hardly damages the humoral immunity; the number of B-lymphocytes in the blood is rather independent from the changes in feeding, the serum IgG and IgM level is stable and the IgA concentration generally is slightly increased (Chandra, 1990).However, the concentration of secretory IgA in the produced nasal, conjunctival, pulmonary discharges and intestinal secretion decreases.In the latter the species differences are great and a low lachrymal IgA level and subsequent chromorhinodacryorrhea in rat is a very sensitive indicator of the protein deficiency or discomfort (Morton and Griffith, 1985; Meek *et al.*,2003; Zoukhri, 2006). Undernutrition fundamentally affects the cell-mediated immune response and the complement production.Under the influence of juvenile malnutrition the lymphoid organs, like thymus, lymph nodes, tonsils and spleen become atrophied. Number and activity of circulatory T-lymphocytes decrease, which can be demonstrated by skin hypersensitivity reaction, although the number of leukocytes does not change, their intracellular bacterial and fungal killing activity decreases. The susceptibility of the individual immune function is different and the manifestations are also individual according to the species.Obesity is a special form of malnutrition.Leptin acts as a cytokine-like immune regulator that has complex effects in both overnutrition and in the inflammatory response in malnutrition (Cunningham-Rundles *et al.,2005*). Dietary fat is capable of activating cholinergic anti-inflammatory pathway, which may explain the failure of immune system to react to feed antigens and commensal bacteria (Tracey, 2005). The role of individual amino acids is also peculiar in immune response. Provision of arginine (Lewis and Langkamp-Henken, 2000), glutamine and branched-chain fatty acids (Leucin, isoleucin, valin) are essential in the APR (Calder, 2006). Dietary methionine is a precursor of the anti-inflammatory glutathione and taurine, but also of the proinflammatory homocysteine.Because the predominance of actual metabolic pathway depends also on the genetic background of the individuals, the methione overdosage may be harmful (Grimble,2006). The injury of the immune system depends not only on the degree of malnutrition, but also on the presence of other metabolic troubles and also on the age. Thus, the malnutrition is only an important member of the factors,besides infective agents,

stress, surgical intervention,tumours,endocrine and metabolic troubles debilitating the patients immune system. Improvement in feeding enhances the appropriate immune response and decreases susceptibility against infection.

2.1.13 Energy and Protein deficiency in Haemopoiesis

The energy metabolism of the ruminant is focused on the utilization of the volatile fatty acids produced by rumen fermentation rather than on carbohydrates as in the nonruminant. The low levels dietary energy causes ineffective erythropoiesis with prominent disorder of heme, a pigment component of haemoglobin in the developing erythroid cells in the marrow. Heme is a planar molecule composed of the tetrapyrrole protoporphyrin 1X, containing a central ferrous molecules. The initial rate controlling step in heme synthesis, the delta aminolevulinic acid (ALA) synthase (5-amino levulinate synthase) reaction occurs with in mitochondria. The energy level of feeds depends on Glycine and the krebs cycle intermediate succinyl-coA (as substrates) and vitamin B6 as pyridoxical phosphate is required as a cofactor. The ALA formed is transported to the cytoplasm where a series of reactions results in the formatiom of coproporphyrinogen 111, which must enter the mitochondria for the final steps in the heme synthesis (Harvey,2008). Following synthesis, heme must be transferred from mitochondria to the cytoplasm for combination with the globin chains to complete the synthesis of haemoglobin. Heme, therefore affects erythroid cell metabolism in different ways depending on the stage of maturation. The succinyl .coA is a key subsrate in the heme synthesis and it is furnished by glucose breakdown. Its avalability varies with the levels of energy in the goats feed, therefore if the levels of dietary energy is low, the succinyl.coA produced from krebs cycle will be low and hence heme synthesis is affected and the production of haemoglobin in the erythroid cell will be affected and the levels of red blood cell production will be affected.

RELATIONSHIP OF THE DIETARY ENERGY TO ERYTHROPOIESIS



(Courtesy of Kaneko,2008)

2.2 African trypanosomosis.

African trypanosomosis is broadly categorized in to Human African Trypanosomosis (HAT) and African Animal Trypanosomosis (AAT) based on the susceptibility of the hosts and the aetiological agents involved. HAT is caused by hemoflagellates of the Trypanosoma genus, Trypanozoon subgenus and brucei species which classically include three sub species; *Trypanosoma brucei brucei*, *T.b. gambiense* and *T.b. rhodesiense.* (Hoare,1972;Smith *et al.*, 1995).

2.2.1 African Animal Trypanosomosis (AAT) is a disease complex caused by *Trypanosoma congolense*, *Trypanosoma simiae*, *T. vivax*, or *T. brucei*, all transmitted by tsetse-fly or simultaneous infection with one or more of these trypanosomes. African animal trypanosomosis is most important in cattle but can cause serious losses in pigs, camels, goats, and sheep (Mare, 1998). Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea, and rapid loss of condition and often terminates in death. In South- Africa the diseases is widely, known as Nagana, which is derived form a Zulu term meaning "to be in low or depressed spirit" – a very apt description of the disease.(Hoare,1972; Ikede and losos 1972; Goodwin *et al.*, 1972; Finelle,1973a; Itard, 1989)

2.2.2 Aetiology:

The pathogenic trypanosomes in the genus *Trypanosoma* are divided into two sections namely, salivarian and stercorarian according to their development and transmissions by vectors. Each section is further sub-divided into sub-genera and species (Hoare, 1972; Itard, 1989). Those of veterinary importance are :

Salivarian type which include all pathogenic trypanosomes in Africa

Subgenus	**Typical species**
Duttonella	*Trypanosoma vivax and T. uniforme*
Nannomonas	*T.congolense* and *T. simiae*
Trypanozoon .	*T.brucei rhodesiense, T. gambiense, T. b. brucei, T.b. evansi* and *T. equiperdium*
Pycnomonas	*T. suis*

Stercoraria include

Subgenus	**Typical species**
Schizotrypanum	*T. cruzi*
Mega trypanum	*T. theileri*
Herpertosoma	*T. lewis*

African animal trypanosomosis is caused by protozoa in the family *Trypanosomatidae* genus *Trypanosoma* (Hoare,1972;Itard,1989;Mare,1998). *T. congolense* belongs to the subgenus *Nannomonas*, a group of small trypanosome with medium-sized marginal kinetoplasts, no free flagella, but with poorly developed undulating membranes. In East Africa, *T. congolense* is considered to be the single most important cause of AAT. *T.vivax* is a major cause of the disease in cattle in West Africa. Sheep, goats, horses and pigs may also be seriously affected. In domestic dogs, chronic infection often results in a carrier state.

T. vivax is a member of the sub-genus *Duttonella*, a group of trypanosomes with large terminal kinetoplasts, distinct free flagella, and inconspicuous undulating membranes. *T. vivax* is a large (18-26µm long) monomorphic organism that is very active in wet-mount blood smears. Cattle, sheep and goats are primarly affected. Although this organism is considered to be less pathogenic for cattle than *T. congolense*, it is nevertheless the most important cause of AAT in cattle. This trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (Itard,1989).

T. brucei brucei is a member of the sub-genus *Trypanozoon*. *T. brucei brucei* is an extremely pleomorphic trypanosomes occurring as short, stumpy organisms, long slender organisms with distinct flagella, and intermediate forms that are usually flagellated. Horses, dogs, cats, camels and pigs are very susceptible to *T. brucei brucei* infection. Infection of cattle, sheep, goats and sometimes pigs results in mild or chronic infection.

2.2.3 Host range

Cattle, sheep, goats, pigs, horses, camel, dogs, cats and monkeys are susceptible to AAT and may suffer syndromes ranging from subclinical mild or chronic infection to acute fatal disease. Rats, mice, guinea pigs, and rabbits are useful laboratory species (Itard, 1989). More than 30 species of wild animals can be infected with pathogenic trypanosomes, and many of these remain carriers of the organism. Ruminants are widely

known to be active reservoirs of the trypanosomes. Wild *Equidae*, lions, leopards, and wild pigs are all susceptible and can also serves as carriers of trypanosomes. (Itard, 1989)

2.2.4 Geographic distribution

Of the three African animal trypanosomes, only *T. vivax* occurs in the Western Hemisphere in at least 10 countries in the carrribean and South and Central America. Trypanosomosis of small ruminant is prevalent in ecological zones of Nigeria i.e in Ebonyi State with prevalence rate of 6.7% for sheep and 3.5% in goat, (Agu and Amadi, 2001). In Gboko Local Government of Benue State prevalence rate of 51.6% in sheep and 33.3% in goats was reported (Kalu *et al*, 1991). In Nsukka area of eastern Nigerian, prevalence rate of 13.6% in WAD sheep and goat was reported (Fakae and Chijenia, 1993), studies across all agroecological zone of Nigeria indicated a prevalence rate of 8.6% in sheep and 8.1% in goats (Onyia, 1997), in the southern humid region, ILCA, (1983) reported a prevalence rate of 0.1% for sheep and 3.5% for goat, in Zaria northern zone of Nigeria Ahmed and Agbede,(1993) reported 1.2% for goats, in Kano state of Nigeria (Kalu and Lawani, (1996) reported a prevalence rate of 1.2±1.6% for sheep and 0.7±1.3%for goats in northern Nigeria, Daniel *et al.*, (1994) reported a prevalence rate of 3.7% for sheep and goats while Joshua and Ige, (1982) reported the predominance of *T. vivax* in the Nigerian Red Sokoto goats.

2.2.5 Transmission

In Africa, the primary vector of *T. congolense*, *T. vivax*, and *T. brucei brucei* is the tsetse fly. These trypanosomes replicate in the tsetsefly and are transmitted through tsetsefly saliva when the fly feeds on an animal.Eleven different species of tsetse flies have been found in Nigeria (Onyia,1997),but the three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favours the open woodland of the savanana; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favours the high, dense forest areas. Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys* and *Chrysops* flies have also been implicated. In Africa, both *T. vivax* and *T. brucei brucei* have spread beyond the 'tsetse fly belts" (Roder *et al.*, 1984), where transmission is principally by tabanid and hippoboscid flies. The vector for *T. vivax* in the Western Hemisphere remains speculative, but several species of hematophagous (especially tabanid and hippoboscid) flies are believed to serve as mechanical vectors (Itard,1989).

2.2.6 Incubaton period

The incubation period for *T. congolense* varies from 4 to 24 days for *T. vivax* from 4 to 40 days; and for *T. brucei brucei*, from 5 to 10 days (Mare, 1998).

2.2.6 Pathogenesis

According to Morrison *et al.*, (1981), the pathogenesis of trypanosomosis is linked with chancre, anaemia, antigenic variation, tissue damage and immunosuppression.Initial replication of trypanosomes is at the site of inoculation in the skin; this causes a swelling and a sore (chancre) (Hoare, 1972). After the bite by a trypanosome-infected tsetse fly, metacyclic forms establish in the skin where they differentiate into bloodstream forms and spread to the vascular system (Lukins and Gray, 1978; Akol and Murray, 1982; Dwinger *et al.*, 1988). The major route of dissemination is via the draining lymph node, and trypanosomes bearing metacyclic antigens are observed in the afferent lymph, but not in the efferent lymph where only bloodstream forms are detected (Lukins *et al.*, 1994). The first visible sign of a response to the parasite in the mammalian hosts (ruminants, man, rabbits) is the appearance a few days later of a local skin reaction or chancre at the infected bitesite (Fairbairn and Godfrey, 1957; Roberts *et al.*, 1969), which always precedes the presence of parasites in the blood. In cattle, the local skin reactions can measure up to 10 cm in diameter and are red, hot, oedematous and painful. The swelling is caused initially by a massive influx of polymorphonuclear cells at the bite site, followed by infiltration of lymphoid and macrophage cells, and reaches a peak during the second week, after which time it subsides to undetectable levels a week later. Lymphocyte subpopulations have been monitored in the skin at the site of the chancre in sheep, and initially a rise in B-and all T-cell populations (CD4, CD5, CD8) was observed, with a marked increase of the CD4/CD8 ratio (Mwangi *et al.*, 1990). Degranulating mast cells have been observed 5 days after infection (Mwangi *et al.*, 1995). Chancres are the consequence of a local inflammatory response against the parasite, but not tsetse salivary products, as trypanocides abrogate the chancre (Akol and Murray, 1985) and intradermal inoculation of in-vitro cultured metacyclic forms will induce chancres (Akol and Murray, 1982; Lukins *et al.*, 1981, Dwinger *et al.*, 1987; Mwangi, *et al*, 1996). The onset, size and duration of the chancre correlate with the number of metacyclic parasite forms that are inoculated into the skin. As tsetse flies inject a higher number of metacyclics of *Trypanosoma brucei* strains than they do of *Trypanosoma congolense* and *Trypanosoma vivax* (Otieno and Dargi, 1979), the most severe chancres are observed with *T. brucei* infections, followed by *T. congolense*, while only a small nodular reaction is seen with *T.*

vivax (Emery and Moloo, 1980;1981). Intradermal inoculation of bloodstream forms can also induce detectable chancres, suggesting that the inflammatory reaction is not due to any specific metacyclic product (Dwinger *et al.*, 1987). Antibody developed to the glycoprotein coat of the trypanosome kills the trypanosome and results in the development of immune complexes. Antibody however, does not clear the infection, for the trypanosome has genes glycoprotein to evade the antibody. Thus, there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immune complex development, and changing surface-coat glycoprotein Immunologic lesions are significant in trypanosomosis, and it has been suggested that many of the lesions (e.g anemia and glumerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent, normal organ function. The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary diseases, which greatly complicates both the clinical and pathological features of trypanosomosis.

2.2.8 Clinical signs

Because simultaneous infections with more than one trypanosome species are very common (Nyeko *et al.*, 1990) and simultaneous infection with trypanosomes and other hemoparasites (*Babesia spp, Theilleria spp, Anaplasma spp and Ehrlichial spp*) frequently occurs, it is difficult to conclude which clinical signs are attributable to a given parasite. Few adequately controlled studies have been made, and thus a "typical" clinical response to each trypanosome is difficult to reconstruct. What follows is a summation of the syndromes observed in field and experimental cases of trypanosomosis caused by each of three African animal trypanosomes.

The cardinal clinical sign observed in AAT is anaemia. Within a week of infection with the hematic trypanosomes (*T. congolense* and *T. vivax*) there is usually a pronounced decrease in packed cell volume, hemoglobin, and red blood cell than the preinfection values (Joshua *et al.*, 1996; Mare, 1998). Also invariably present are intermittent fever, edema and loss of condition. Abortion may be seen and infertility of males and females may be a sequel. The severity of the clinical response is dependent on the species and the breed of affected animal and the dose and virulence of the infecting trypanosome. Stress, such as poor nutrition or concurrent disease, plays a prominent role in the disease process,

and under experimental conditions, where stress may be markedly reduced, it is difficult to elicit clinical disease (Stephen, 1970; Robertson, 1978, Mare, 1998).

T. congolense is a haematic trypanosome found only in the blood vessels of the animals it infects (Itard,1989). It does not localize and multiply outside blood vessels (Mare,1998). Infection with *T. congolense* may result in peracute, acute, or chronic disease in cattle, sheep, goats, horses and camels. Pigs often develop a milder disease; chronic disease is common in dogs. The incubation period is followed by intermittent febrile episodes, depression, lethargy, weakness, loss of condition, anemia, salivation, lacrimation, and nasal discharge. As the disease progresses, loss of condition and hair colour changes from black to metallic brown are seen. The back is often arched and the abdomen "tucked up". Accelerated pulse and jugular pulsation occur and breathing is laboured. Anemia is a prominent sign. Early in the disease progresses to its acute and chronic forms, organisms are most readily demonstrated in lymph node smears (Joshua, *et al.*, 1996 , Mare, 1998).

T. vivax has a variable incubation period, and although it is considered to be less virulent for cattle than *T. congolense*, mortality rates of over 50 percent can occur. There seems to be a marked variation in the virulence of different strains of *T.vivax*, but it remains the most important cause of trypanosomosis of cattle, sheep, and goats in West Africa. It causes mild disease in horses and chronic diseases in dogs. *T.vivax* is often difficult to find in blood smears and can also be demonstrated in lymph node smears (Mare,1998).

T. brucei brucei has a relatively short incubation period and causes severe to fatal infection. This is followed by recurrent febrile response occurs in the horse 4-14 days after infection.The heart beat and respiration may be accelerated, and loss of condition and weakness are seen, whereas the appetite remains good (Mare, 1998). Progressive anemia and icterus, and oedema of the ventral regions, especially the male genitalia, are characteristic. The organisms are not always easily perceived in blood smears and are best demonstrated in tissue smears or sections, such as lymph nodes. Infected animals die in a few weeks or several months, depending on the virulence of the strain of *T. brucei brucei* (Itard, 1989; Mare, 1998).

The marked immunosuppression resulting from trypanosome infection lowers the host's resistance to other infections and causes in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomosis.

2.2.9 Gross lesions

There is no pathognomonic sign in AAT but in the acute stage there is loss of condition grossly, anaemia, oedema and serous atrophy of fat are commonly observed (Itard, 1989). Subcutaneous edema is particularly prominent and is usually accompanied by ascites, hydropericardium, and hydrothorax. The liver may be enlarged, and edema of lymph nodes is often seen in the acute disease, but they may be reduced in size in the chronic diseases. The spleen and lymph muscle and subserous petechial hemorrhages commonly occur. Gastroenteritis is common and focal polioencephalomalacia may be seen. A localized lesion (chancre) may be noted at the site of fly bite, especially in goats. The microscopic examination of blood will show that the haemopoietic system is actively trying to compensate for the loss of red cells with regenerative changes such as anisocytosis, poikilocytosis, polychromasia, and punctuate basophilia. All, some, or none of the above may be seen in some outbreak (Itard, 1989).

The lesions caused by the trypanosomes in susceptible host species vary considerably, depending on species and strain of trypanosomes and the species and breed of host animal affected. The haematic trypanosomes (*T. congolense* and *T. vivax*) cause injury to the host mainly by the production of severe anaemia, thrombocytopenia. In the terminal stages of the disease caused by the haematic trypanosomes, focal polioencephalomalacia probably results from ischemia due to massive accumulation of the parasites in the terminal capillaries of the brain from those seen with the haematic trypanosomes. Anaemia is an important lesion, but much more dramatic are the inflammation, degeneration, and necrosis resulting from cellular invasion of various organs. Marked proliferative changes reflecting immunologic response are observed in most body tissues.In chronic trypanosomosis the pathological changes seen at post mortem are more striking, without being typical.The carcass is emaciated and often dehydrated.The skin may show pressure sores and ulcers, when the animal has been unable to stand up for some time.The fat reserves under the skin have been used up and the skin is hide bound (Itard,1989).

2.2.10 Diagnosis:

2.2.10.1 Field Diagnosis

Trypanosomosis should be suspected when an animal in an endemic area is anemic and in poor condition. Confirmation depends on the demonstration of the organism in blood or lymph node smears.

In the early phases of infection, especially with *T. vivax* and *T. congolense*, the parasite can readily be observed by microscopic examination of a wet-mount of blood slides. Thick blood films and stained with Giemsa are also a good technique, but in thin fixed blood films, which are favoured for species identification, the parasites may be hard to demonstrate. When parasitemia is low, smears of buffy coat (obtained by microhematocrit centrifugation) can be useful for demonstration of the parasites. Because *T.congolense* tends to associate with the erythrocytes, it is essential that buffy coat and adjacent erthrocytes be included in the smear to ensure demonstration of the parasite.

Stained lymph node smears are a very good method for diagnosis, especially for *T. vivax* and *T. brucei brucei*. In chronic *T.congolense* infection, the parasites localize in the microcirculation of the lymph nodes and in other capillary beds, allowing diagnosis by examination of lymph node smears made with blood collected from the ear. Early in infection, blood smears are optimal for the demonstration of *T. congolense*.

These conventional techniques of microscopic examination for the presence of trypanosomes are still widely used, but newer and far more sensitive methods are beginning to supplement them. The antigen-detecting enzyme-linked immunosorbent assay (ELISA) is extremely sensitive for the detection of trypanosomosis in cattle and goats (Masake and Nantulya, 1991; Trail *et al.*, 1991a,b), and species-specific DNA probes have been shown to detect simultaneous infection of cattle with *T. vivax, T. b. brucei,* and *T. congolense* when conventional methods revealed only single infections (Nyeko *et al.,* 1980). The diagnosis is to demonstrate the parasites either in a white film or in a thin or thick blood smear stained with Giemsa. This is easiest if the animal is in the early stage of disease and is febrile. After the initial stage, parasitemia occurs in cycles of high peaks followed by varying intervals when parasites are few or not detectable even though the animal is still infected and may be sick. To increase the accuracy of clinical diagnosis, special techniques for the detection of parasites are now used routinely in addition to the above standard methods. The most commonly used methods involve concentrating the parasites in a buffy coat layer of a microhematocrit capillary tube and examining for parasites in the tube at a low power or preferably with a dark ground/phase contrast microscope in a wet preparation (Woo *et al.,* 1969). The efficiency of the test varies with the species but it is simple, sensitive and applicable to field use on individual animals as well as in herds. The blood should be examined fresh or refrigerated for up to 25 hours, beyond which most parasites will die and disappear

from the sample. One alternative to direct parasite detection needed the less active and chronic stages of the disease is the inoculation of blood into experimental animals, usually rodents, but this is cumbersome and accurate for only *T. brucei* and possibly *T. congolense* but not *T. vivax*. Another alternative is a series of serological test to detect anti trypanosome antibodies in the sera of the body fluids. The three tests often used are the indirect immunosorbent antibody test (IFAT), the Capillary Agglutination Test (CAT) and the Enzyme Linked Immunosorbent Assays (ELISA). The tests have the advantage that they indicate past as well as present infections but they are difficult to standardize for different laboratories and are not species specific. The ELISA technique was recently modified to detect circulating trypanosome antigen (Rae *et al.*, 1984). By using monoclonal antibodies that distinguish between T*rypanosoma vivax, T.congolense* and *T.brucei*, the antigen, ELISA technique can theoretically detect only current very recent infections and can identify the species involved (Nantaluya *et al.*,1987). The method is currently under trial in several African countries and even though the initial results indicate that the technique may be four to five times more sensitive than the buffy coat technique (Anon. 1992), there are concerns about its specificity.

2.2.10.2 Necropsy Findings

The post mortem lesions are, like the clinical findings, not definitive. The carcass is marked by anemia, emaciation, anasarca and enlargement of the liver, spleen and lymph nodes. Body fat stores are depleted or show marked serous atrophy especially around the heart and in the bone marrow. There may be corneal capacity and testicular degeneration in acute cases, there will be a general congestion of the visceral and extensive hemorrhage in all tissues. As infected animals frequently succumb to secondary bacterial or other parasitic infections, the post mortem picture of trypanosomosis may be masked.

The lesions are also not specific except in very acute infections in which clumps of trypanosomes may be found in blood vessels. Lymphoid organs are usually hyperplastic and may show varying degree of erythrophagocytosis or hemosiderosis. 3Bone marrow may engulf red cells as well as other immature and matured cells. Anosa *et al*.,(1992). The interstitial tissues of various parenchymatous organs may contain a lymphoplasmacytic infiltrate and this tends to be most marked with *T. brucei* in which

the parasites often localize extravascularly in the interstitial tissues. A severe non supportive meningoencephalitis or myocarditis may result. Degenerative changes may also be present in the liver, testis and pituitary gland (Akol *et al.*, 1986).

2.2.10.3 Specimens for the laboratory

To perform the preceding and more sensitive procedures, the following specimens should be submitted to the laboratory from several animals: Serum, blood with the anticoagulant EDTA, dried thin and thick blood smears, and smears of needle lymph nodes biopsies.

2.2.11 Control and eradication.

Control of trypanosomosis has been attempted since hundred years ago and early methods involved a combination of clearing vegetation, extermination of wildlife hosts ,and spraying of bushes.These methods involved destruction of valuable resourses.The new and enviromentally safer methods were developed.These are tsetse fly control and trypanosome control (FAO,2003).

2.2.11.1 Vector control

Fly eradication and drug prophylaxis are the only effective trypanosomosis control methods now available. Several approaches to fly control have been used with varying degrees of success. Discriminative bush clearing, extensively used in early tsetse fly eradication campaigns, has been locally useful because it eliminates the breeding places of the tsetse. But, to be completely effective, bush clearing requires ecologically unacceptable destruction of vast areas of bush and forest. It is still a useful procedure when used locally in conjunction with other control methods (FAO, 2003).

Game elimination, and thus elimination of the main source of blood meals for the tsetse, was used in early eradication campaigns.This was an ineffective, wasteful and contentious control method.

Application of the sterile male technique (as used in screwworm eradication in the United States) received considerable attention in the 1980's.Early problems with breeding of the male flies have been overcome, and field trials have been done in both East and West Africa to determine the effectiveness of this approach in vector control. In limited trials, this procedure has reduced fly populations (FAO, 2003).

Ground and aerial spraying with insecticides and the use of synthetic pyrethroids on cattle have lowered fly densities in some areas, but widespread use would require considerable international cooperation and expense. Widespread application of insecticide has the tremendous disadvantages of also eradicating many other arthropods, several of

which are desirable.The recent introduction of odour-baited targets impregnated with insecticides is proving promising as a means of reducing the tsetse fly (FAO, 2003).

2.2.11.2 Chemotherapy and chemoprophylaxis

The use of drugs for the prevention and treatment of trypanosomosis has been important for many decades, but the rapidity with which the trypanosomes have developed resistance to each drug introduced has tremendously weakened this approach to controlling the disease. The drug pyrithidium bromide (Prothidium and AD 2801) is useful in the prophylaxis of *T. vivax* and *T. congolense* infections in cattle, sheep and goats and can give protection for up to 6 months. The most widely used of the newer chemoprophylactic drugs (and also the least expensive) is isometamidium chloride (Ogunyemi and Ilemobade, 1989). This drug, in use for over 20 years and sold under the trade names Samorin^{R}, Trypamidium^{R}, and M and B 4180A, is excellent for the prophylaxis of all three African animal trypanosomes, and give protection for 3-6 months. The development of resistance to this drug has been reported in both east and West Africa. Homidium bromide has also been found to be an effective chemoprophylactic drug in Kenya, and the newly introduced arsenial malarsamine hydrochloride (Cymelarsan$^{R)}$ is effective in treatment of *T. brucei brucei* infection.

A very widely used chemotherapeautic drug is diminazine acetate (Berenil^{R}), which is effective against all three African animal trypanosomes (Geerts *et al.*, 2001). The isometamidium chloride is also an excellent chemotherapeutic agent. Although extensively used in trypanosomosis control, chemoprophylaxis is an expensive, time-consuming, and thus unsatisfactory long-term solution to the problem of African animal trypanosomosis

Chemotherapy in livestock currently depends upon the salt of six compounds, several of which are chemically closely related. These groups are the phenanthridines, isometamidium and homidium, and the aromatic diamidine, diminazene (Radostitis *et al.*, 2007). After the introduction of isometamidium in 1961 (Berg *et al.*, 1961) the development of new trypanocidal drugs has made little progress. The incidence of resistance to these drugs is apparently on the increase (Peregrine, 1994) and the main means of controlling the disease is therefore under threat.

2.2.11.2.1 Diminazene aceturate

Diminazene is probably the most commonly used therapeutic agent for trypanosomosis in livestock in SSA (Geerts and Holmes, 1998). Sensitive populations of *T. congolense* and *T. vivax* are eliminated by intramuscular treatment of the host with diminazene aceturate

at a dose of 3.5 mg/kg bw. Diminazene aceturate was thought to have some prophylactic activity, providing protection against natural infection for up to 3 weeks when used at a dosage of 7 mg/kg bw. However, the drug is quickly excreted (Bauer, 1962; Karanja *et al.*, 2002) and is not used as a prophylactic. Treatment of domestic livestock with standard therapeutic doses of diminazene aceturate (3.5-7.0 mg/kg bw) rarely results in signs of toxicity. Since its therapeutic index in most animals is relatively large, cattle for instance can tolerate doses as high as 21 mg/kg b.w without exhibiting signs of systemic toxicity (Fairclough, 1963). In camels, a single dose of 7.0 mg/kg b.w can be highly toxic (Leach, 1961). Diminazene is also relatively toxic in dogs (Losos and Crockett, 1969).

2.2.11.2.3 Isometamidium chloride (ISMM)

Isometamidium (Berg *et al.,* 1961) is a phenanthridine-aromatic amidine marketed as both a therapeutic and prophylactic agent. In the prophylactic recommended dose (0.5-1.0 mg/kg bw), the compound has been successfully used to maintain the productivity of Zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Moloo *et al.*, 1987). On Mkwaja ranch in Tanzania where there was a high tsetse challenge, cattle maintained under isometamidium prophylaxis were 80% as productive as high quality Boran cattle on a trypanosomosis-free ranch in Kenya (Trail *et al.*, 1985). This form of mass treatment exerts a strong selection pressure on the trypanosome population (Geerts and Holmes, 1998). As a result, multiple drug resistance later developed on this ranch (Fox *et al.*, 1993; Peregrine, 1994; Geerts and Holmes, 1998). There however, is considerable variation in the prophylactic activity of ISMM (2-22 weeks) even at a dose rate of 1.0mg/kg (Kirby, 1964; Pinder and Authie, 1984; Peregrine *et al.*, 1991). Variation in drug susceptibility between different trypanosome populations appears to be the major factor determining the duration of prophylaxis (Peregrine *et al.*, 1991). Reduction of the number of trypanocidal treatments by integrating drug treatment with other control measures may help alleviate the problem (Geerts and Holmes, 1998).

2.2.11.2.4 Homidium

Homidium (chloride salt: Novidium; bromide salt: Ethidium) is a phenanthridium whose antitrypanosomal activity was demonstrated more than 60 years ago (Browning *et al.*, 1938). Homidium was extensively used in the 1960s and 1970s but its usefulness has been greatly reduced due to widespread resistance (Scott and Pegram, 1974) and possible mutagenic effects.Over the years it has remained essentially a curative drug in the field, despite claims that the 14 drug has prophylactic properties (Dolan *et al.*, 1990).

Homidium is active against *T. congolense* and *T. vivax* infections and is recommended for intramuscular use at a dose of 1 mg/kg b.w.

2.2.12 Trypanocidal resistance

There is growing concern that the future effectiveness of trypanocidal drugs may be severely curtailed by widespread drug resistance (Geerts and Holmes, 1998). This is compounded by the fact that no new drugs have been produced for treatment of animals over the last 40 years, except for melarsomine. The use of the same drugs over such long periods has resulted in the widespread development of drug-resistant strains of trypanosomes (Peregrine,1994).Resistance of trypanosomes to trypanocidal drugs has been reported in many parts of Africa (Jones, 1967; Authie 1984; Peregrine *et al.*, 1988,Joshua *et al*., 88;1995). In Kenya, drug resistant *T. congolense* (Gitatha, 1979, Joshua *et al*., 1995) and *T.vivax* (Schönefeld *et al.,* 1987) have been described. Work carried out by *Clausen et al.,* (1992) in Burkina Faso indicated that diminazene aceturate at 7.0 mg/kg b.w cured infections of *T. vivax,* but was ineffective against *T. congolense* in 20 Zebu cattle that were naturally infected. Several reports from Southwest Ethiopia (Codjia *et al*., 1993; Leak *et al.,* 1993; Rowlands *et al*., 1993) also indicated that there is a severe drug resistance problem to both diminazene aceturate and isometamidium chloride. Rowlands *et al.,* (1993) demonstrated that in the Ghibe Valley in Ethiopia, the mean prevalence of diminazene resistant infections increased significantly from 6% in 1986 to 14% in 1989. Codjia *et al.,* (1993) subsequently determined the drug-resistance phenotype of trypanosome isolates from this site and confirmed the occurrence of drug-resistant populations. Furthermore, development of multiple-drug resistant strains of *T. congolense* isolates has been detected in several areas: in the Bobo-Dioulasso region of Burkina Faso (Authie, 1984; Sones *et al.,* 1988; Moloo and Kutuza, 1990; Clausen *et al.,* 1992) and Ethiopia (Codjia *et al*., 1993; Mulugeta *et al.,* 1997; Afewerk *et al.,* 2000). This suggests that the concept of the sanative pairs might no longer be working in certain regions. The 'sanative pairs' of trypanocides are ones in which induction of resistance to one drug does not lead to resistance in the other (Whiteside, 1960). Examples of these pairs are: isometamidium and diminazene or homidium and diminazene. Thus, integration of tsetse and trypanosomosis control methods is deemed necessary (Holmes, 1997).

2.2.13 Immunization

No vaccine is currently available for African animal trypanosomosis .

2.2.14 Trypanotolerance

It has long been recognized that certain breeds of African cattle are considerably more resistant to African trypanosomosis that others. This is especially true of the West African short-horned cattle (Muturru, Baoule, Laguna, Samba and Dahomey) and the N'Dama, which is also of West Africa. These cattle breeds have existed in the region for over 5,000 years. Susceptibility studies have shown the N'Dama to be the most resistant breed followed by the smaller West African short-horned cattle, but the large and more recently introduced Zebu is the most susceptible (Murray *et al.*, 1979). The mechanisms of trypanotolerance have been extensively studied, and it is now well established that trypanotolerance has a genetic basis (Moulton and Sollod, 1976; Murray *et al.*, 1984). Trypanotolerance in sheep and goats has also been described (Kumshe, 2005), but the mechanisms of the tolerance phenomenon have not been defined.

2.2.14.1 Genetic Markers of Trypanotolerance

Till now, there is no genetic marker that can be used for selecting trypanotolerant cattle, sheep or goats. This means that selective breeding for trypanotolerance has to rely on laborious and time-consuming progeny-testing programmes and on subjective evaluation of the host phenotype. The phenotypic assessment may also be a prerequisite for the identification of genetic markers. The animal industry can greatly benefit from the knowledge gained as a result of research studies carried out in humans. The problems facing animal breeders are significantly different from those encountered by human geneticists, genetic markers can be used to rapidly identify genes of economic importance, thereby enabling cloning and characterisation. In addition, it is possible to define with these techniques a series of DNA markers closely linked to major genes which determine production traits or disease resistance. Such markers can be used to predict at birth or even in pre-implantation embryos (for example, immediately prior to embryo transfer) the phenotype of an animal, which until now could not be assessed until maturity. Pleomorphic markers will allow the "tagging" of specific segments of chromosomes. One can then follow the segregation in pedigrees of the tagged chromosomal regions and associate the marker with the presence of a desirable trait such as high productivity or disease resistance. Gelderman *et al.*, (1985) demonstrated the feasibility of this approach for milk production in dairy cattle and Beever *et al.*, (1989) for certain carcass characteristics in beef cattle. The development of a series of DNA markers

that will identify genes controlling traits of production or disease resistance requires a coordinated, international research effort. Given the rapid advancement of linkage analysis in man, the establishment of a gene linkage map in domestic animals is now realistic and possible. The use of "reverse genetics", i.e. the identification of genes without prior knowledge of the causal mechanisms involved, has provided dramatic results in recent years. But we must not be too optimistic for the prospects in the detection of the genes of trypanotolerance. The problem in practice is that measuring objectively and accurately a milk production or carcass characteristics is much easier than measuring trypanotolerance. At the same time, there has been considerable advancement in our knowledge of the immunological processes involved in disease resistance. It is vitally important that studies aimed at elucidating the pathways determining disease resistance and trypanotolerance in particular be undertaken in conjunction with the molecular genetics.

2.2.14.2 Estimation of trypanotolerance by measuring phenotypical traits.

As stated above, the estimation of trypanotolerance by measuring phenotypical traits by using different parameters is probably a prerequisite to further progress in selective breeding or the development of polymorphic markers and DNA markers (Baderha *et al.*,1990). Since there are different degrees of trypanotolerance, one must not only identify the phenomenon but also measure it both between animal breeds and within breeds. For that purpose, different parameters are used, but most of them present different problems of objectivity and/or accuracy, and/or sensitivity and/or feasibility.In the practice, five different types of criteria could be used in an attempt to "measure" the trypanotolerance during natural or experimental trypanosomosis: (i) direct pathological criteria e.g. parasitaemia, anaemia, survival time duration, prepatent period, (ii) indirect production criteria e.g. body weight and meat production, milk production, working capacity, reproductive performance, (iii) immunological parameters e.g. trypanolytic antibodies production, activation of complement by the classical pathway (CPW) and the alternative pathway (APW), cofactors of complement e.g. C_3 factor, B factor, different other effectors of immunity, e.g. interleukine ˙, conglutinin, immunocompetent cells and the local cellular reactivity at the inoculation site of the trypanosomes, etc… (iv) non-immunological factors of resistance against trypanosomosis e.g. trypanotoxin, (v) treatment index: frequency of trypanocide treatment necessary to maintain more or less

trypanotolerant animals in optimal healthy condition under natural trypanosome challenge (e.g. Berenil index) (Baderha *et al.*,1990)..

2.2.15 Public health

Trypanosoma brucei brucei, although not causing human disease, is closely related to *T. b. gambiense* and *T. b. rhodesiense*. The latter is the cause of human sleeping sickness, a very debilitating and often fatal disease considered to be of major public health significance in 36 Sub-Saharan countries of West, Central, and East Africa with 50 million people at risk (Nyeko *et al.,* 1990). In west and central Africa, a chronic form of human sleeping sickness is caused by *T. b. gambiense*, which uses humans as its major host but also infects pigs. In east and southern Africa, *T. b. rhodensiense* is the cause of a much more acute form of human sleeping sickness. This trypanosome also infects cattle, bushbuck (*Tragelaphus scriptus*) and probably many other wild animals that may serve as reservoirs of the parasite (Nyeko *et al.,* 1990).

2.3.0 COMPLEMENT SYSTEM

The complement system comprises a group of serum proteins and cell membrane receptors that function primarily to fight infection. Clinically, measurement of complement pathway activity and individual component levels is of value in cases of immunodeficiency and inflammatory conditions that involve complement activation. These components interact in three activation pathways and one final pathway (Jonathan and Whaley, 2001).

2.3.1 Pathways of complement activation

Complement activation can be divided into three pathways and one final pathway: the classical pathway (CPW), the lectin pathway(LPW), the alternative pathway(APW) and the membrane attack (or lytic) pathway(MAPW). Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is essential for the activation of the membrane attack pathway (Roitt *et al.,* 1989; Jonathan and Whaley, 2001).

2.3.2 Classical pathway (CPW)

1 C1 activation C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have

interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. In some cases C1 can bind to aggregated immunoglobulin [e.g. aggregated IgG] or to certain pathogen surfaces in the absence of antibody). The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.(Roitt, *et al.*, 1989;Arinola, 2004)

11. C4 and C2 activation (generation of C3 convertase)

The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released into the micro-environment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b (Roitt *et al*., 1989; Jonathan and Whaley 2001; Arinola, 2004)

111. C3 activation (generation of C5 convertase)

C3b binds to the membrane in association with C4b and C2a, and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway. Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Some of these products may also have detrimental effects if produced in an unregulated manner (Roitt, *et al.*, 1989;Arinola, 2004).

2.3.3 Lectin pathway(LPW)

The lectin pathway is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated

MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway. The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway (Roitt *et al.*, 1989; Jonathan and Whaley, 2001).

2.3.4 Alternative pathway (APW)

The alternative pathway begins with the activation of C3 and requires Factors B and D and Mg^{++} cation, all present in normal serum (Jonathan and Whaley 2001).

1. Amplification loop of C3b formation

In serum there is low level spontaneous hydrolysis of C3 to produce C3i. Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb. The C3iBb complex acts as a C3 convertase and cleaves C3 into C3a and C3b. Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D. The resulting C3bBb complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. If this process continues unchecked, the result would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled (Roitt *et al.*,1989; Jonathan and Whaley,2001; Arinola,2004)

11. Control of the amplification loop

As spontaneously produced C3b binds to autologous host membranes, it interacts with DAF (decay accelerating factor), which blocks the association of Factor B with C3b thereby preventing the formation of additional C3 convertase. In addition, DAF accelerates the dissociation of Bb from C3b in C3 convertase that has already formed, thereby stopping the production of additional C3b. Some cells possess complement receptor 1 (CR1). Binding of C3b to CR1 facilitates the enzymatic degradation of C3b by Factor I. In addition, binding of C3 convertase (C3bBb) to CR1 also dissociates Bb from the complex. Thus, in cells possessing complement receptors, CR1 also plays a role in controlling the amplification loop. Finally, Factor H can bind to C3b bound to a cell or in the in the fluid phase and facilitate the enzymatic degradation of C3b by Factor I. Thus, the amplification loop is controlled by either blocking the formation of C3 convertase, dissociating C3 convertase, or by enzymatically digesting C3b. The importance of

controlling this amplification loop is illustrated in patients with genetic deficiencies of Factor H or I. These patients have a C3 deficiency and increased susceptibility to certain infections (Roitt *et al.,* 1989, Arinola, 2004)

111. Stabilization of C convertase by activator (protector) surfaces

When bound to an appropriate activator of the alternative pathway, C3b will bind Factor B, which is enzymatically cleaved by Factor D to produce C3 convertase (C3bBb). However, C3b is resistant to degradation by Factor I and the C3 convertase is not rapidly degraded, since it is stabilized by the activator surface. The complex is further stabilized by properdin binding to C3bBb. Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram-negative bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3(JonathanandWhaley,2001).

1V. Generation of C5 convertase

Some of the C3b generated by the stabilized C3 convertase on the activator surface associates with the C3bBb complex to form a C3bBbC3b complex. This is the C5 convertase of the alternative pathway. The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The alternate pathway pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents. Many gram negative and some gram positive bacteria, certain viruses, parasites, heterologous red cells, aggregated immunoglobulins (particularly, IgA) and some other proteins (e.g. proteases, clotting pathway products) can activate the alternative pathway. One protein, cobra venom factor (CVF), has been extensively studied for its ability (Jonathan and Whaley 2001;Arinola, 2004).

2.3.5 Membrane attack (lytic) pathway

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane. Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. Lysis is not an enzymatic process; it is thought to be due to physical damage to the membrane. The complex consisting of C5bC6C7C8C9 is referred to as the membrane attack complex (MAC). C5a generated in the lytic pathway has several potent biological activities. It is the most potent anaphylotoxin. In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages. Its activities are controlled by inactivation by carboxy-peptidase B (C3-INA).Some of the C5b67 complex formed can dissociate from the membrane and enter the fluid phase. If this were to occur it could then bind to other nearby cells and lead to their lysis. The damage to bystander cells is prevented by Protein S (vitronectin). Protein S binds to soluble C5b67 and prevents its binding to other cells (Jonathan and Whaley, 2001; Arinola, 2004).

2.3.6 Biologically active products of complement activation

Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.

i. Kinin production

C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin. Excess C2b production is prevented by limiting C2 activation by C1 inhibitor (C1-INH) also known as serpin which displaces C1rs from the C1qrs complex. A genetic deficiency of C1-INH results in an overproduction of C2b and is the cause of hereditary angioneurotic edema. This condition can be treated with Danazol which promotes C1-INH production or with ε-amino caproic acid which decreases plasmin activity (Jonathan and Whaley, 2001).

ii. Anaphylotoxins

C4a, C3a and C5a (in increasing order of activity) are all anaphylotoxins which cause basophil/mast cell degranulation and smooth muscle contraction. Undesirable effects of these peptides are controlled by carboxypeptidase B (C3a-INA).

Chemotactic Factors

C5a and MAC (C5b67) are both chemotactic factor with C5a being also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells (Jonathan and Whaley, 2001).

iii. Opsonins

C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.

iv. Other Biologically active products of C activation

Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their functions.In summary, the complement system takes part in both specific and non-specific resistance and generates a number of products of biological and pathophysiological significance .There are known genetic deficiencies of most individual C complement components, but C3 deficiency is most serious and fatal. Complement deficiencies also occur in immune complex diseases (e.g., SLE) and acute and chronic bacterial, viral and parasitic infections (Jonathan and Whaley, 2001).

2.3.7 Interaction between Trypanosomes and complement

Complement is involved in the defense against a number of pathogens and recently there has been an increasing interest in the interaction between complement and different protozoa and trypanosomes in particular e.g. *T. lewisi* (Allbright and Allbright, 1985; Sturtevant and Balber, 1987) *T. cruzi* (Kipnis *et al.,* 1985; Sher *et al.,* 1986), *T. brucei gambiense* (Devine *et al.,* 1986) *T.congolense* (Tabel,1982;Malu and Tabel, 1986).Experimental trypanosomiasis in susceptible cattle is characterised by persistent and marked hypocomplementaemia (Kobayaski and Tizard, 1976; Nielsen *et al.,* 1978; Rurangirwa *et al.,* 1980). Authié and Pobel (1990) investigated the kinetics of serum complement and C_3 during natural infection with *T. vivax* and *T. congolense* in cattle of different susceptibility, in order to determine whether there was a relationship to trypanotolerance. They observed a significant correlation between minimum complement activety, C_3 and minimum PCV in early infection. These three parameters correlated with individual resistance and might therefore, be useful criteria for the identification of the most resistant individuals within a trypanotolerant breed. These findings are of highest

interest for the future selective breeding for trypanotolerance. The problems remaining in the practice are: difficulties to set up the experimental design, by keeping cattle out of trypanosome risk for a certain period of time and moving them into a region with natural challenge, the duration of the experiment, death of highly susceptible animals, necessity of treatment of some animals during the experiment, the use of time-consuming techniques for the assessment of the total haemolytic activity of the alternative complement pathway as developed by Barta and Hubberts (1978), the extreme variability of the complement activity (APW and CPW) in different animals before the challenge influencing greatly the minimum complement activity values during the infection, and finally the classical disadvantages of a natural challenge, e.g. exact time of infection, characteristics of the infecting trypanosomes (species, serodemes, virulence, doses, possible cross-reactivity with trypanosomes of previous contacts).Theinvestigation on the kinetics of APW and CPW in Saanen goats was done in Belgium(Baderha *et al.*,1990) and in Borgou and in West African Dwarf Shorthorn cattle in Benin (Doko *et al.*, 1991), during artificial infection with *T. b. brucei* AnTat 1/1 associated or not with four other VATs of the same repertoire. A number of other parameters were evaluated at the same time, e.g. clinical symptoms, PCV, VAT-specific trypanolytic antibodies and heterologous agglutinating antibodies using the Testryp-CATT to evaluate the multiplication rate of infecting trypanosomes.Highly susceptible animals demonstrate a severe decrease of complement in APW and a complete exhaustion in the CPW some days prior to death. In tolerant animals, APW and CPW are decreasing during the parasitaemia phase but are restored to initial values a few days after the trypanosomes have been eliminated and may rise above the initial values in highly trypanotolerant animals. First results seem to indicate that all non-resistant animals may be identified as early as 15–20 days after inoculation, allowing early treatment with trypanocide. To discriminate between the trypanotolerant animals, the “Trypanotolerance Test” has to be continued till day 30–40. Recovery time (days) of APW and CPW are easy parameters to use. The mean PCV decrease is evident for the total group of animals being tested, but seems to be of limited value to discriminate between trypanotolerant individuals, nor to predict death in an early stage. Parasitaemia (duration of the first AnTat 1/1-specific wave, duration of parasitaemia, cumulated parasitaemia values) is a parameter that is directly linked with the ability of the animal to control or to eliminate the trypanosomes. The AnTat 1/1-specific trypanolytic antibody titers display a most characteristic curve, but awkward for interpretation as mentioned above. Since antibodies against AnTat 1/1

do not occur naturally in farm animals in Africa (Demey, 1987), an adequately standardized test can be repeated at any time and at any place, either in tsetse infested areas, without moving cattle, or rearing in a tsetse proof stable or embryo transfers. A new method has also been developed for an easy assessment of APW and CPW in cattle, goat and sheep.

2.4 IMMUNITY

Immunity is defined as the resistance of the body against the pathogenic agents or abilility of the body to resist different types of foreign bodies like bacteria, viruses, toxic substances (Sembulingam and Sembulingam , 2005) and parasites(Halliwell and Gorman ,1989) which enter the body. Immunity is of two types namely, Innate and acquired immunity.

2.4.1 Innate immunity.

Innate immunity is otherwise called natural immunity.It is present from birth and it is the inborn capacity of the body to resist the entry of microorganisms into the body, by chance if the organisms enter the body, it eliminates them before they cause any diseases, this type of immunity represents first line of defense against any pathogens and it is therefore called non specific immunity (Halliwell and Gorman,1989;Sembulingam and Sembulingam, 2005),Innate immunity involved some activities of white blood cells and tissue macrophages,which destroy the foreign bodies by means of phagocytosis,the enzymes of gastrointestinal tract and the acids in the stomach, which destroy the toxic substances or organisms entrying digestive tract through feeds,Protective function of keratinized stratum corneum of skin against bacteria or other organisms, lysozymes and some polypeptides,which destroy or inactivate the bacteria (Halliwell and Gorman, 1989;Sembulingam and Sembulingam, 2005).

2.4.2 Acquired immunity.

This is otherwise known as specific immunity and it is the resistance developed in the body against any specific foreign body like bacteria,viruses,toxins vaccines or transplanted tissues.It is the most powerful immune mechanism that protects the body from the invading organisms or toxic subtances.Lymphocytes are responsible for acquired immunity (Halliwell and Gorman, 1989;Sembulingam and Sembulingam, 2005).

2.4.3 Stage-specific immunobiological changes that occur during infection

2.4.3.1 Macrophages and innate immunity.

Cells of the macrophage lineage provide the first line of host defense against infectious disease, and also modulate downstream events that impact on the development of acquired immunity that eliminates many microbes. It is well established that recognition of microbes by macrophages results in cellular activation following the uptake or binding of microbial components to specific membrane receptors (Mosser, 1992; Hoffmann *et al.*, 1999; Mosser and Karp, 1999). Receptor-mediation activation of macrophages represents one of the first events in the innate immune response to many microbial infections, leading to the production of pro-inflammatory cytokines that initiate an inflammatory response and affect the downstream development of activated T-cells as well as other parameters of the host immunity. Cytokines produced by activated T-cell, primarily IFN-γ provide additional activation signals for macrophagea, unhealthy, effect or functions that can destroy a wide range of intra- and extracellular microorganisms (Fearon and Locksley, 1996; Bendelac and Fearon, 1997; Medzhitov *et al.*, 1997). Thus, the processes of innate resistance and acquired immunity are intimately interdependent,with macrophages playing a dual role as the initiators of acquired response and as a major efector component of cell-mediated immunity.

Macrophage activation in trypanosomosis is one of the hallmarks of infection with the African trypanosomes (Bancroft *et al.*, 1983; Askonas, 1984; Askonas, 1985; and Beschin *et al.*, 1998). There is extensive evidence that the numbers and activity of macrophages increase dramatically in the tissues of trypanosome infected animals, and are associated with tissue pathology. Within the first two weeks of experimental *Trypanosoma brucei rhodesiense* infection, for example, a large percentage of cells in the enlarged spleen exhibit membrane and functional characteristics associated with activated macrophages. These include: increases in the release of IL-12 and IL-18, known to be important in the development of the early polarized Th1 cell responses to tryapanosome antigens (Schleifer *et al.*, 1993;Mansfield, 1994; Schopf *et al.*, 1998; Mansfield , 2001); an enhanced ability to serve as antigen processing cells coupled to increases in expression of membrane I-A, B7-1 and B7-2 and B7-2 (Imboden *et al.*, 2001;); and, upregulation of mRNA or proteins for other markers that include TNF, IL-1, IL-6, iNOS, prostaglandins and IL-10 (Beschin *et al.*, 1998; Magez *et al.*, 1999;Imboden *et al.*, 2001). More importantly, the expression or release of several of these activation markers is associated with modulation of host immunity and resistance. For example NO, prostaglandins and

TNF have been implicated in the suppressor cell activity exhibited by macrophages at different time points of infection (Beschin *et al.*, 1998; Hertz and Mansfield, 1999) although NO and TNF have been shown to kill trypanosomes in vitro and are thought to be important for trypanosome control in extravascular tissue sites (Magez *et al.*, 1997; Mnaimneh *et al.*, 1997), neither factor alone has been linked definitely to protection in vivo (Hertz and Mansfield 1999; Magez *et al.*, 1999) and the expression of these factors may be linked to pathological changes during infection, However, the pro-inflammatory pattern of macrophage activation appears to change over the course of infection to become a counter-inflammatory pattern of activation in which IL-10 predominates and Type 2 cytokine responses appear to emerge (Namangala *et al.*, 2000; 2001) these events have been associated with late stage disease.

2.4.3.2 Role of the variant surface glycoprotein gpi anchor in macrophage activation

The source (s) and mode of activating factors delivered to macrophages during trypanosome infection are only partially understood. Often major activation factor is of parasite origin; this is the glycosylphosphatidylinositol (GPI) membrane anchor of the trypanosome VSG molecule. The GPI anchor precursor is synthesized in the endoplasmic reticulum and subsequently is covalently attached to newly synthesized VSGs after proteolytic cleaveage of a VSG C-terminal GPI attachment signal sequence (Bangs *et al.*, 1998; Menon 1999; Sharma *et al.*, 1999). After further modifications in both the glycoprotein and GPI anchor residue, the mature VSG is transported to and anchored in the trypanosome plasma membrane as membrane-form VSG (GPI-mfVSG). During the course of infection, a trypanosome membrane-associated phospholipase C (GPI-PLC) becomes activated and cleaves the GPI anchor; this results in the release of the original GPI anchor and leaves the dimyristoyglycerol (DMG) lipid component remaining behind in the membrane (Butikofer *et al.*, 1996; Armah and Mensa-wilmot, 2000; Paturiaux-Hanocq *et al.*, 2000). Parasite numbers routinely fluctuate during infection, both in the blood and extravascular tissues, primarily as the result of host B and T-cell responses to variant determinants of the VSG molecule. Since (a) trypanosome numbers may approach 10^8 organisms per ml blood and may also be quite high in the extravascular tissues during peak parasitemias, (b) there are approximately 10^7 VSG molecule per cell, (c) GIP-sVSG is clipped and released from both viable and stressed/damaged trypanosomes, and (d) trypanosomes are episodically destroyed by Ab-Th1 cell/macrophage-dependent effector mechanisms, the amounts of GPI substituents (GPI-mfVSG, GIP-sVSG and DMG) saturating host tissues during infection are quite

substantial. Conservative calculations estimate that experimentally infected animals may be exposed to 15-20μM VSG with each wave of parasitaemia.

Limited in-vitro laboratory studies have begun to characterize the activating effect of GPI subsitutents on macrophages. It appears that GIP-sVSG and GPI-mfVSG (containing the DMG lipid substituent) have similar macrophage activating capabilities in terms of TNFα, IL-6 and NO production, but that there may be subtle differences in the ability of GPI-mfVSG to more effectively induce IL-1 and IL-12 production (Schofield *et al.*, 1996;Magez *et al.*, 1998). An extension of these initial studies regarding the ability of GIP-s VSG to interact with macrophages demonstrates that the GIP-sVSG component binds directly to macrophages induces expression of a specific subset of activation genes in an IFN-γ independent manner (Imboden *et al.*, 2001; Paul nock and Coller, 2001). Studies have also shown that GPI substituents exhibit signaling activities (Tachado, *et al.*, 1997).

During trypanosome infection, it is clear that host cells are exposed to biologically active levels of GIPsVSG, DMG and GPI-mfVSG, although the timing of exposure to these molecules and the nature of their delivery to the macrophage membrane could be very different. While the effects of such GPI substituents on uninfected macrophages have been partially characterized in vitro, the impact of tissue saturation with these activating agents in vivo during infection is just being fully appreciated (Imboden *et al.*, 2001; Paul nock and Coller, 2001). Also, macrophages are not the only cell type that are targeted by GPIs specific for GPI determinants and that B cells may be stimulated to undergo cell differentiation by GPIs (Bento *et al.*, 1996; Schofield *et al.*, 1999). Thus, GPI substituents released by pathogens such as the African trypasomones may have broad effects on the host immune system on the host immune system that surpass central activating effects on macrophages .

2.4.3.3 Role of interferon gamma (IFNγ)- in macrophage activation and early host protection and /or pathology

The other major macrophage activation factor produced during trypanosomosis is IFNY-, which is of host origin. Small amounts of IFN-γ may be produced very early during infection as the result of T-lymphocte trypanolytic factor (TLTF) activation of CD8 T cells (Bakhiet *et al.*, 1996; Schleifer and Mansfield, 2001). First described by Bakhiet, Olsson and colleagues (Bakhiet *et al.*, 1993) and subsequently cloned (Vaidya *et al.*, 1997), TLTF is a protein with potentially important biological effects TLTF was

discovered when researchers noted that rodents injected with *T. b. brucei*, or lymphoid cells cultured with trypanosomes in vitro, exhibited an increase in the number of antigen non-specific IFN-γ secreting cells; depletion of CD8+ T cells in animals or cultures abrogated the effect and, interestingly, also resulted in less trypanosome growth (Bakhiet *et al*., 1990). Use of a chamber system separating lymphoid cells and trypanosome showed that a soluble factor was responsible for induction of IFN- γ synthesis .

Several *Trypanosoma* species appears to express TLTF but may possess different IFN-γ stimulating abilities as measured by the relative increase of IFN-γ producing cell numbers in the presence of extracts or culture filtrates of species including *T. evans*, *T. b. rhodesiense*, and *T. b. gambiense* (Bakhiet *et al.*, 1996). Subsequent characterization of CD8+ T cell IFN-γ activation by TLTF showed that tyrosine protein kinases are necessary for activation but protein kinase C and protein kinase A specifically are not (Bakhiet *et al.*, 1993). Interestingly, TLTF may stimulate other cells to release IFNY-γ, such as rat dorsal root ganglia and this secretion apparently also experimental extension over the past decade have led investigators to posit the TLTF which binds to CD8 molecules expressed on CD8+ T cells, thereby inducing antigen non-specific activation and production of IFN-γ; TLTF-induced release of IFN-γ subsequently serves as a growth factor that promotes trypanosome growth (Bachiet *et al*., 1996; Hamadien *et al.*, 1999). Thus a factor secreted from the parasite, TLTF, is visualized as inducing an essential trypanosome growth factor, IFN-γ, from host cells.

Interestingly, TLTF may stimulate other cells to release IFN-γ, such as rat dorsal root ganglia and this secretion apparently also is dependent on tyrosine kinase (s) (Eltayeb *et al*., 2000). These types of studies and their experimental extension over the past decade have led investigators to posit the following hypotheses with respect to the role of TLTF: trypanosomes secrete TLTF which binds to CD8 molecules expressed on CD8+ T cells, thereby inducing antigen non-specific activation and production of IFN-γ,; TLTF – induced release of IFN-γ subsequently serves as a growth factor that promotes trypanosome growth (Bakhiet *et al.*, 1996; Hamadien *et al.*, 1999). Thus a factor secreted from the parasite, TLTF, is visualized as inducing an essential trypanosome growth factor, IFN-γ, from host cells.

To date there is no clear evidence that TLTF is a secreted protein, though the predicted protein does possess unique internal targeting sequences. More recent studies on the cell biology of TLTF have suggested an alternate (or concident) role for the

protein. The gene sequence identified as TLTF is expressed in both insect and bloodstream forms of *T. b. brucei* and the protein appears to be tightly associated with the flagellar cytoskeleton (present in detergent-resistant and Ca^{2+} resistant cytoskeletal fractions of trypanosomes extracts) (Hill *et al*., 2000), modification of TLTF gene expression in the procyclic form resulted in an unusual motility defect, suggesting that TLTF may be an integral part of the trypanosome cytoskeletal architecture. Surprisingly, TLTF-like genes are present in a number of divergent eukaryotes including *Drosophila* and zebra fish. Notably,the human tumor suppressor molecule with a submolecular region that may localize to cellular microtubules.

Thus, it is difficult to see how TLTF, a tightly bound cytoskeleton-associated molecule, would be secreted or released in biologically active levels during infection or in cell cultures containing viable trypanosomes to affect the release of IFN-γ from host cells. Yet, it is clear that trypanosome infections and trypanosome extracts are capable of inducing IFN-γ release from naïve host lymphoid cells in an antigen specific induction of IFN-γ from Th1 cells (Mansfield, 2001). Thus, IFN-γsecretion induced by parasite material (s) has been a repeatable phenomenon and is clearly of some interest; there is the distinc possibility that release of biologically active TLTF (or a similar molecule with closely related effects) occurs during periods of cataclysmic elimination of trypanosome VATs by host Ab and Th1/macrophage cell response throughout infection.

The IFN-γ /IFN-γ receptor interaction and downstream subscellular signaling pathways have already been well characterized in other model systems, and macrophages activation events triggered by IFN-γ have been extensively studied (Adam and Hamilton, 1986; Hamilton, 1989). In African trypanosomosis, it is clear that some macrophage activation events are dependent on IFN-γ exposure and others on exposure to trypanosome-derived molecules, including GIP-vVGS (Imboden *et al.*, 2001). The activation patterns observed in the presence of both factors are different from either one alone, are dependent on genetic background of the infected host, and may be important in the control of infection. Why trypanosomes induce such broad macrophage activation effects in unknown. Why trypanosomes induce such broad macrophage activation effects is unknown. It may be important for trypanosomes to induce early temporal protection against the infection regardless of the genetically-based resistance status of the host (e.g, so the host is not killed by infection before natural transmission of the disease can effectively occur). Alternatively, it might be linked to the early generation of suppressor macrophage activity so as to depress host T cell responses to parasite antigens, or it may

result in deregulation of IFN-γ induced activation events in macrophages in order to avoid parasite elimination.

2.4.3.4 Tissue specific immune control mechanisms in early infection

There are clear differences in the ability of various host species, and strains within species, to display relative resistance to African trypanosomosis (Levine and Mansfield, 1981). Studies over the past twenty years have revealed that the host antibodies response plays only a great role in such relative resistnce against trypanosomes. While VSG-specific antibody clearly is responsible for the catalysmic elimination of VATs from the bloodstream of infected hosts, it is now known that this event is not linked, functionally or genetically, to overall host resistance (De Gee *et al.,* 1988; Mansfield, 1990; Mansfield and Olivier, 2001). The seminal studies were those in which H-2 compatible radiation chimera mice, reconstituted with reciprocal bone marrow cell transplants from relatively resistant or susceptible donors, revealed the following: that susceptible mice, which normally do not make a sufficient antibody response to VSG and do not clear VATs from the blood, were afforded by donor cells from resistant mice a functional B cell response that enabled them to clear parasitemia during infection; however, depiste the ability to eliminate trypanosomes from the blood, these animals were just as susceptible as mice receiving susceptible donor bone marrow cells that failed to make protective VSG-specific B cell responses (De Gee and Mansfield, 1984). Subsequent genetic studies with crosses between Ab+ resistant and Ab-susceptible mouse strains showed that the F1 hybrids all were able to make VAT-specific Ab responses and control parasitemias, but all such hybrids were as susceptible as the susceptible parental strain (De Gee *et al.,* 1988). Taken together, these types of results showed that the VSG-specific B cell response, although linked to trypanosome clearance from the blood, was not by itself functionally or genetically linked to overall host resistance.

2.4.3.5 The cell responses to trypanosome antigens

A nonspecific immunosuppression of T-cell responses in trypanosomosis had been recognized for many years , and, although earlier studies revealed that T cell responses to trypanosome antigens could be induced in immunized animals (Campbell *et al.,* 1982), such responses were not readily detectable in infected animals (Paul nock *et al.,* 1989). For example, not only were spleen or lymph node T cells from infected mice unable to proliferate in response to mitogens or antigen, they also failed to produce significant amounts of IL-2 or IL-4, and these events could be shown to impact on T-independent B cell responses to a variety of antigens (Mansfield and Bagasra, 1978). This generalized

immunodeficiency was shown to result in part from the presence of macrophage "suppressor cells" in lymphoid tissues (Sacks *et al.*, 1982) in fact, macrophages from infected mice had the capacity to actively suppress the proliferative responses of normal T cells to mitogens and antigens in vitro and in vivo. A breakthrough in recognizing that Th cell responses to trypanosome antigens occurred during infection came with the finding that functional compartmentalization of such responses occurred (Schleifer *et al.*, 1993). It was revealed that Th cells reactive with VSG were predominant in the peritoneal T cell population; when stimulated with VSG, these cells made a substantial IL-2 and IFN-γ cytokine response but failed to proliferate. Subsequently, it was discovered that Th cells in the peripheral lymphoid tissues also made an IFN-γ response but little IL-2, when stimulated with VSG. Thus, it was apparent that VSG-reactive T cells were present in infected animal tissues but that they exhibited a restricted cytokine response and minimal evidence for clonal expansion (Schleifer *et al*., 1993). Since these VSG-reactive T cells displayed a $CD4^+$ γ δT TCR^+ membrane phenotype, expressed Type 1 cytokines, were MHCII restricted and APC dependent (Schleifer *et al*., 1993), it was clear that they represented a classical Th1 subset of T cells that recognized VSG during infection. More recent work has begun to elucidate the submolecular targets of VSG-reactive Th cells. In preliminary studies it has been shown that Th cell specificities are directed against a defined hypervariable subregion of VSGs that is not exposed when VSG homodimers are assmbeld into the surface coat structure (Mansfield *et al.*, 2001), fulfilling earlier predictions that VSG sequence variability in nonexposed regions of the molecule might be driven by T cell selection (Field and Boothroyd, 1996).

The extreme polarization of the Th1 cell cytokine responses seen in some experimental systems is due in part ot the early production of IL-12 by macrophages exposed to trypanosome GPI substituents (Mansfield *et al.*, 2001). That IL-12 is not the only polarizing factor is seen from preliminary studies with IL-12 KO mice and mice exposed to Abs against IL-12; in each case, early temporal depression of the Type 1 cytokine response did not result in a compensatory Type 2 cytokine response and, after a period of 10 days or so, the Th1 cell response emerged in both groups (91). Thus, there are complex features of infection that promote the production of Type 1 cytokines and the outgrowth of antigen-reactive Th1 cells. While reasons for the relative tissue compartmentalization of Th cell cytokine responses (e.g IL-2 and IFN-γ production by peritoneal Th cells, but mostly IFN-γ production by the cells in the peripheral lymphoid tissues) have not been elucidated, the reason for inhibition of T cell clonal expansion has

now been resolved. Suppressor macrophages were shown to elaborate several factors that inhibited the proliferative (but not the cytokine) responses of VSG activated Th1 cells: NO, prostaglandins and TNF (Darji *et al.,* 1996). Macrophages were activated to produce these suppressive factors primarily as the result of exposure to GIP-sVSG and to IFN-γ released by parasite antigen stimulated Th cells (Hertz and Mansfield, 1999). The full implcat of NO and prostagladins on host immunity to trypanosomes has not been completely resolved, but studies with iNOS KO mice have shown that, although NO is the main "suppressor" factor that limits clonal expansion of T cells (and may be also modulates cytokine responses to a degree) the absence of NO didnot affect overall host resistance (Hertz and Mansfield, 1999).

Early and strong trypanosome-specific Th1 cell responses may provide an essential component of host resistacne; this realization emerged from studies with cytokine gene knockout mice. The central finding in one study was that mice with resistant genetic background but which lacked a fucntioanl IFN-γ gene were as susceptible as aids mice to trypanosome infection, even though those mice produced Abs sufficient to control parasitemia (Hertz *et al.*, 1998). In contrast, the same genetic strain of mouse with IL-4 instead of the IFN-γ gene knoced out were as demonstrating that the VAT-specific Ab response and control of parasitemia were not capable of providing resistance alone, and that the production of a single cytokine, IFN-, in response to infeciton was found to be a critical element in host resistance. The mechanism{s} associated with IFN-y- mediated resistance are not yet clear, but seem to involve macrophage factors induced by IFN-y activation. Several candidate factor have been proposed, such as NO and TNFa, both of which have been shown to kill trypanosomes in vitro (Lucas, *et al.*, 1994;Magez, *et al.*, 1999) Recent studies suggest, however, that neither factor alone is capable of mediating resistance in vivo, results with trypanosome infected iNOSKO mice and TNFa KO showed that such mutations on a resistant mouse genetic background do not significantly affect the course of infected (Hertz and Mansfield, 1999; Magez *et al*., 1999), although it is possible that the combination of NO and TNFa is required for functional resistance. Clearly, IFN-y inducible events in macrophages must carefully be evaluated for their impact on trypanosomes during early stages of infection. Since these events occur independently of B cell mediated resistance mechanisms that are known to control trypanosomes in the vasculature, since IFN-y activated macrophage control mechanisms are presumed to be important in regulating trypanosome numbers in the extravascular tissue space, but this by itself is inadequate to

provide protection; (Mansfield *et al.*, 2001), it appears that multiple arms of the host immune system are required to control trypanosomes and to provide relative resistance during infection. Thus, relative resistance to African trypanosomes may be mediated by two major components of host immunity, neither one of which by itself is adequate to provide resistance. First, VSG specific Ab responses control trypanosome present in the blood. Second, T cell production of IFN-γ and subsequent macrophage actiation events are necessary to control trypanosomes in the extravascular tissues. Animals that make weak B cell and/or T cell response to trypanosome variant antigens invariably will demonstrate relative susceptibility; in contrast, animals making pronounced B and T cell response (including appropriate macrophage activation events) will display relative high resistance.

2.4.3.6 Changes in parasite cell biology during infection that impact on host resistance

The African trypanosomes display considerable biological variation during their life-cycle. This biological variation is directed by specific patterns of gene and protein expression. For example, bloodstream trypanosomes display a number of different surface antigenic phenotypes during infection of their mammalian hosts; this phenotypic variation has as its basis the differential expression of VSG genes and molecules (Cross,1990a,b;Borst and Rudenko, 1994). Additionally, the differentiation of long slender (LS) trypomastigote forms to short stumpy (SS) trypomastigotes during infection results in profound morphological and functional changes in these cells. Such changes include mitochondrial biogenesis and the acquisition of new metabolic pathways; it is the differential expression of specific genes and proteins that preceed these biological changes. Furthermore, the differentiation of SS forms to procyclic forms in the insect vector also results from the differential expression of specific genes and proteins (Roditi, 1996;Butikofer *et al.*, 1997). Finally, the transformation of insect forms to metacyclic trypomastigotes results in different morphological and functional changes in the parasites that permit infection of a new mammalian host; this changes also occur as a result of differential gene and protein expression (Turnaz *et al.*, 1986;Vanhamme and Pays, 1995).

The molecular mechanisms that regulate changes in VSG phenotype and stage of cellular differentiation are shared in common among African trypanosomes; however, these are the only mechanisms that regulate biological differences in these parasites. New evidence is emerging that differential susceptibility of brucei group trypanosomes to host factors such as trypanosome lytic factor (TLF) (De Greef and Hamers, 1994; Hager *et al.*,

1994) occur as the result of clonal variation among trypanosomes (Hajduk *et al.*, 1995) the basis for such changes has not yet been defined, but is believed to encompass specific clonal modifications in gene or protein different transferring receptor genes (Bitter *et al.*, 1998). While these examples reflect on trypanosome infectivity for a host species, related observations and mathematical modeling predict that considerable biological variation occurs among trypanosomes during infection that is not related directly to infectivity or cyclical differentiation in the life cycle (Vassella *et al.*, 1997). For example, it is well known that different isolates, species and subspecies of trypanosomes exhibit remarkable variation in pathogenicity and virulence for genetically defined host species (Herbert and Parratt, 1979; Joshua, 1988,1990). A key question has been whether such differences in virulence are immutable characteristics associated with genetically distinct populations of trypanosome populations that impacts on the course of disease in a genetically defined host.

This question was addressed in an earlier study in which mice of a relatively resistant phenotype were infected with a single mouse; these VATs were subcloned and characterized as to VSG phenotype. Three different VATs which represented antigenetically distinct daughter cell populations clonally derived from a signle LouTat 1 parental cell, populations clonally derived from a single LouTat 1 parental cell, were used to infect the same mouse strain; the course of infection were monitored in comparison with mice infected with LouTat 1. The interesting result was that each of the daughter cell populations exhibited different virulence profile compared to the parental clone (Inverso and Mansfield, 1983). For example, LouTat 1 caused death in approximately 62 days post-infection, while LouTat 1.3, LouTat 1.4 and LouTat 1.5 caused death in approximately 44, 30, and 28 days, respectively. These results demonstrated that VATs arising during infection expressed virulence phenotypes different from the infecting VAT. In essence, daughter cells arising within a trypanosome population expressed the capacity to transcend host genetic resistance characteristics and render a relatively resistant animal into a more susceptible one.

The related observations on clonal heterogeneity among trypanosomes have been made in other studies (Joshua, 1990). Additionally, the general observation has been made that many other daughter cells/VATs arisng naturally from LouTat 1 also expressed differences in virulence and that the most virulent VATs seemed to arise at later time points in infection, just prior to host death. Thus, the apparent result of infection with relatilvey low virulence trypanosomes is a progressive increase in

population virulence with time, rather like the turning up of a "virulence rheostat". Such a virulence rheostat may have evolved as a programmed mechanism to overcome different levels of host resistance that might be encountered in nature, where there is a pool of genetically disparate mammalian hosts available for infection. Implicit in this speculation, however, is the idea that a virulence rheostat must somehow be reset, perhaps upon cyclical passage through the intermediate host and vector, the tsetse fly.

This was achieved by infecting irradiated mice with LouTat 1 **a**nd subpassaged the trypanosomes into different mice every three days for approximately 6 months. At the end of this time, trypanosome stabilates were made from sublines and subclones, and were assessed for their virulence characteristics. One representative subclone, designated LouTat 1A, was examined in some detail. These trypanosomes displayed a single uncontrolled peak of parasitemia and were to kill a resistant mouse strain (as well as all other resistant or susceptible strains of mice) in approximately 4 days post-infection; in contrast the parental clone LouTat 1 gave rise to multiple peaks of parasitemia and a prolonged survival time of over 60 days in the same mouse strain . Thus a model system of comparative trypanosome virulence was developed from this approach, in which the relatively low virulence clone LouTat 1 and the relatively high virulence LouTat 1A represent different ends of a virulence spectrum, with the virulence of other naturally arising VATs existing somewhere between these two extremes

A natural question that arose from these types of studies was whether the VSG molecules expressed by virulent VATs acted as virulence factors, with specific VSG isotypes exerting defined biological effects on the host. This idea was not unfounded since several biological traits associated with VSG molecules have been described in the literature (Mathias *et al.,* 1990; Schofield *et al.,* 1999). Alternatively, one could also speculate that expression site-associated genes (ESGAs) co-transcribed with certain VSG genes at specific chromosomal expression sites may be responsible for virulence expression or regulation.This idea was based on observations of others concerning potential growth or differentiation regulatory roles associated with ESAGs (Cross, 1990a,b; Vickerman *et al.,* 1993). However, an analysis of the LouTat1/LouTat 1A model system revealed that both organisms displayed the same antigenic surface coat structure, transcribed identical VSG genes and expressed their VSG genes by a duplicative transposition event from the same chromosomal telomeric expression site .

Therefore, it is unlikely that either VSG molecules or the active VSG gene expression site are important for virulence regulation in trypanosomes confirmation that

VSG genes and VSG gene expression sites are not involved in virulence came from additional experimental approaches . New sublines and subclones were derived by rapid subpassage of LouTat1 through irradiated mice, as above, and examined for VSG phenotype and gene expression. Many of the subclones expressed the same VSG gene as LouTat1 and all were highly virulent like LouTat 1 infected controls, they did not die as early as mice and trypanosomes were able to undergo antigenic variation. The VATs generated from LouTat 1A in these animals were isolated and subsequently used to infect mice; the results showed that the LouTat 1A-derived VATs were as virulent of mice as LouTat 1A (Inverso *et al.,* 1988;). Thus, high levels of virulence, once expressed in mammalian hosts, appear to be a constitutive trait that is unaffected by further VSG gene switching. Subsequently LouTat1was examined for non-VSG related cellular differences (Mansfield *et al.,* 2001). Comparative analysis of several traits revealed significant differences between the two clones.A few chromosomes were altered in size, as determined by pulsed-field gel electrophoresis; however, there was no net loss of cellular DNA and no apparent differences in RFLP patterns utilizing a number of random and known non-VSG cDNA probes were found. Thus, the chromosomal size variations observed may largely be subtelomeric or simply do not involve chromosome regions to which the probes hybridized. Two-dimensonal gel electrophoresis of ^{35}S-labeled proteins showed not only that different proteins were expressed in LouTat 1 compared to LouTat 1A. Competitive Northern analyses in which labeled total cDNA from LouTat1A, in the presence of excess unlabelled LouTat 1 competitive total cDNA, was hybridized to mRNA from LouTat 1A showed that there were numerous mRNA species unique to LouTat 1A. These observations suggested that a subset of genes and proteins was being expressed inLouTat 1A that was not being expressed at the same level in LouTat 1. Overall, preliminary observations on the biological behaviour of LouTat 1 and LouTat 1A, and on the subcellular differences detectable between LouTat 1 LouTat 1A, as well as biological variation observed with other subspecies of *T. b. brucei* in terms of TLF susceptibility or TNF sensitivity have led to the hypothesis that Africal trypanosomes have the capacity to regularly clonal expression of virulence**.** Specifically, it has been proposed (a) that trypanosomes have evolved the programmed capacity for clonal upregulation of host genetics background: (b) that this capacity to modify virulence phenotype occurs independently of changes in VSG gene expression and (c) that the level of virulence expressed is determined by differential gene and /or protein expression during trypanosomes growth in an infected host.

2.4.4 Innate immunity in trypanosomosis

2.4.4.1 Natural immunity

Normal human sera injected into *T.b. brucei*-infected mice caused a dramatic reduction in parasitemia (Laveran and Mesnil, 1912). This phenomenon was not reproduced with the human trypanosome strains *T.b. gambiense* and *T.b. rhodesiense.* Trypanolytic factors (TLF) contained in normal human serum were identified as high-density lipoproteins (Rifkin, 1978). Recently, two TLFs have been characterized in human serum. The first one (TLF1) belongs to a subclass of high-density lipoproteins and is inhibited by haptoglobin. In contrast, the second factor, TLF2, has a much higher molecular weight and does not appear to be a lipoprotein. Probably, the main trypanolytic effect is due to TLF2, which is not inhibited by haptoglobin (Raper *et al.,* 1996).The trypanocidal effect of Cape buffalo serum has been attributed to xanthine oxidase (Muranjan *et al*., 1997). Recently a trypanosome lysosomal protein (SRA) was found to be associated with resistance to normal human serum. SRA is a truncated form of VSG and interacts with serum apolipoprotein L-I in the parasite lyzosome (Vanhamme and Pays, 2004).

2.4.4.2 Natural killer cells

Natural killer (NK) cells have been identified as an important defence mechanism against tumour cells and intracellular pathogens, especially viruses.They are considered to belong to the lymphocyte lineage and have functions in both innate and acquired immune responses. NK cells lyse extracellular parasites. NK cells from *T. cruzi*-infected mice have been shown to exhibit significant activity against trypomastigotes of *T. cruzi* (Hatcher and Kuhn, 1982). NK cells secrete cytokines and especially IFN-γ and TNF-α, which play major roles in trypanosomiasis and are regulated by cytokines which can activate or inhibit NK cell functions. NK cells also participate in the initiation of the inflammatory response, through the synthesis of chemokines.In *T. brucei*-infected mice, NK activity was not modified in the early stages of infection, but was severely reduced from day 9 onwards (Askonas and Bancroft, 1984). By contrast, NK cells were activated in mice infected with a natural extracellular trypanosome (*T. musculi*) and their critical role was demonstrated by the effects of their depletion by antiserum against asialo GM1 or their activation by polycytidylic copolymer (Albright *et al.,* 1997).

2.4.4.3 T cells

Initial studies have evidenced alterations in T cell functions in trypanosomosis, both *in vivo* and *in vitro* (Mansfield and Wallace, 1974). Histological examination revealed a

massive B cell expansion in the lymph nodes and spleen, which replaced the thymus-dependant area in *T.b. brucei* TREU 667-infected mice. These changes were seen within 7 days post-infection and persisted for at least 70 days. Moreover the role of T cells in controlling infection was not clear (Askonas and Bancroft, 1984). Trypanosome-specific T cell response was difficult to identify. In several studies, a transient proliferative T cell response to trypanosome antigens was noted in the first days of the infection followed by an absence of response (Gasbarre *et al.,* 1980). The kinetoplastid membrane protein 11 of African trypanosomes is a potent stimulator of T lymphocyte proliferation (Tolson *et al.,* 1994). In *T. b. brucei* - infected mice, an increased proliferation of T cells was noted in the first days of infection in spleen and bone marrow, T blasts disappeared very rapidly. In *T. congolense*-infected cattle, antigen-specific proliferation of T cells was obtained with more or less difficulty according to the antigen, the T cell population and the time used. However, a strong trypanosome-specific T cell proliferation occurred in infected cattle following treatment (Emery *et al.,* 1980; Lutje *et al.,* 1995). Most T cells in humans and mice bear Tαβ antigen receptors. These cells possess surface markers, which allow the discrimination of CD4+ T cells (helper T cells) and CD8+ T cells (cytotoxic T cells). The knowledge of T cell subsets has been deeply modified by the discovery of two subsets of T helper cells, Th1 and Th2 cells. Th1cells expressing a functional T cell response directed to VSG is generated in *T.b. rhodesiense*-infected mice. VSG specific T cells were found predominantly in the peritoneum. These cells did not proliferate but made a substantial IFN-γ and IL-2 cytokine response (Schleifer *et al.,* 1993). The cellular phenotype of VSG-responsive T cells (CD4+CD3+) indicates that the VSG appear to preferentially stimulate a Th1 cell subset during infection. Analysis of lymphocyte subsets in regional lymph nodes of *T. congolense*-infected N'Dama (trypanotolerant) and Boran (trypanosusceptible) were performed by flow cytometry. In both breeds, a significant decrease in the percentage of CD2+and CD4+ T cells was observed, associated with an increase in the percentage of CD8+ T cells, B cells and γ δT cells. VSG and two invariant antigens (33 kDa cysteine protease and 66 kDa antigen homologous to immunoglobulin heavy chain binding protein hsp70/Bip) induced *in vitro* proliferation and synthesis of IL-2 and IFN-γ (Authié *et al.,* 1992; Boulangé and Authié, 1994; Lutje *et al.,* 1995). No significant differences in the *in vitro* proliferation of lymph node cells to VSG, Concanavalin A (ConA) or hsp 70/Bip were observed between the two breeds. However, IFN-γ production in response to ConA was higher in Boran at 35 days post infection. Human and mouse immune systems contain few γ δT cells, in marked

contrast to those of ruminants (Hein and Mac Kay, 1991). Functions of γ δT cells remain largely unknown. Involvement of γ δT cells in malaria and leishmanosis has been observed (Rosat *et al.,* 1995; Rzepczyk *et al.,* 1997). A proliferative response of CD8+ T cells and γ δT cells from trypanotolerant N'Dama to an antigen complex containing immunodominant epitopes was observed whereas a quasi absence of response was observed in trypanosusceptible Boran. The role of this γ δT cell response in parasite resistance remains unclear. So, γ δT cells, as CD4+ or CD8+, do not proliferate when stimulated with soluble VSG *in vitro* (Flynn and Sileghem., 1994). It would be interesting to determine the role of cytokines synthesized by γ δT cells. Indeed, although specific T cells do not act on trypanosomes in the same way as the cytotoxic T cells in several infectious diseases such as viral infections, they markedly modify immune responses, specially by the secretion of cytokines. They greatly modify functions of B cells (antibody synthesis, isotype switch) and macrophages (antigen presentation, effector mechanisms).

2.4.4.4 B cells

In African trypanosomosis, the main feature is a dramatic increase in immunoglobulin (Ig) levels (especially IgM), including trypanosome-specific antibodies and non-specific Ig production induced by cytokine activation of B cells. Some of these antibodies are also raised against autoantigens, corresponding to non-specific polyclonal activation of B-cells producing natural autoantibodies and also to antigen-driven antibodies induced by molecular mimicry. DNA from *T.b. brucei* stimulated B cell proliferation (Shoda *et al.,* 2001). In *T.b. brucei*-infected mice, T lymphocytes display an aberrant activation phenotype (Sacco *et al.,* 1994). Antibodies specific to trypanosomes are induced by several parasite antigens, including variant and invariant VSG epitopes, as well as membrane, cytoplasmic and nuclear antigens, through T-dependent and T-independent pathways (Reinitz and Mansfield, 1990). Antibodies directed against trypanosome VSG components appeared in sera and their binding to the surface coat of the trypanosomes was able to induce a decrease in parasitemia, both in the blood and extravascular spaces, specifically by immune lysis of parasites and their destruction by the Kupffer cells in the liver. Only heterologous antigenic variants (<0.1%) remain to repopulate the blood and tissues. Parasites are eliminated due to VSG-specific IgM (appearing at high levels, 3-4 days after infection). In contrast, VSGspecific IgG does not seem to be involved in the destruction of trypanosomes, as they appeared after the disappearance of this VAT population. Another induction of antibodies, linked to the new VSG epitopes, appeared in

sera and also contributed to decrease the new VAT-specific population. The VATspecific antibodies therefore decreased to low levels, whereas antibodies, belonging predominantly to the IgM class specific to invariant epitopes, remained at high levels. During infection, B cell nonspecific stimulation was enhanced as T-independent B cell responses to the VSG successive parasitemias. In contrast, specific trypanosome B cell response, depending on T cell regulation, was depressed. Several factors may contribute to this immunosuppression. Macrophages may become unable to present antigens to T cells (by defects in antigen processing and association of epitopes with MHC Class II) and produce immunosuppressive factors as nitric oxide (NO), prostaglandins (PG), and cytokines. An increase in immunosuppressive cytokines, such as INF-γ and transforming growth factor (TGF)-β, was also detected during infection. However, TGF-β is known to inhibit the production of IL-4, IL-5, IL-6, the major cytokines implied in B cell proliferation and differentiation (Fargeas *et al.*, 1992). Several autoantibodies are detected during African trypanosomiasis. High levels of polyclonal Igs were a marked feature of HAT. The specificity of these Igs is frequently characterised against a large range of autoantigens. Autoantibodies were directed against red blood cells (Kobayakawa *et al.*, 1979), liver and cardiolipids (MacKenzie and Boreham, 1974), nucleic acids: DNA and RNA (Kobayakawa *et al.*, 1979; Hunter *et al.*, 1992b), intermediate filaments (Anthoons *et al.*, 1986) and rheumatoid factors (Kazyumba *et al.*, 1986). Autoantibodies directed against components of CNS myelin have also been reported. They are specific for the major glycosphingolipids of myelin, the galactocerebrosides, and were detected in sera from both experimentally infected animals (Jauberteau *et al.*, 1991) and patients from the Ivory Coast (Amevigbe *et al.*, 1992). Other autoantibodies directed against not yet characterised proteins have been described in HAT patients (Asonganyi *et al.*, 1989) as well as antibodies directed at myelin basic protein in experimentally infected animals (Hunter *et al.*, 1992a). Other antibodies were raised against an epitope containing L-tryptophan, a precursor to the neurotransmitter serotonin, (Okomo-Assoumou *et al.*, 1995b) or recognised some neuronal components of the cytoskeleton, neurofilament proteins. In some cases, these autoantibodies (anti-galactocerebrosides and anti-neurofilaments) are associated with the neurological stage of the disease and their detection in sera and CSF could contribute towards defining the neurological involvement of HAT (Courtioux *et al.*, 2005). *In vivo* demyelinisation has been produced by purified antibodies to galactocerebroside (Saida *et al.*, 1979). There are several hypotheses for the origin of these antibodies. They may be induced by a non-specific stimulation of B cells

producing natural autoantibodies (Arneborn *et al.,* 1983; Mortazavi- Milani *et al.,* 1984). In other cases, antigen-driven autoantibodies are specific to epitopes of the causative infecting agent with molecular mimicry to self antigens, inducing a cross-reactivity to intermediate filaments (Dales *et al.,* 1983; Fujinami *et al.,* 1983; Davies,1997) as demonstrated for anti-neurofilament and anti-galactocerebroside antibodies which recognised respectively a flagellar component and a proteolipidic epitope of trypanosomes, and epitopes expressed by neurones (Ayed *et al.,* 1997; Girard *et al.,* 2000). A subpopulation of B cells identified by the expression of high levels of surface Igs and of CD5 in humans and Ly-1 in mice is responsible for most serum IgM (Kipps, 1990). These CD5 cells produce autoantibodies, and antibodies to thymus-independent antigens. In cattle infected with *T. congolense*, a dramatic increase in these cells (more than four times the control value in blood) was measured and correlated with increases in serum Igs and in the absolute number of B cells (Naessens andWilliams, 1992). An induction of these CD5 B cells (directly by parasite products or indirectly through the cytokine network) could account for the alteration in immunoglobulin synthesis and antibody production observed in trypanosomosis.

2.4.4.5 Macrophages

Mononuclear phagocytes play a key role in all steps of immune response in the inflammatory phase, an antigen presenting cells, in specific immunity, in synergy with antibodies and cytokines. They also can be involved in immunosuppressive and immunopathological phenomena. Qantitative, biochemical and functional changes of mononuclear phagocytes are observed in trypanosomiasis. In *T. brucei*- infected mice, histological examination showed a marked expansion in macrophages of the liver, spleen and bone marrow. The Kupffer cells in the liver increased in number and were often found in mitosis. The cells contained abundant phagolysosomes (vacuolated cytoplasm). An increase uptake of intravenously injected sheep red blood cells was also noted. Macrophages are highly sensitive to environmental factors, especially microorganisms, microorganism- derived products and cytokines. A clear reduction in mannose receptors, Fc receptors, C3bi receptors (Mac-1) and F4/80 occurs by day 4 post *T. b. brucei* infection. The expression of MHC Class II molecules (Ia antigens)was reported to have increased in *T. b. brucei*-infected mice and decreased in *T.b. rhodesiense*-infected mice. The antigenpresenting function was reported to be unmodified in *T. b. brucei*-infected mice and defective in *T. b. rhodesiense*-infected mice. Macrophages react to stimuli by adapted response. They secrete many factors with various functions. They synthesise

cytokines and effector mediators. Macrophages may play an important role in protection against trypanosomes, particularly in the presence of homologous antiserum. The immunological clearance of [75Se]-methionine-labelled *T. brucei* in mice has been conducted to investigate the respective roles of antibodies, macrophage activation and complement in the removal of circulating parasites. The clearance was largely accomplished by antibody-mediated hepatic phagocytosis. C3 is necessary for the full opsonic activity present in murine clearance in passively immunized mice (MacAskill *et al.,* 1980). These *in vivo* studies extend previous studies on the *in vitro* phagocytic function of macrophages in the presence of immune serum (Takayanagi *et al.,*1992) As the existence of receptors for the Fc region of IgM on the macrophage membrane is still controversial, the role of IgM antibodies on trypanosome phagocytosis in the absence of complement remains unlikely. Receptor-mediated phagocytosis is enhanced during infection. It is possible that trypanosomes phagocytosed through receptors (C3b receptors, Fc receptors, etc.) or after destruction by complement-mediated lysis trigger macrophage suppressor activity, although the participation of soluble factors or another cell types cannot be ruled out. Furthermore, macrophages from *T.b. brucei*infected mice are able to synthesise reactive oxygen intermediates (ROI) after triggering by phorbol myristate acetate. Oxygen-derived species are among the most toxic products produced by macrophages.Trypanosomes are highly sensitive to these species, and in peculiar to hydrogen peroxide and hypochlorous acid, synthesised during phagocytosis. Macrophages from trypanosome-infected mice also synthesise reactive nitrogen intermediates (RNI). Trypanosomes are highly sensitive to the cytostatic / cytotoxic effects of these compounds (Vincendeau and Daulouède, 1991; Vincendeau *et al.,* 1992). They are highly reactive radicals with short half-lives, which can react together to form potent and more stable effector molecules able to act on distant targets such as extracellular parasites. We have recently shown that *T.b. gambiense* are highly sensitive to S nitroso-compounds, which are new effector molecules synthesised by activated human macrophages *in vitro* (Mnaimneh *et al.,* 1997). Nitrosylated compounds could represent new effector molecules with a potent effect on targets distant from macrophages. In a recent study DNA from *T.b. brucei* have increased macrophage production of IL-12, TNF-α and NO (Shoda *et al.,* 2001). Macrophages are also active in secreting PGs which modulate lymphocyte and macrophage functions. During a *T. b. brucei* infection, the ratio of PGE2/PGF1a is reversed, with an overproduction of PGE2 (Fierer *et al.,* 1984). Macrophages are involved in immunosuppressive mechanism, and

VSG can also inhibits macrophage functions (Flynn and Sileghem,1991; Schleifer and Mansfield, 1993;Coller *et al.,* 2003). Macrophages respond to, and synthesise, a large number of cytokines. The production of IL-1 is increased in *T.b. brucei*-infected mice, but this increase may be due to release rather than synthesis (Sileghem *et al.,* 1989). In murine macrophages, VSG induces IL-1 and TNF-α synthesis. Human monocytes can also be induced by trypanosomes and secreted factors from trypanosomes to express TNF-α RNA transcripts and secrete TNF-α in culture supernatants (Daulouède *et al.,* 2001). Classical and alternative states of macrophage activation are observed in trypanosomiasis. Classical activation precedes alternative activation in murine trypanosomosis. However, both activation states are expressed in these mice. By inducing alternative macrophage activation, trypanosomes induce host arginase which both decrease trypanocidal nitrosylated compound synthesis and increases L-ornithine production (Gobert *et al.,* 2000). L-ornithine is the first step of polyamine synthesis, essential for parasite growth and trypanothione synthesis (Vincendeau *et al.,* 2003).

2.4.4.6 Cytokines and chemokines

A profound dysregulation of the cytokine network is observed in trypanosomiasis. The first evidence of overproduction of TNF-α/cachectin was shown in *T.b. brucei*-infected rabbits (Rouzer and Cerami, 1980). TNF-α is known to induce fever, asthenia, cachexia and hypertriglyceridemia. High levels of TNF-α are associated with the presence of patent inflammatory signs in the early phase of human trypanosomiasis and of major neurologic signs in the late phase (Okomo-Assoumou *et al.,* 1995a). A persistently increased serum TNF-α level could contribute to the hypergammaglobulinemia observed in trypanosomiasis because the role of TNF-α on activation, proliferation and differentiation of B cells has already been shown (Roldan *et al.,* 1992). Nevertheless, TNF-α participates in the mechanisms leading to trypanosome elimination: TNF-α acts indirectly in a cascade of events leading to cell activation or directly on parasites due to its cytotoxic properties (Lucas *et al.,* 1994). Initial control of parasitemia in *T.b. brucei*-infected mice was diminished by the injection of anti-TNF-α antibodies (Lucas *et al.,* 1993). VSG can trigger TNF-α production by macrophages, which are the cells, which produce the most of this molecule. Moreover, TNF- α production can be stimulated by IFN-γ . IFN-γ and TGF-β can be produced by CD8 T cells activated by TLTF released by *T.b. brucei* (Vaidya *et al.,* 1997). TGF-β has immuno-suppressive effects. An interesting fact is that IFN-γ stimulates parasite growth (Olsson *et al.,* 1991). The binding of epidermal growth factor (EGF) on *T.b. brucei* receptors favoured parasite growth and was

one of the first cytokine-parasite interactions noted (Hide *et al.*, 1989). All these data show that by interfering with the cytokine network and by using cytokines as growth factor, trypanosomes can completely modify the effector functions of the immune system. The effects of cytokines could also be completely different according to the presence of co-stimulators and the time period during which they are produced in trypanosome infected animals. Chemokines also play essential roles in infectious disease control. They induce cell recruitment and activation. They induce adhesion molecules on cells of the immune system, which can bind to various cells, mainly endothelial cells, which express adapted ligands (Hickey, 1999). Cytokines and chemokines can also be involved in neurological disorders (Sorensen *et al.*, 1999). So, TNF-α has been reported to contribute to the pathophysiology of cerebral malaria. Mice chronically infected with *T.b. brucei* develop inflammatory lesions of the CNS after treatment with subcurative doses of a trypanocidal agent (Hunter *et al.*, 1991). Chemokines favour macrophage and lymphocyte recruitment in CNS of *T.b. brucei*-infected animals. The activity of these cells in precise and selective ar- eas of CNS might induce alterations leading to various disorders, such as sleep and endocrine disorders (Buguet *et al.*, 1993, 2001; Lundkvist *et al.*, 2004). The presence of TNF-α RNA transcripts in the CNS of these mice suggests that TNF-α production could play a role in these lesions. Also, TNF-α and other cytokines contribute to the generation of somnogenic molecules such as IL-1 (Pentreath, 1994). In a recent study an intra cerebral infusion of soluble type I TNF-α receptor reduced trypanosome-induced neurodegeneration (Ning *et al.*, 2003). High levels of plasmatic IL-10 are also found in human trypanosomiasis. A number of aspects deserve further investigation: the study of all the various cytokines and soluble cytokine receptors, the possible existence of membrane or soluble cytokine receptors synthesised by the parasite, and the interaction and modulation of all these elements. Cytokines have been shown to play an essential role in the synthesis of NO, whose effects on several features of immune response have been observed over the past few years.

2.4.4.7 Nitric oxide

Nitric oxide (NO) is a short-lived diatomic free radical synthesised from L-arginine byNOsynthase (NOS). Calcium-dependent constitutive NOS (cNOS) release small amounts (picomoles) of NO within a short time, whereas calcium-independent inducible NOS (iNOS) release high levels (nanomoles) of NO for a long time. Expression of iNOS in macrophages, neutrophils, hepatocytes, endothelial cells and epithelial cells is regulated at transcription level by a number of agents, including microbial products and

cytokines. *In vitro*, murine cells produce large amounts of NO after exposure to a combination of stimuli: lipopolysaccharide (LPS), IFN-γ, IL-1, TNF-α etc. Human monocytes treated by IL-4 express CD23 antigen. The crosslink of CD23 induces iNOS expression, the release of NO and various other molecules (IL-6, TNF-α, oxygen radicals, lipid mediators). Nitric oxide is involved in the inflammatory response mediated by endotoxin, cytokines or physiochemical stress. NO produced by cytokine-activated macrophages is important in host defence and plays a crucial role in controlling infections *in vivo*. The role of NO and cytokines has been studied in detail in mice infected by intracellular parasites as *Leishmania*. In this murine model, IFN-γ synthesized by Th1 cells leads to iNOS activity, whereas IL-4 and IL-10 synthesised by Th2 cells have a suppressive effect. NO or other nitrogen intermediates can also react with the oxygen intermediates and form peroxynitrite and hydrogen radicals. Moreover, NO can form nitrosylated compounds which are able to transport and liberate NO on targets, distant from NO producing cells. Nitrosylated compounds can not only act on extracellular parasites, but also modify parasite antigens and host cell function. These compounds may have various effects (parasite killing, alteration of tissue functions such as neurotransmission, etc.) according to their localization (spleen, liver, peritoneum, CNS, etc.). By selective inhibition of Th1 cells, NO exerts a negative feedback effect. The altered production of NO, induced by dysregulation of the cytokine network, may lead to alteration of immune response and may also be involved in pathophysiological mechanisms. Nitrotyrosine, a marker of peroxy-nitrite formation, and iNOS are immunodetected in the brains of *T.b. brucei*-infected mice. Nitrotyrosine staining is associated with the appearance of neurological signs (Keita *et al.*, 2000). In HAT, nitrite production is increased at first. NO can also be stored as nitroso compounds. These NO-adducts are indirectly detected, as they induce the appearance of antibodies directed to nitrosylated antigens (Semballa *et al.*, 2004). However, in trypanosome-infected mice, a decrease in plasmatic L-arginine leads to a decreased NO production. L-arginine is consumed by arginase, which synthetics L-ornithine and urea. L-ornithine is the precursor of polyamines and trypanothione. By inducing arginase, trypanosomes bypass NO production and benefit growth factor production. Arginase induction by parasites might be considered as a new strategy elaborated by parasites to escape host defence and benefit growth factors.

2.5 Immuno-suppression

The increased susceptibility of *T. gambiense*-infected patients to secondary infections was pointed out in the initial observations and reports of the Sleeping Sickness Commission (Low and Castellani, 1903). Cellular immunity (skin tests to PPD, *Candida* or streptococcal antigens and sensitization with DNCB) and humoral immunity (response to the H antigen of *Salmonella typhi*) were depressed in patients with HAT (Greenwood *et al.,* 1973). In a recent study, no statistical difference was found between the prevalence of HIV infection in HAT patients and controls (Meda *et al.,* 1995). Immunosuppression was also observed and investigated in experimental trypanosomiasis and trypanosome - infected cattle (Ilemobade *et al.,* 1982). In these models, immunosuppression was attributed to polyclonal B cell activation as well as the generation of suppressor T cells and suppressor macrophages. General B cell activation was noted in trypanosomiasis (hypergammaglobulinemia and a large increase of B cells in the spleen, as well as the presence of numerous Mott cells in cerebral spinal fluid and plasma cells in perivascular infiltrates), whereas specific antibody response to trypanosome antigens were reduced (Sacks *et al.,* 1980). A marked suppression of antibody response to *Brucella abortus* was reported in cattle infected with *T. congolense* (Rurangirwa *et al.,* 1983). *Trypanosoma evansi* infection in sheep delayed and depressed the increase in total cell and lymphoblast output from a lymph node draining the site of a *Pasteurella haemolytica* vaccine administration. These reduced outputs may limit the dissemination of antigenic specific cells (Onah *et al.,* 1997). Cells, cytokines and prostaglandins have been studied in order to know their contribution, alone or in synergy, and with or without parasitic elements, to immunosuppression mechanisms. Trypanosome membrane fragments have been found to mimic the immunosuppressive effects of living parasites (Clayton *et al.,* 1979). A deficient production of IL-2 and of IL-2 receptor expression has been shown in several models (Alcino and Fresno, 1985; Sileghem *et al.,* 1989). The roles of macrophage-derived factors, especially prostaglandins and IFN-γ secreted by CD8+ T cells in the suppression of IL-2 receptor expression on CD4+ and CD8+ T cells, were also shown (Darji *et al.,* 1993). Besides its action on the Th1 subset, rather than the Th 2 subset, NO also acts on other elements, favouring immunosupression.

2.6. Immuno-intervention

The resistance of mice to African trypanosomes can be increased non-specifically by immunostimulants such as Calmette-Guérin bacilli and *Propionibacterium acnes* (Murray and Morrison, 1979; Black *et al.,* 1989). These immunostimulants are considered

to activate macrophages. *P. acnes*treated macrophages inhibited *T. brucei* growth *in vitro* (Black *et al.,* 1989). An acquired resistance has been observed in trypanocide-treated cattle. In a cohort study in Zaire during the 10 year observation period of adults previously diagnosed and treated for HAT, the risk of a second episode of HAT was greatly reduced compared to the risk of a first episode in previously undiagnosed adults (Khonde *et al.,* 1995). Induction of protective immunity by vaccination is an important goal to control infectious diseases. However, a vaccine must be very effective and not only delay development of the disease, but also include a large number of antigenic variants. A major aim is to identify antigen(s) that elicit a protective immune response in trypanosomiasis. Identifying molecules inducing durable protection may lead to their production as recombinant antigens. Nucleic acid vaccines represent a new promising approach. They are able to induce all the elements of the specific immune response unlike killed microorganisms or defined protein. Studies using dead or living trypanosomes, soluble-released antigens, purified VSG and irradiated parasites have shown that protection is restricted to the VSG-specific epitopes (Pays, 1995). Strategies using invariant antigens (particularly those in the flagellar pocket) may be very worthwhile (Olecnick *et al.,* 1988). Furthermore, in experimental murine trypanosomiasis, vaccination based on glycosylphosphatidyl (GPI) anchor of VSG can prevent TNF-α associated immunopathology and decrease disease severity (Magez *et al.,* 2002). TNF-α associated immunopathology may also be prevented by selective inhibitors of macrophage functions (Mamani-Matsuda *et al.,* 2004).

CHAPTER THREE

3.0 PRELIMINARY SURVEY ON SEASONAL INFLUENCE ON PREVALENCE OF TRYPANOSOMOSIS IN WEST AFRICAN DWARF GOATS IN OGBOMOSO AREA OF OYO STATE, NIGERIA

3.1 INTRODUCTION

Ogbomoso is one of the major towns in Oyo state and it lies on longitude $8^0$15 North East ward from Ibadan capital of Oyo state. The altitude is between 800-600mm above sea level and the mean annual temperature is about 27^0C while that of annual rainfall is 1247mm. The vegetation of the study area is derived savanna (Oguntoyinbo, 1978). There are five local governments in Ogbomoso area. These are Ogbomoso North, Ogbomoso South, Orire, Ogo Oluwa and Surulere, all in Ogbomoso land.

Trypanosomosis is one of the major constraints to livestock productivity in Sub-Saharan Africa. Only trypanotolerant breeds survive, reproduce and remain productive without treatment in tsetse-infested area (Murray *et al*., 1982). In West African, ruminants play a crucial role in providing protein (milk, meat) and non-food commodities (manure, hides). They equally serve as a cash reserve and a form of saving for rural population and as protection against agricultural failure, therefore, the number of animals is more important than individual productivity (Panin and Mahabile, 1997; Litty *et al*., 1997). In the Sub-Saharan Africa, domestic ruminant population compos ced of 162 million cattle, 127 million sheep, and 147 million goats. Africa has 11% of the total world cattle population and 26% of the world small ruminant population. Eighty-two percent of the total livestock biomass in Africa is ruminant, thus the ruminant population forms the most important group on the continent (WIIDA, 1992).

Prevalence of trypanosomosis has been reported by various workers (using different diagnostic, parasitological and Elisa techniques). In the epidemiology studies of trypanosomosis in different part of Nigeria, a number of workers have reported different rate of prevalence. In Zaria, northern Nigeria,(Ahmed and Agbede, 1993) reported 1.2% in goats,(Kalu and Lawani, 1996) showed a prevalence rate of 1.2±1.6% in sheep and 0.7 ± 1.3% in goats in Kano state Nigeria. Daniel *et al*., (1994) reported a prevalence rate of 7.4 % in sheep and 5.0% in goats in Bauchi state. In the Eastern part of Nigeria,

especially in Abakaliki the prevalence rate of 4.6% was reported for small ruminant but prevalence rate of sheep -6.7% and goats-3.5%. In Nsuka area, Fakae and Chiejina, (1993) reported 61.6% in small ruminant. In recent studies across all agro-ecological zone in Nigeria, Onyia, (1997) reported a prevalence rate of 8.6% in sheep, 8.1% in goat and 10% in cattle while survey by EEC- trypanosomosis control project reported 4.3% in cattle, 1.6% in sheep and 1.0% in goats. There was no information on the seasonal influence on the prevalence of trypanosomosis in West African Dwarf goats in Ogbomoso land, therefore this study focused on investigation on seasonal influence on trypanosomosis in West African Dwarf goats in Ogbomoso area of Oyo state, Nigeria.

3.2 MATERIALS AND METHODS

3.2.1 Study Areas: The study was carried out in five local government area of Ogbomoso area of Oyo state, namely; Ogbomoso North, Ogbomoso South, Orire, Surulere and Ogo Oluwa. The study was done during the rainy season (October-April, 2005) and dry season (May-November, 2006) in all the five local governments. These five local governments were chosen because of the availability of West African Dwarf goats in these areas, presence of some ponds which made the area more conducive for tsetse-animal contact, seasonal variation in availability of pasture and these areas linked south western zone to northern zone. The animals investigated were peri-domestic West African Dwarf goats.

3.2.2 Blood Examination: Random sampling was employed in bleeding the animals. In a group all the animals were bled, but where they are more than five, every other animal was bled. About 2ml of blood was collected from the jugular vein of each animal using EDTA as anticoagulant. Trypanosomes were detected using the haemotocrit centrifugation of Woo (1971), wet blood film and stained thin blood smears. Parasites were examined by observing 60 fields of a wet film preparation microscopically (x100 eye pieces and x 100 oil immersion objective) for identification of trypanosomes species. PCV was estimated using a haematocrit centrifuge and reader (Schalm *et al.*,1975, Jain 1986) and in all, some total of 675 WAD goats were investigated and examined.

3.2.3 Statistical Analysis: Descriptive and Student's t-test was used to analyze the results of the PCV of the infected and non-infected animals. The statistical significance of prevalence in both rainy and dry seasons for goats in all the five local governments was analyzed using chi-square test.

3.3 RESULTS :

Out of 675 goats examined, 24 were infected with trypanosomes, with total prevalence rate of 3.5%. *Trypanosome congolense* accounted for all the infections. (Table 3.1 and 3.2). The mean PCV values for non infected goats were 27.6% while the values for dry and rainy season were 13.3% and 16.2% respectively for infected goats (Table 3.3). Infection caused significantly decrease in mean PCV ($P < 0.05$) in blood of infected WAD goats in these areas.

In the study area in all the five local governments during dry and rainy seasons, the results showed that out of 374 goats examined in dry season, 17 were infected with prevalence rate of 4.5%, while out of 301 goats examined during rainy season, 7 were infected with prevalence rate of 2.3% (Table 3. 4). Infection was found to be significantly higher in the dry season in all these WAD goats ($P < 0.05$) than in the rainy season.

Table 3.1: Prevalence of Trypanosomosis in West African Dwarf goats in Ogbomoso area of Oyo State, Nigeria.

LGA Locations	No of animal Examined	No of animal Positive *T. congolense*	*T. vivax*	*T. brucei*	*% Prevalence*
Ogbomoso North	170	8	0	0	4.7
Ogbomoso South	190	3	0	0	1.6
Orire	90	4	0	0	4.4
Surulere	110	3	0	0	2.7
Ogo Oluwa	115	6	0	0	5.2
TOTAL	**675**	**24**	**0**	**0**	**3.5**

Table 3.2: Seasonal Prevalence of Trypanosomosis in West African Dwarf goats in Ogbomoso area of Oyo State, Nigeria.

LGA Locations	Dry season Nov-April			Rainy season May-October		
	No animal examined	No positive	%Prevalence	No Animal examined	No positive	% Prevalence
Ogbomoso North	70	5	7.1	100	3	3.0
Ogbomoso South	99	2	2.0	91	1	1.1
Orire	50	4	8.0	40	0	0.0
Surulere	70	2	2.9	40	1	2.5
Ogo Oluwa	85	4	4.7	30	2	5.7
TOTAL	**374**	**17**	**4.5**	**301**	**7**	**2.3**

Table 3.3: Seasonal influence on Mean PCV(%) of West African Dwarf goats (WAD) in Ogbomoso Area of Oyo State, Nigeria.

LGA	WAD Goats (PCV%)			
	Dry season		Rainy season	
	Infected	Non-Infected	Infected	Non-Infected
Ogbomoso North	14.0(2)	28.0(5)	15.0(1	27.6(5)
Ogbomoso South	16.0(3)	27.5(5)	17.0(1)	27.0(5)
Orire	12.0(3)	27.5(5)	16.0(2)	27.5(5)
Surulere	12.0(3)	27.0(5)	16.0(2)	28.0(5)
Ogo Oluwa	12.5(2)	27.5(5)	17.0(1)	28.0(5)
TOTAL	**13.3(13)**	**27.6(25)**	**16.2(7)**	**27.6(25)**

The values in the parenthesis indicated the number of animals.

3.4 DISCUSSION

Trypanosomosis is one of the major constraints for livestock productivity in sub-Saharan Africa (Murray *et al.*,1982).The prevalence of trypanosomosis have been studied by various workers in all agro-ecological zones in Nigeria. In this study the total prevalence rate of 3.5% was obtained for all WAD goats in Ogbomoso area of Oyo State, Nigeria, this rate is not similar to reports in Ebonyi state with prevalence rate of 4.6% but is not as high as in some reports in other parts of the Nigeria. In Gboko local government area of Benue state, prevalence rate of 51.6% was reported in sheep and 33.3% in goats (Kalu *et al*, 1996), while in Nsukka area of Eastern Nigeria 13.6% was reported by(Fakae and Chiejina,1993) in both WAD sheep and goats.

In Zaria prevalence rate of 1.2% was reported (Ahmed and Agbede, 1993).In recent studies across all agro-ecological zones in Nigeria, Onyia (1997) showed a prevalence rate of 8.6% in sheep, 8.1% in goats and 10.0% in cattle with *T. congolense* accounted for 15.6%, 6.3% and 18.2% for sheep, goat and cattle respectively. The differences in level of prevalence depend in a number of factors like husbandry practiced (Macleannan, 1970), the climate and presence of tsetse flies. In the study area, all the ruminants undergo pastoral management due to the fact that sheep, goats and cattle were grazed together (Kalu *et al.*, 1991), so they were exposed to the same level of infection. Infection rate in WAD sheep is higher ($p < 0.01$) than goats and cattle even though they were maintained under the same husbandry system.

The predominance of *T. vivax* infection had been reported by many epizootiological studies in Nigeria (Joshua and Ige, 1982; Anosa *et al.*, 1995; Kalejaiye *et al.*, 1995; Kalu *et al.*, 1996; Onyia, 1997; Agu and Amadi, 2001). The reverse is the case in this study, *T. congolense* was the only trypanosomes that accounted for the infection. The reasons for this may be due to the fact that *T. congolense* may well be adapted to development in the tsetse flies in this region than others. The infection rate in the study in dry season was higher ($P < 0.05$) than that of rainy season in all the goats. This agreed with Anosa *et al.*, (1995) in South Western Nigeria, Kalu and Lawani, (1996) in Kano state, Nigeria and Agu and Amadi, (2001) in Ebonyi state, Nigeria. The reasons for higher prevalence in dry season in the present study could be due to presence of few ponds during dry season which were available for many animals to drink in the immediate surrounding of the surviving ponds and also provided enabling environments for the tsetse flies with the high result of the fly- animal contact. A similar situation was

observed in Ebonyi state by Agu and Amadi (2001), although *Trypanosoma vivax* was incriminated (Jordan, 1965; Hoare, 1972; Agu and Amadi, 2001), which was contrary to what was observed in this study. There was a significant differences ($P<0.05$) in the PCV of both infected and non-infected goats in the area of study. This conformed with report of Anosa and Isoun, 1983; Anosa, 1988 a,b;Ogunsanmi *et al.*,1994) that anaemia was a cardinal symptom of trypanosomosis. Poor nutrition and intercurent gastro-intestinal helminth infection could also contribute to the general low PCV.

In the Southern part of Nigeria, goats are of great economic importance, particularly in Ogbomoso area. These animals serve as sources of income for their owners .They sell them for use in traditional ceremonies such as funerals, marriages and other social functions. They serve as sources of animal protein and farm yard manure. With the prevalence rate of 3.5% and relatively low PCV recorded during this investigation, this may constitute huge economic loss to the people in the areas. The efforts should therefore be made to do further investigation as the Ogbomoso area is one of the area that links the south western part to the Northern region, however, further investigation should involve the influence of graded dietary energy on dynamics *Trypanosoma congolense* infection in WAD goats.

CHAPTER FOUR

EXPERIMENT 1

4.0 CHANGES IN LIVE WEIGHTAND HAEMATOLOGICAL PARAMETERS IN *TRYPANOSOMA CONGOLENSE* INFECTED WEST AFRICAN DWARF GOATS FED THREE DIFFERENT GRADED DIETARY ENERGY

4.1 INTRODUCTION

Trypanosomosis is an important disease of man and animals (Radostitis *et al.*, 2007). It has long been recognized as a major constraints to livestock production in tropical Africa and it is transmitted by tsetseflies (*Glossina*) and is endemic in many parts of tropical Africa including Nigeria. The death of affected animals, stunting and debilitation which occurred during infection resulted in great economic losses. It is well documented that patho-physiological alterations occur in the cellular and plasma components of blood during infection (Makinde *et al.*, 1991). African trypanosomosis are generally characterized by haematological and serum biochemical alteration and the severity of which are often determined by the strain of infecting trypanosomes and the host animals (Anosa, 1988a, b). Dietary energy is known to modulate the severity of trypanosomal infection in animals, (Fagbemi *et al.*,1990; Otesile *et al.*, 1991) but this has not been fully substantiated in West African Dwarf goats. This trial now investigated the influence different levels of dietary energy on body weight changes and haematology of WAD goats experimentally infected with *Trypanosoma congolense*.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Site: The experiment was carried out at the large animal ward II, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ibadan, Ibadan,Oyo state, Nigeria.

4.2.2 Experimental Animals:

A total of fifty four (54) male West African Dwarf (WAD) goats were used, they were purchased at a local market in Ibadan, and were acclimatized for four weeks at the experimental site (Plate I).They were treated against worm infestation,

haemoprotozoan diseases such as babesiosis and trypanosomosis as clinically indicated *Pestes des petit ruminantum* (PPR) vaccine was administered to all goats.Antibiotics (Long acting oxytetracycline at 1ml/10kg body weight),antihelmintics (mebendazole at 12.5mg/kg body weight) and acaricides dips were also administered as clinically indicated (Plate 1).

4.2.3 Grouping of Animals

The goats were randomly divided into three experimental groups (A, B and C) based on weight and sex. Each group contained eighteen goats. The animals were on these rations below for 4 weeks before experimental infections were carried out. This was to create the desired differences in the nutritional status of the animals before experimental infection. In each group (AI, BI and CI), twelve goats were infected with trypanosomes while the remaining four served as non-infected controls (AC, BC and CC).

4.2.4 Feeding of experimental animals

Animals in group A, B, and C were placed on low, medium, and high plane rations respectively based on different dietary energy but isonitrogenous (the crude protein levels were the same). The composition of rations and proximate analyses of the feeds are given in Tables 4.1and 4.2 respectively. Feed was given based on 4.0% of their body weight (maintenance ration) and water was provided ad libitum.

Table 4.1: Composition of rations offered to WAD Goats

Feed ingredients	Percentage of ration		
	A	B	C
Panicuum maximum	20.00	15.00	10.00
Air dried cassava	50.00	55.00	60.00
GNC	10.00	10.00	10.00
PKC	4.00	4.00	4.00
Fish meal 65%	4.25	4.25	4.25
Wheat offals	10.00	10.00	10.00
Oyster shell	1.00	1.00	1.00
Vitamin premix	0.50	0.50	0.50
Salt	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**

Table 4. 2 : Proximate Analysis of ration feeds offered to the WAD Goats

Components	RATION		
	A	B	C
Dry matter %	72.95	75.25	77.57
Crude protein %	3.17	13.26	13.35
Ether extract %	24.64	26.92	29.20
Crude fibre %	16.82	16.82	16.81
Nitrogen free extract %	62.40	54.50	45.80
Total Ash %	3.60	11.60	15.20
Gross Energy(kcal/kg)	2467.29	2548.57	2670.40

4.2.5 Infection with Trypanosomes

The *Trypanosoma congolense* parasites used in this experiment was obtained from the National Institute for Trypanosomosis Research (NITR), Vom, Nigeria. *Trypanosoma congolense* (Binchi Bassa Strain) was obtained and subjected to six passages in albino mice, prior to use (Plate 11).The infected animals were inoculated intraperitoneally with equal number of *Trypanosoma congolense* at the rate of 1.0×10^{6}/ ml in sterile normal saline as used by Talabi, (2006). This experiment lasted for a period of 10 weeks .

4.2.6 Blood Collection Techniques

Five millilitre of blood was taken weekly from the jugular veins of each goats and dispensed inside bijou bottles containing ethylene diamine tetra acetate (EDTA) as anticoagulants. Blood samples were taken to the laboratory in the ice pack for haematology.

4.2.7 Haematology

The blood was analysed for packed cell volume (PCV), haemoglobin concentration (HB), red blood cell (RBC) counts, total and differential leucocyte counts by standard haematological techniques (Schalms *et al,* 1975).Erythrocytic indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated using standard formulae (Jain,1986).

4.2.8 Determination of liveweight

Each animal was put in a light bag and weighed weekly on a hanging weighing balance (Salter suspended Model 285, Made in England).Prior to that, the weight of the light bag was determined. The weight of the animal was then obtained by a method of difference.

4.2.9 Post treatment phase: At the end of five weeks, all the infected goats on graded dietary energy were treated with diminazene aceturate (Berenil^{R}) .

4.2.10 Statistical Analysis and Experimental Design

The parasitaemia, liveweight and haematological changes were observed at pre-infection and post infection and post treatment phase and data were statistically compared with the respective control groups by a one –way analysis of variance (ANOVA) using soft ware SPSS Vs 10. Duncan's multiple range tests were applied to compare the significance of differences of groups. Duncan's multiple range tests were applied to compare the significance of differences of groups and parasite counts were correlated with haematology. $p < 0.05$ and $p < 0.001$ were considered significant and very highly significant difference respectively.

Plate 1. The experimental animals (WADgoats) during the period of acclammatization

4.3 RESULTS

4.3.1 Parasitaemia:

Parasitaemia was first detected at day 7 post-infection (p.i) in the three groups with log parasitaemia of 4.0±0.13, 3.6±0.05 and 3.2±0.02 in all infected animals on low ,medium and high levels of dietary energy respectively with lowest parasitaemia level in goats placed on high level of dietary while highest level was observed in goats on low level of dietary energy.The serial buffy coat examination revealed that all infected goats in the three groups became parasitaemic by day 7 after infection. The parasitaemia persisted with peak parasitaemia on day 35 post-infection. (Tables 4.3 and Plate II).

Table 4.3: The trypanosomal parasitaemia (log_{10}^{6})Trypanosomes /ml blood in goats fed three different levels of dietary energy.

Days (post infection)	Low plane(A)	Medium plane(B)	High plane©
Day 7	4.0±0.13	3.6±0.05	3.2±0.02
Day 14	2.9±0.02	3.0±0.04	2.7±0.05
Day 21	6.8±0.02 [a]	6.2±0.03 [b]	6.0±0.03[c]
Day 28	8.0±0.05 [a]	7.0±0.02 [b]	6.8±0.03[c]
Day 35	8.5±0.03 [a]	8.1±0.03 [b]	7.6±0.02[c]

Values are expressed as means±standard errors of mean.

a,b,c means in the same column followed by different superscript differ significantly ($p < 0.05$).

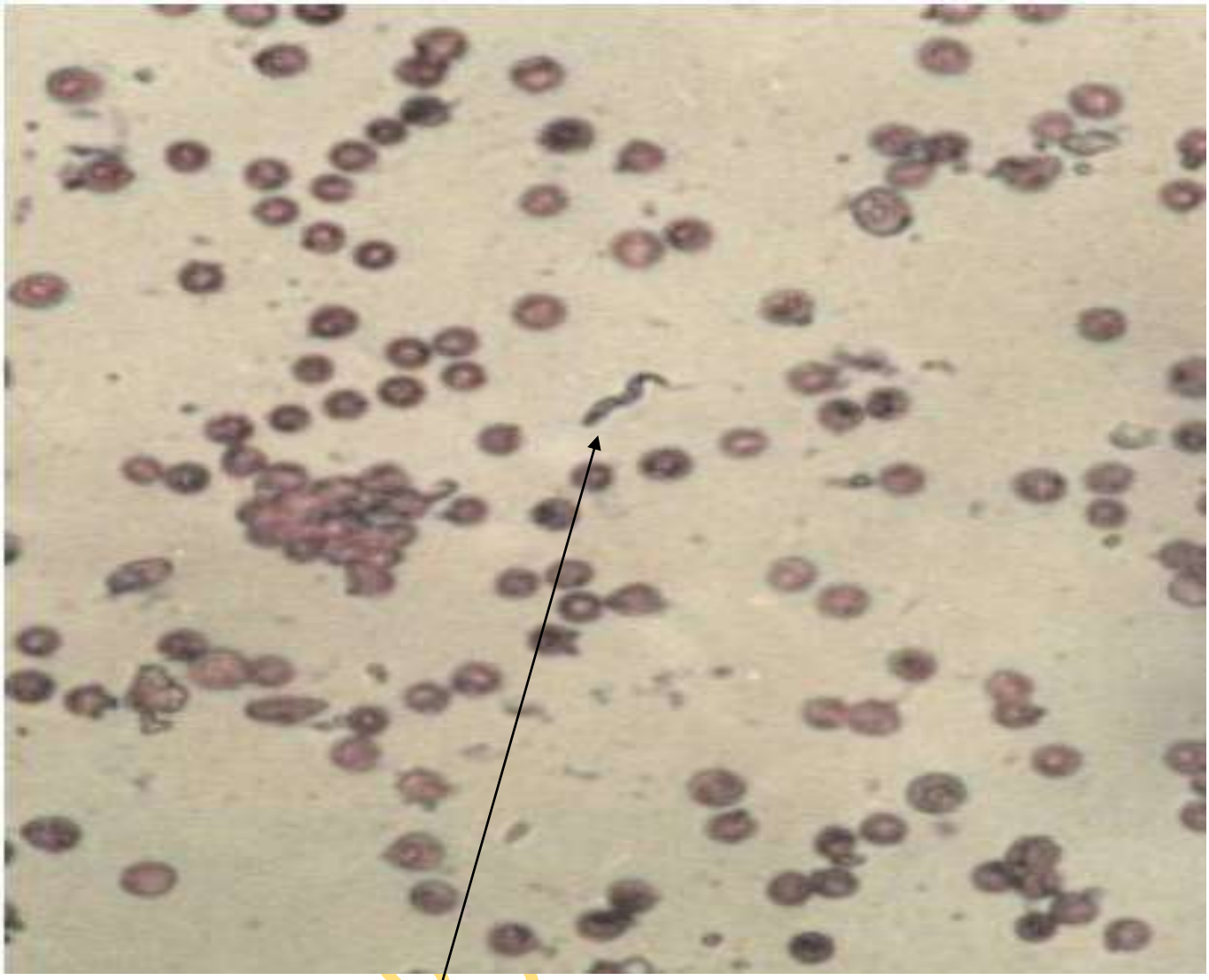


Plate 11: Parasitaemia on week 2 Post- infection with *T.congolense* parasites (x 40) around the centre of the field of view.

4.3.2 Live weight assessment

In group A the mean weekly liveweight gain of infected animal was 0.24±0.04kg while the control group was 0.50±0.03kg (48%), in group B, the mean weekly liveweight gain was 0.44±0.04kg while that of control was 0.72±0.05kg(61%) , in group C the mean weekly liveweight was 0.9±0.06kg while that of control was 1.0±0.06kg (90%),liveweight gain in the infected and their uninfected controls in A, B and C were significantly different ($P<0.05$).Following treatment with diminazene aceturate, group A mean weekly liveweight gain was 0.36±0.02kg while the control group was 0.50±0.02kg (72%), group B mean weekly liveweight gain was 0.46±0.01kg while the control was 1.10±0.02kg(41.8%) , group C mean weekly liveweight gain was 0.50±0.03kg while that of control was 1.4±0.02kg(35%), the liveweight gain in treated and their uninfected controls in A, B and C were significantly different ($P<0.05$). (Tables 4.3 and 4.4).

Table 4.4: The effect of experimental *T. congolense* infection on WAD goats fed at three different planes of nutrition: pre-treatment liveweight changes.

GROUPS	STATUS	NO	LIVE WEIGHT (KG) Wk 1	Wk 5	Weekly Mean weight gain	Overall weight gain
Group A	Infected	14	8.0±0.07	9.2±0.07	0.24±0.04[a]	1.2±0.07
	Control	4	7.5±0.04	10.0±0.07	0.50±0.03[b]	2.5±0.08
Group B	Infected	14	8.0±0.06	10.2±0.04	0.44±0.04[a]	2.2±0.04
	Control	4	7.5±0.04	10.5±0.04	0.72±0.05[b]	3.0±0.08
Group C	Infected	14	8.0±0.05	12.0±0.05	0.90±0.06[a]	4.5±0.06
	Control	4	7.5±0.04	12.5±0.07	1.00±0.06[b]	5.0±0.08

Values are expressed as mean±standard errors of mean.

a,b,c means in the same column followed by different superscript differ significantly

($p < 0.05$)in infected and control in groups A,B and C.

Table 4.5: Effect of experimentally *T. congolense* infection on WAD goats fed at three different planes of nutrition: (iii) post treatment liveweight changes.

GRP	Status of WAD goats	No of WAD goats	Mean liveweight/ kg			
			Week 6	Week 10	Weekly Weight gain	Overall weight gain
A	Infected	14	9.20±0.05	11.0±0.06	0.36±0.02[a]	1.8±0.08
	Control	4	12.50±0.05	15.0±0.08	0.50±0.02[b]	2.5±0.12
B	Infected	14	10.20±0.03	12.5±0.03	0.46±0.01[a]	2.3±0.04
	Control	4	10.50±0.04	16.0±0.08	1.10±0.02[b]	5.5±0.12
C	Infected	14	12.50±0.10	15.0±0.07	0.50±0.03[a]	2.5±0.13
	Control	4	10.00±0.20	17.0±0.12	1.40±0.02[b]	7.0±0.08

Values are expressed as mean±standard errors of mean.

a,b,c means in the same column followed by different superscript differ significantly ($p < 0.05$)in infected and control in group A, B and C.

4.3.3 Group Mean Haematocrit Changes

Following the appearance of the parasites in the peripheral blood there was a decline in group mean PCV values of infected goats in all the three groups. At post infection phase (week 0-5 p.i), the percentage group mean fall in PCV values in group A was from pre-infection value of 27.13±0.08 to 13.39±0.10, while those of the corresponding controls maintained relatively thesame values. In group B, the percentage group mean PCV values declined from preinfection value of 28.13±0.025 to 17.50 ±0.14, while those of the corresponding controls maintained relatively thesame values.In group C the percentage group mean PCV declined from preinfection value of 27.38±0.16 to 18.05±0.09, while those of the corresponding controls maintained relatively the same values. When infected animals in groups A, B and C were statistically compared with their corresponding controls there were statistical very highly significant differences ($p < 0.001$),when infected animals in group A were statistically compared with infected animals in groups B and C, there were significant differences ($p < 0.05$). At the post treatment phase (week 6-10) in group A, the percentage group mean PCV values increased from 14.00±0.41 to 25.00±0.40, in group B, the percentage group mean PCV values rose from 18.00±0.41 to 28.00±0.10 while in group C rose from 18.00±0.41 to 28.25±0.25. All the control groups maintained values which ranged from 26.50±0.00 to 28.50±0.25. When group A, B and C were compared with their corresponding controls, there were very highly significant difference ($p < 0.001$) up to week 10 in group A, while in group B up to week 9 and in group C up to week 8 .When group A, B and C were compared with one another there were significant differences ($p < 0.05$) as shown in Table 4.6.

4.3.4 Group Mean Haemoglobin Concentration Changes

At post infection phase (week 0-5 p.i), the percentage group mean haemoglobin concentration of infected animals dropped from pre-infection value of 9.45±0.02 to 5.15±1.00 and from preinfection value of 9.55±0.20 to 6.05±0.03 in groups A and B respectively while in group C, the percentage group mean haemoglobin concentration of infected animals dropped from pre-infection value of 9.08±0.02 to 6.10 ± 0.05. The fall in group mean haemoglobin concentrations in the three groups when statistically compared to their controls, there were very highly statistical significant differences ($p < 0.001$).When infected animals in groups A, B and C were compared statistically with one another, there were significant differences ($p < 0.05$). At post treatment phase (week 6-10), the percentage group mean Hb concentration in group A increased from 5.23±0.09 to 7.80±0.00), group B increased from 6.60 ±.04 to 8.80±0.04 while group C rose from 6.55±0.05 to 8.83±0.03. All the control groups maintained relatively their normal values but when compared with treated goats at the end of week 10,there were significant differences ($p < 0.05$) between the groups as shown in Table 4.7.

4.3.5 Group Mean Red Blood Cell Counts

At post infection phase (week 0- 5 p.i),.there was a fall in group mean RBC counts of all infected goats the three groups. In group A, the percentage group mean RBC counts dropped from preinfective value of 12.38±0.20 to 7.00±0.20. In group B, the percentage group mean RBC counts dropped from preinfective value of 12.45±0.31 to 7.30±0.12 while in group C, the percentage group mean RBC counts dropped from preinfective value of 12.60±0.02 to 7.70±0.06 ,when compared with their corresponding control groups there were very highly significant differences ($p < 0.001$) ,when infected groups A, B and C were compared with one another there were significant differences ($p < 0.05$). At post treatment phase (week 6-10) the percentage group mean RBC counts in group A increased from 6.53±0.05 to 9.53±0.03, in group B ,the percentage group mean RBC counts rose from 7.10±0.00 to 10.63±0.05 while group C rose from 7.35±0.05 to 11.78±0.03,when compared with their corresponding control groups there were very highly significant difference ($p < 0.001$). At this phase, the RBC values in all treated did not return to normal values as shown in Table 4.8.

4.3.6 Mean Group Mean Corpuscular Haemoglobin (MCH) Changes

The effect of three different levels of dietary energy was observed on mean group mean MCH of all infected goats. In group A, the mean values of MCH in the infected goats fell from pre-infection level of 8.58±0.20pg to 6.68±2.50 3WPI and later rose to 7.35±0.23pg. In group B, the mean values of MCH on the infected goats declined from 7.68±0.23 pg to 7.20±0.09 and then increased to 9.88±0.17 pg and then declined to 9.30±0.15pg at the end of week 5. In group C, the mean value of MCH on the infected goats fell from pre infection level of 10.58±0.15 to 7.90±0.11pg.The mean group mean MCH values of all infected goats and their uninfected control groups were within the normal range and they were not significantly different ($p > 0.05$) but there were significant differences between infected animals in group A, B and C ($p < 0.05$).When the infected animals in group A, B and C were treated with diminazene aceturate, the group mean MCH values response to treatment in group A wererelatively inconsistent and in group B the mean MCH values declined from 9.30±0.03 to 7.81±0.61 and then rose 8.28±0.06,while the percentage group mean MCH values response to treatment in group C fell from 8.91±0.17 to 6.84±0.10 by week 7 and then rose 8.19±0.05 by week 10. All the treated and un-infected control groups were significantly different ($p < 0.05$).At the end of week 10, there were no significant differences ($p < 0.05$) between infected A, B and C. The normal values of MCH range from 6.88- 10.00 (pg). All infected animals and their controls were within the normal ranges of anaemia i.e normochromic anaemia as shown in Table 4.9.

4.3.7 Group Mean Mean Corpuscular Volume Changes

Following infection, there was no significant difference in mean group MCV of infected animals in group A while their uninfected control fluctuated between 22.00 ± 0.01 to 22.50 ± 0.03 fl at the end of the week 5. In group B, the MCV of infected animals declined from pre-infective values of 22.48± 0.74 to 21.25 ± 0.21 fl at the end of week 2 and then increased to 27.33 ± 1.38 fl at the end of the week 5 post infection while their uninfected control groups kept on undulating from pre infective MCV values of 21.85± 0.35 to 22.30± 0.00 at the end of week 5. In group C, the MCV values of infected declined from pre infective values of 31.98± 0.25 to 24.43 ± 0.29 fl. In group A,there was no significant difference ($p > 0.05$) between infected and uninfected control group, while in group B and C there was a significant difference between infected and uninfected control groups ($p < 0.05$). Following treatment with diminazene aceturate, the infected animals in group A maintained their MCV values while their infected control group declined from 23.90± 0.20 to 22.95± 0.35fl.In group B, the MCV values of infected animals increased from 25.35± 0.73 to 26.38± 0.19 fl while their uninfected control group declined from 25.10 ± 0.03 se to 23.00± 0.40 fl. In group C, the MCV values of infected animals increased from 24.85± 0.40 to 28.38 ± 0.38 fl while their uninfected control group kept on undulating from 22.35±0.02 to 25.60± 0.3 fl .There was no significant difference between infected and uninfected control in group A ($p > 0.05$) while there was significant difference ($P < 0.05$) between infected and uninfected control in Group B and C. The normal MCV values range from 19-37 (fl) .All infected animals in groups A, B and C and their controls were within these ranges i.e normocytic anaemia as shown in Table 4.10.

4.3.8 Group Mean MCHC Values (%):

At post-infection phase (week 0-5 p.i), the group MCHC values in group A declined from pre-infective value of 34.84 ± 0.29 to 32.06± 0.60% at week 1 and then rose to 35.65± 0.32 % after week 3, then decreased to 33.58 ± 0.58 % at week 4 and then rose to 38.50± 1.08 % at week 5 while uninfected corresponding controls had their values between 34.55± 0.02 to 34.81±0.02 and then decreased to 33.33 ± 0.01 % at the end of week 5. In group B, the group mean MCHC values decreased from preinfective values of 34.20± 0.60 to 35.38 ± 0.40 % at week 3 and then decreased to 32.70 ± 0.48 % at week 4 and then increased to 34.10 ± 0.10% at the end of week 5, while uninfected corresponding controls declined from pre-infective values of 35.25±0.35% to 33.80± 0.01 %.In group C, the group mean MCHC values increased from pre-infective values of 30.13 ±0.44 to 33.80 ± 1.00% while uninfected corresponding controls declined from 33.10 ± 0.50 to 32.30± 0.01%. When groups A, B and C were paired and compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when compared with one another they were statistical significant differences ($p < 0.05$). At post-treatment phase (week 6-10), the group mean MCHC values in group A declined from 37.45± 1.3 to 31.23 ± 0.51% , while uninfected corresponding controls fluctuated between 33.30± 0.01 and 34.25± 0.45%. In group B, the group mean MCHC values decreased from 36.73± 1.00 to 31. 43± 0.20%, while their uninfected corresponding controls declined from 31.60± 0.00 to 31.30± 0.30 %. In group C, the group mean MCHC values decreased from 36.43± 0.73 to 31.50± 0.00 %, while their uninfected corresponding controls increased from 29.80± 0.70 to 31.20± 0.00 %. When groups A, B and C were paired and compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when compared with one another there were statistical significant differences ($p < 0.05$).The normal MCHC values range from from 30.34 to 38.40%. All infected animals and their controls were within the normal ranges of anaemia i.e normochromic anaemia as shown in Table 4. 11.

4.3.9 Group Mean Total White Blood Cell Count Changes:

At post-infection phase (week 0-5 p.i), the group mean total white blood cell (TWBC) values of group A increased from preinfective values of 11.80±0.12 to 14.50±0.40/mm^3 at the end of week 5, in group B, the group mean TWBC values declined from pre-infective values of 12.23± 0.13 to 11.10 ± 0.06/mm^3 while in group C, the group mean TWBC increased from pre- infective values of 12.80±0.13 to 14.00± 0.78/mm^3. When infected and their corresponding controls were paired and compared in group A, B andC, there was no statistical significant difference ($p > 0.05$) between them.At post treatment phase (week 6-10) , the group mean TWBC in group A increased from 12.03± 0.18 to 13.53± 0.02 mm^3 , in group B, the group mean TWBC increased from 10.53± 0.00 to 11.75± 0.02 /mm^3 while in group C, the group mean TWBC increased from 13.70± 17 to 16.58± 0.07/mm^3,when all treated and corresponding controls in group A, B and C were paired and compared there were very significant differences ($p < 0.001$) as shown in Table 4.12.

4.3.10 Group Mean Neutrophil Count Changes

At post-infection phase (week 0-5 p.i), the group mean neutrophils values in group A decreased from pre-infective values of 65.00± 0.05% to 59.00± 0.08%, while their uninfected control increased from 65.00± 0.01 to 68.50± 0.05%. In group B, the group mean neutrophil values decreased from pre-infective values of 65.30±0.09 to 56.00±0.05, while their uninfected control values increased from 64.50± 0.05 to 68.50± 0.15%. In group C, the group mean neutrophils values declined from pre-infective value of 64.50± 0.10 to 59.50 ± 0.33, while their uninfected control values increased from 65.00± 0.10% to 67.50± 0.05%. When the group mean neutrophil values of group A, B and C were paired and compared with their corresponding controls, there were very highly significant differences ($p < 0.001$),but when the group mean neutrophil values of groups A, B,and C were paired and compared,there was no significant difference ($p > 0.05$)in group B and C,but signicant difference ($p < 0.05$) was observed in groupA . At post treatment phase (week 6-11p.i),the group mean neutrophil values relatively increased from 59.75± 0.48 to 61.00± 0.05 in group A, while the uninfected controls decreased from 68.10 to 70.15± 0.50 %. In group B, the group mean neutrophil values increased from 56.00± 0.14 to 62.00± 0.03 while uninfected controls increased from 68.50± 0.50 to 70.20± 0.02. In group C, the group mean neutrophil values increased from 58.25±0.45 to 66.25± 0.25 while unfected controls increased gradually and steadily from 63.50 ± 0.50 to 66.00± 0.00. When groups A, B and C and their corresponding controls were paired and compared, there were very highly significant difference ($p < 0.001$), but but when paired and compared with one another there were significant difference ($p < 0.05$) as shown in Table 4.13.

4.3.11 Group Mean Lymphocyte Count Changes:

At post-infection phase (week 1-5 p.i), in group A, the group mean absolute lymphocyte counts declined from 61.00 ± 0.71 to 37.00± 0.58,while unfected control declined steadily from pre-infective values of 67.00± 0.04 to 63.00 ±1.00. In group B, the group mean absolute lymphocyte counts decreased from pre-infective values of 58.50±1.50 to 41.40 ±0.65 while unfected control increased from 62.00 ± 0.02 to 64.00 ±0.03. In group C, the group mean absolute lymphocyte counts declined from 61.75 ±0.63 to 50.50 ±0.39 while the uninfected control declined from 63.50± 0.50 to 61.50± 1.50.When groups A, B and C were paired and compared with their corresponding controls, they were highly very significant ($p < 0.001$)but when compared with one another they were statistically significant ($p < 0.05$).At post-treatment phase (week 6-10), in group A, the group mean absolute lymphocyte counts increased from 40.00±0.06 to 54.75±0.02,while the corresponding uninfected controls maintained relatively thesame group mean absolute lymphocyte counts .In group B, the group mean absolute lymphocyte counts increased from 41.75± 0.25 to 54.75±0.60, while corresponding uninfected controls increased from 60.00 ± 0.02 to 64.90 ± 2.50. In group C, the group mean absolute lymphocyte counts increased from 42.35 ± 0.25 to 55.38± 0.41 while, the uninfected control decreased from 64.50± 0.50 to 63.50 ± 2.50.When groups A, B and C were paired and compared with the corresponding controls, they were highly very significant ($p < 0.001$) but when compared with one another group C were statistically significant difference ($p < 0.05$)from group A and B as shown in Table 4.14.

4.4 DISCUSSION

The result of the present study showed that dietary energy had a marked influence on parasitaemia, body weight changes and haematology during infection and post re-infection phase and also on the rate of recovery from anaemia following administration of a trypanocidal drug to the WAD goats.The different levels of dietary energy influenced the susceptibility of goats to *T.congolense* infection and the infected goats in group A fed low levels of dietary energy had significant high parasitaemia, retardation of growth and high levels of anaemia than infected goats in group B and C fed medium and high levels of dietary energy and this agree with (Katunguka-Rwakishaya *et al.*,1999). The groups of infected goats fed different levels of dietary energy became parasitaemic at the same time during infection and post reinfection period. It was observed that differences in dietary energy had no effect on the prepatent period to parasitaemia, this agreed with observation made by Little *et al.*, (1990), who found no differences in the prepatent periods in N'Dama cows inoculated with bloodstream forms of *T. congolense* when on either a low or a high plane of nutrition. In contrast, Hecker *et al.*, (1991) observed that sheep supplemented with cotton seed cake and maize bran showed a delayed onset of parasitaemia compared to the animals that were only grazing natural grasslands. However, it should be noted that these animals were infected by exposure to high tsetse challenge in the field, although Trypanosomal infections were characteriesd by undulating parasitaemia and the host goats did not reveal parasites in their peripheral blood for several days.This observation agreed with (Vickerman, 1978). All infected animals became aparasitaemic after treatment with Berenil® at dose rate of 3.5mg/kg body weight.This observation agreed with (Otesile *et al*., 1992) who treated pigs infected with *T.brucei* and fed different levels of dietary energy with Isometadium chloride (Suramin®) . Following the establishment of infection, infected animals fed different levels of dietary energy experienced greater retardation of growth than their control groups. These findings were in agreement with those of Hecker *et al.*, (1991) on Djallonke sheep and Agyemang *et al.*, (1990) on N'Dama cattle exposed to natural fly challenge. The observation of more pronounced retardation of growth in infected animals on a low dietary energy level in the ration cannot be attributed to a decrease in feed intake alone but due to infection, as this was significantly lower than that of control animals. It is of interest to note that by 5 weeks infection and re-infection goats on low ,medium and high energy rations had started to lose body weight, indicating that the feed consumed was no longer sufficient to meet the maintenance requirement in the state of infection with the parasite.This

observation was similar to Fagbemi *et al.*,(1990) in boars placed on different dietary energy and infected with *T.brucei.*Ilemobade and Balogun (1981) made a similar obser vation on pigs infected with a chronic disease-inducing strain of *Trypanosoma simiae* and ascribed it to a combination of lowered voluntary feed intake and worsened feed conversion efficiency. This finding is in contrast with reports of reduced feed intake in goats infected with *T. brucei* (van Miert *et al.*,(1990) or *T. vivax* (Zwart *et al.*, 1991). Experiments in goats infected with *T.vivax* (Verstegen *et al*., 1991) have demonstrated that development of fever during a course of trypanosome infection is associated with increased heat production and increased metabolisable energy for maintenance. Thompsen, (1987) showed that parasitism alters some aspects of carbohydrate metabolic pathways in the host. There is apparently no specific report on carbohydrate metabolism in *Trypanosoma-infected* animals. As for proteins, however, it is known that there is excessive protein catabolism (Wellde *et al.*, 1974) which is often masked by a corresponding hyperimmunoglobulinaemia (Hudson *et al.*, 1976). This increased synthesis of immunoglobulins and essential amino acids occurs at the expense of muscle protein synthesis and results in loss of weight and with the carcass having the appearance of non-specific cachexia (Dargie,1980). There were declines in PCV, RBC count and Hb concentration in this study, it is obvious from the results that the reduction of these erythrocytic values were more pronounced in the group of goats on a low level of dietary energy and this agree with observation of Fagbemi *et al.*,(1990) in boars placed on different dietary energy and infected with *T.brucei.* It is well known fact that trypanosomosis causes anaemia (Stephen, 1966;Anosa,1988a,b) and that the impairment of the re-utilisation of i3ron from degraded erythrocytes, as a result of blockage of reticuloendothelial iron release, results in decreased RBC values (Dargie *et al.*, 1979a, b). It is therefore possible that (adequate) dietary energy is a requirement for the re-utilisation of iron in trypanosomosis.Another reason why the infected goats on low dietary levels resulted in pronounced reduction in erythrocytic values was that the kinetics of erythroid cells were affected due to changes in biochemical and metabolic pathways during erythropoiesis.The low levels dietary energy causes ineffective erythropoiesis with prominent disorder of heme,a pigment component of haemoglobin in the developing erythroid cells in the marrow.Heme is a planar molecule composed of the tetrapyrrole protoporphyrin 1X,containing a central ferrous molecules.The initial rate controlling step in heme synthesis,the delta aminolevulinic acid (ALA) synthase (5-amino levulinate synthase) reaction occurs with in mitochondria.Glycine and the krebs cycle intermediate

succinyl-coA are utilized as substrates and vitamin B6 as pyridoxical phosphate is required as a cofactor.The ALA formed is transported to the cytoplasm where a series of reactions results in the formatiom of coproporphyrinogen 111, which must enter the mitochondria for the final steps in the heme synthesis (Harvey,2008).Following synthesis,heme must be transferred from mitochondria to the cytoplasm for combination with the globin chains to complete the synthesis of haemoglobin.Heme, therefore affects erythroid cell metabolism in different ways depending on the stage of maturation.The higher the levels of succinyl.coA produced from krebs cycle the the higher the levels of heme formed, therefore if the levels of dietary energy is low, the succinyl.coA produced from krebs cycle will be low and hence heme synthesis is affected and the production of haemoglobin in the erythroid cell will be affected and the levels of red blood cell production will be affected. The present study has shown that a high dietary energy diet ameliorates the effect of trypanosome infection on growth rate. Following patency, all infected animals in groups A, B and C on different dietary groups developed anaemia. In group B and C infected goats, the anaemia was characterized by normocytic-normochromic during infection . The MCH, MCV and MCHC of all infected animals and their control groups were within the anaemic value ranges. This agrees with Little *et al.*, (1990) who observed that the rate of development of anaemia in N'Dama cattle inoculated with *T. congolense* and supplemented with extra groundnut was slower than in unsupplemented cattle and but disagrees with observation of similar degrees of anaemia in the low and high plane infected groups by the findings of Agyemang *et al*., (1990) in N'Dama cattle. In agreement to these reports, the present study showed that trypanosome establishment and the rate of development of anaemia in trypanosome infected WAD goats is influenced by different dietary energy. The leucocytes, neutrophils and lymphocyte levels decreased drastically in infected animals in all the groups but effect was pronounced in group A with low dietary energy.This is an indication of immunodepression .The mechanisms of immunosuppressive effects of trypanosomosis had been attributed to activation and exhaustion of of poly clonal B-cells (Esuruoso,1976;Hudson *et al.*,1976),release of large quantities of of free fatty acids by trypanosomes,(Tizard *et al.*,1978) activation and hypocomplementamia of classical and alternate pathways(Kobayashi and Tizard,1976;Kumshe,2005;Talabi,2006),defect in functional macrophage population,inhibition of increased levels of suppressor of T-cells (Jayawardena and Waksman,1977) and production of soluble suppressor of factors (Moulton and Coleman,1977).These result in immunosuppressive effects on the host

animals and serve as an indication of lymphocyte malfunction (Molyneux and Ashford,1983) and this agree with observation of Fagbemi *et al.*,(1990) in boars placed on different dietary energy and infected with *T.brucei.* Following chemotherapeutic treatment with berenil® , all infected goats became aparasitaemic ,there were improvement relatively in the body weight gain of treated animals,the haematological parameters returned back to normal pre infection values and the adverse effects of immunosuppression in trypanosomosis tend to be reversed immediately following chemotherapy in this study . From this study it was observed that the higher the level of dietary energy the lower the level of susceptibility in caprine experimental trypanosomosis.

CHAPTER FIVE

EXPERIMENT 2

5.0 CHANGES IN BIOCHEMICAL PARAMETERS IN *TRYPANOSOMA CONGOLENSE* INFECTED WEST AFRICAN DWARF GOATS FED THREE DIFFERENT GRADED DIETARY ENERGY

5.1 INTRODUCTION

In a previous study in (experiment 1), it was observed that the level of dietary energy in goats influences the haematological responses to *T.congolense* infection. Infected goats on high-energy diets had higher liveweight gains and tended to develop lower parasitaemias than those on lower energy feeds. Serum biochemical changes during trypanosomosis have been reported in cattle (Finnes *et al.*, 1946), and in sheep and goats (Anosa and Isoun, 1976). These changes have been extensively reviewed (Anosa, 1988a,b). Abnormalities in lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosomes. Thus when rabbits were experimentally infected with *T. brucei* (Rouzer and Cerami, 1980), an increase in plasma cholesterol and total lipids occurred. In contrast, *T. rhodesiense* infection in cattle (Wellde *et al*., 1989) and *T. congolense* infection in sheep (Katunguka-Rwakishaya *et al*., 1992b), were both associated with a drop in plasma cholesterol concentration. Changes in other biochemical parameters, for example plasma proteins (Holmes, 1976) and serum iron (Tartour and Idris, 1973), have also been reported in animals infected with trypanosomes Otesile *et at.*, (1991) reported changes in serum biochemistry as influenced by different levels of dietary energy in boars infected with *T.brucei.*There were changes in transaminases,serum total protein,albumin,globulin and serum testosterone as influenced by dietary energy during trypanosomosis. Biochemical changes in humans and animals during trypanosomosis have been documented (Anosa, 1988a, b). It has been suggested that serum biochemistry might give an indication of the degree of damage to the host tissue as well as the severity of infection (Otesile *et al.*, 1991; Cano *et al.*, 2004). Although the biochemical changes have been used as indicators of tissue damage, specific organ functional defect and response to therapy during disease (Otesile *et al.*, 1991; De Souza *et al.*, 2000; Manga-Gonzalez *et al.*, 2004), have not been exhaustively studied in the *T.congolense* in goat model. Although *T.congolense* parasites are located in blood while *T.brucei* parasites invade host tissues and the pathology induced by the organisms is related to the site of localization (Losos and Ikede, 1972). It

is, therefore, conceivable that serum biochemistry might give an indication of the degree of damage to host tissues and, hence, the severity of infection. This study evaluated the serum levels of some biochemical analytes in goats experimentally infected with *T.congolense* when on three different levels of dietary energy feeds.

5.2 MATERIALS AND METHODS:

5.2.1 The experimental site, experimental animals, infection techniques, animal grouping and feeding, Statistical analysis and feeds proximate analysis were as described for Experiment 1.

5.2.2 Serum biochemistry

Five milliliters (5ml) of blood sample was collected in anticoagulant free plastic tube and allowed to clot at room temperature within 3 hours of collection. The serum samples were separated and stored at – 20^0C for biochemical analysis.

The serum sodium, potassium, calcium, inorganic phosphate, bicarbonate, urea, total protein, albumin, globulin, alanine amino transaminases and aspartate amino transaminases in infected and non-infected goats were measured during the phases of the experiment.

Serum sodium and potassium concentrations were determined by flame photometry (Hawk *et al.*, 1954). Serum calcium concentration ,phosphate and bicarbonate were estimated according to Schales and Schales 1941; Toro and Ackerman, 1975 respectively. The serum concentration of aspartate amino transferase (transaminase AST) and Alanine amino transferase (transaminase ALT) were determined by colourimetric method as described by Reitman and Frankel, (1957).

Total serum protein (TP) was determined by Biuret techniques using colourimetric method(Toro and Ackerman, 1975), while albumin was determined by using the Bromocresol green (BCG) method as described by Peter *et al.*, (1982) and globulin by subtracting albumin from total proteins.The globulin and albumin:globulin ratio were calculated according to Coles,(1986).

5.3 RESULTS

5.3.1 Group Mean Serum Sodium Concentration (mmol/l):

At post-infection phase (week 0-5 p.i),the group mean serum sodium concentration of in group A goats increased from pre-infective value of 138.00 ± 0.41 to 152.80±0.52 mmol/l,while the uninfected controls increased from 138.00±0.01 to 142.80±0.50 mmol/l .The group mean serum sodium concentrations in group B increased from pre-infective value of 138.50±0.50 to 146.00±0.41 mmol/l,while the uninfected controls maintained relatively the same serum concentration from pre- infective values of 138.00±0.05 to 138.04±0.01 mmol/l. The group mean serum sodium concentration in group C relatively increased from pre-infective values of 138.75±0.63 to 138.80±0.04, while the uninfected controls maintained the same concentration. When groups A, B and C were statistically compared with their corresponding controls, the groups A and B were significant different ($p < 0.001$)while group C had no significant difference ($p > 0.05$)when compared with the control. At the post-treatment phase (week 6-10), the group mean serum sodium concentration in group A decreased from 152.00±0.27 to 138.00±0.26 mmol/l, while the uninfected control A declined from 142.00±0.58 to 140.00±0.01. In group B, the group mean serum sodium concentrations decreased from 145.75±1.25 to 138.75±0.63 mmol/l while the uninfected control B maintained relatively the same mean serum sodium concentration. In group C, the group mean serum sodium concentration of both treated and their uninfected control maintained relatively the same mean serum sodium concentrations. When groups A, B and C were statistically compared with one another and their controls, there were no significant differences ($p > 0.05$) as shown in Table 5.1.

5.3.2 Group Mean Serum Potassium Concentration (mmol/l):

The group mean serum potassium concentrations of goats infected with *T.congolense* and fed three different levels of dietary energy were as shown in Table 5.2. At post-infection phase (week 0-5 p.i), the group mean serum potassium concentration in group A decreased from pre-infective value of 3.64±0.02 to 2.47± 0.01 mmol/l, while the uninfected controls kept on undulating from 3.68±0.02 mmol/l and rose to 3.70± 0.01mmol/l by week 2 p.i and then declined to 3.65± 0.05mmol/l .The group mean serum potassium concentration in group B declined from pre-infective values of 4.76±0.02 to 4.14±0.07mmol/l while the uninfected controls increased from 4.76± 0.01 to 4.80±0.01mmol/l. In group C the group mean serum potassium concentration declined from pre-infective values of 4.76±0.01 to 4.10± 0.03 mmol/l, while the uninfected controls declined from 4.80±0.70 to 4.75± 0.01 mmol/l. When groups A, B and C were statistically compared with their controls, there were very highly statistically significant differences ($p < 0.001$)but when infected goats in groups A, B and C were compared with one another, group A was statistically different from B and C ($p < 0.05$). At the post-treatment phase (week 6-10), the group mean serum potassium concentration in group A increased from 2.44±0.27 to 3.95±0.28 mmol/l,while the uninfected controls increased from 3.63±0.01 to 3.95±0.03 mmol/l. The group mean serum potassium concentration in group B increased from 4.13 ± 0.05 to 4.80± 0.15 mmol/l,while the uninfected controls relatively increased from 4.84± 0.00 to 4.85±0.03 mmol/l. The group mean serum potassium concentration in group C increased from 4.11 ± 0.03 to 4.68± 0.02mmol/l while the uninfected controls relatively maintained the same values from 4.70± 0.01 to 4.70±0.08 mmol/l. When groups A, B and C were compared with their corresponding controls, there were very highly significant differences ($p <0.001$) as shown in Table 5.2.

5.3.3 Group Mean Serum Bicarbonate Concentration (mmol/l):

At post-infection phase (week 0-5),the group mean serum bicarbonate concentration in group A declined from pre-infective values of 24.43± 0.08 to 16.38±0.05 mmol/l, while uninfected corresponding controls declined from 24.60±0.01 to 23.00±0.02 mmol/l. The group mean serum bicarbonate concentration in group B declined from 23.30±0.06 to 20.93±0.25mmol/l, while uninfected correspondin controls declined from 24.35±0.25 to 23.30±0.01 mmol/l. The group mean serum bicarbonate concentrations in group C declined from 25.00±0.14 to 19.58±0.11 mmol/l, while uninfected corresponding controls declined from 25.45±0.15 to 25.00± 0.01 mmol/l. When groups A, B and C were compared with their corresponding controls, there were very highly significant differences ($p < 0.001$) but when compared with one another there were statistical significant differences ($p < 0.05$). At post treatment phase (week 6-10), the group mean serum bicarbonate concentration in group A increased from 16.30± 0.07 to 22.45± 0.01mmol/l, while uninfected corresponding controls increased from 23.30±0.17 to 24.30±0.01 mmol/l. The group mean serum bicarbonate concentration in group B increased from 20.30± 0.17 to 24.30±0.01 mmol/l, while uninfected corresponding controls increased from24.00±0.23 to 24.30±0.02.mmol/l. The group mean serum bicarbonate concentration in group C increased from 19.39±0.03 to 25.53±1.33 mmol/l, while uninfected corresponding controls declined from 25.00±0.01 to 24.05±0.02. When groups A, B and C were compared with their corresponding controls,there were very highly significant differences($p < 0.001$) but when compared with one another there were statistical significant difference ($p < 0.05$) as shown in Table 5.3.

5.3.4 The Group Mean Serum Calcium Concentration (mmol/l):

At post-infection phase (week 0-5 p.i),the group mean serum calcium in group A increased from pre-infective values of 9.00 ± 0.30 mmol/l to 10.63 ± 0.03 mmol/l, while uninfected corresponding controls increased from 8.40 ± 0.01 mmol/l to 8.60 ± 0.03 mmol/l. The group mean serum calcium in group B increased from 8.45 ± 0.03 mmol/l to 8.65 ± 0.03 mmol/l, while uninfected corresponding controls declined from 8.50 ± 0.01 to 8.40 ± 0.03 mmol/l. The group mean serum calcium concentration in group C increased from 8.35 ± 0.03 to 9.15 ± 0.03 mmol/l, while uninfected corresponding controls declined from 8.45 ± 0.05 to 8.40 ± 0.01 mmol/l. When groups A, B and C were compared with their corresponding controls,there were very highly significant difference in groups A, B and C ($p < 0.001$) but when infected A, B and C were compared with one another there were statistical significant difference ($p < 0.05$). At post treatment phase (week 6-10), the group mean serum calcium concentration in group A declined from 10.13 ± 0.38 to 8.49 ± 0.03 mmol/l, while uninfected corresponding controls declined from 8.50 ± 0.05 to 8. 45 ± 0.02 mmol/l. The group mean serum calcium concentration in group B declined slightly from 8.55 ± 0.03 to 8.50 ± 0.02 mmol/l, while uninfected corresponding controls decreased from 8.80±0.17 to 8.40±0.01 mmol/l. The group mean serum calcium concentration in group C declined from 8.95 ± 0.09 to 8.54 ± 0.02 mmol/l,while uninfected corresponding controls kept on slightly increased from 8.35 ± 0.49 to 8.40 ± 0.01 mmol/l. When groups A, B and C were compared with their corresponding controls, there were no significant differences($p > 0.005$) even when compared with one another there were no statistical significant difference ($p > 0.05$) as shown in Table 5.4.

5.3.5 Group Mean Serum Phosphate Concentration (mmol/l):

At post-infection phase (week 0-5 p.i), the group mean serum phosphate concentration in group A increased from pre-infective value of 3.58 ± 0.05 to 4.95 ± 0.03 mmol/l, while the uninfected corresponding controls increased from 3.63±0.03 to 3.67±0.05 mmol/l at week 1, then declined to 3.36±0.06 mmol/l at week 4 and then rose to 3.70±0.01mmol/l at week 5.The group mean serum phosphate concentration in group B increased from pre-infective values of 3.66±0.02 to 3.53±0.05 mmol/l at week 1 p.i.and then rose to 3.89±0.01 mmol/l, while the uninfected corresponding controls increased from 3.60± 0.01 to 3.65±0.03mmol/l. The group mean serum phosphate concentration in group C increased from pre-infective value of 3.75±0.03 to 4.43±0.05 mmol/l, while the uninfected corresponding controls had no significant changes from 3.80±0.01 to 3.85±0.03mmol/l. When groups A, B and C were compared with their corresponding controls,there were very highly significant difference ($p < 0.001$) but when compared with one another there were statistically significant differences ($p < 0.05$). At post-treatment phase (week 6-10), the group mean serum phosphate concentration in group A decreased from 4.75± 0.01 to 3.74±0.04 mmol/l , while the uninfected corresponding controls increased from 3.65±0.01 to 3.85±0.14 mmol/l at week 8 and then declined to 3.70±0.01 mmol/l at week 10. The group mean serum phosphate concentration in group B declined from 3.89±0.30 to 3.63± 0.09 mmol/l, while the uninfected corresponding controls increased from 3.63±0.01 to 3.80± 0.12 mmol/l.The group mean serum phosphate concentration in group C declined from 4.30± 0.02 to 3.73±0.01 mmol/l, while the uninfected corresponding controls increased from 3.70±0.01 to 4.00± 0.01 mmol/l at week 8 and then 3.70±0.02 mmol/l at week 10. When groups A, B and C were statistically compared with their corresponding controls,there were very highly significant differences ($p < 0.001$) but when infected animals were compared with one another there were statistically significant differences ($p < 0.05$) as shown in Table 5.5.

5.3.6 Group Mean Blood Urea Nitrogen (BUN):

At post-infection (week 0-5 p.i), the mean serum blood urea nitrogen concentration in group A increased from pre-infective values of 20.73±0.14 to 22.05±0.03 mg/dl, while the uninfected corresponding controls maintained relatively the same concentration 21.00±0.01 to 21.00 ± 0.04 mg/dl .The group mean serum blood urea nitrogen in group B increased from pre-infective values of 22.13± 0.06 to 23.85±0.05 mg/dl, while the uninfected corresponding controls decreased from 22.03±0.25 to 21.00±0.85mg/dl. The group mean serum of blood urea nitrogen in group C decreased from pre-infective values of 22.10±0.8 to 20.25±0.14 mg/dl, while the uninfected corresponding controls declined from pre-infective values of 21.90±0.10 to 21.25±0.14 mg/dl. When infected animals in groups A,B and C were statistically compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when infected animals in groups A, B and C were statistically compared with one another there were statistically significant differences ($p < 0.05$). At post treatment phase (week 6-10), the group mean serum of blood urea nitrogen in group A declined by from 21.88± 0.03 to 20.65 ± 0.07 mg/dl, while the uninfected corresponding controls had no significant changes from to 21.00 ± 0.02 to 21.25 ± 0.14 mg/dl. The group mean serum of blood urea nitrogen in group B increased from 21.62 ± 0.01 to 23.10±0.68 mg/dl at week 7 and then declined to 21.05±0.05 at week 5, while uninfected corresponding controls maintained relatively the same concentration 21.69 ± 0.01 to 21.50 ± 0.26mg/dl. The group mean serum blood urea nitrogen in group C declined from 20.13±0.80 to 22.10±0.25 mg/dl, while uninfected corresponding controls had no significant changes 21.15±0.26 to 21.90±0.23mg/dl. When groups A, B and C were statistically compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when compared with one another there were statistical significant difference ($p < 0.05$) as indicated in Table 5.6.

5.3.7 Group Mean Serum Total Protein (TP g/dl):

At post-infection phase (week 0-5 p.i),the group mean serum TP in group A decreased from pre-infective value of 6.73 ± 0.01 to 6.58 ± 0.03g/dl, while the uninfected corresponding controls declined from 7.78± 0.03 to 6.80 ± 0.03g/dl .The group mean serum total protein in group B declined from pre-infective value of 7.55 ± 0.03 to 6.85± 0.02 g/dl in week 1 p.i and rose again and decreased to 7.31 ± 0.03g/dl, while the uninfected corresponding controls increased from 7.55 ± 0.05 to 7.60 ± 0.01g/dl, then declined to 7.00 ± 0.05g/dl at week 4 and then rose again to 7.50 ± 0.01g/dl at the end of week 5. The group mean serum total protein in group C declined from pre-infective value of 7.65 ± 0.05g/dl to 7.08 ± 0.05g/dl at week 1 and then rose to 7.60 ± 0.01g/dl at the end of week 5 PI, while the uninfected corresponding controls declined from 7.75 ± 0.05g/dl to 7.60 ± 0.01g/dl at the end of week 5.When groups A, B and C were statistically compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when infected animals in groups A, B and C were compared with one another there were statistically significant differences ($p < 0.05$). At post-treatment phase (week 6-10), the group mean serum TP in group A declined from 6.98 ± 0.04g/dl to 6.71 ± 0.08g/dl, while the uninfected corresponding controls increased from 6.80 ± 0.01g/dl to 8.00 ± 0.01g/dl . The group mean serum TP in group B increased from 7.24 ± 0.02 to 7.80 ± 0.12g/dl , while the uninfected corresponding controls increased from 7.50±0.01g/dl to 8.45 ± 0.02g/dl .The group mean serum TP in group C increased from 7.48 ± 0.03 to 8.10 ± 0.17g/dl, while the uninfected corresponding controls increased from 7.70±0.05 to 8.00 ± 0.17g/dl . When groups A, B and C were statistically compared with their corresponding controls,there were no significant differences ($p > 0.05$) but when infected animals in groups A, B and C were compared with one another there were statistically significant differences ($p < 0.05$) as shown in Table 5.7.

5.3.8 Group Mean Serum Globulin Concentration (g/dl):

At post-infection phase (week 0-5 p.i),the group mean serum globulin in group A increased from pre-infective value of 3.31 ± 0.01g/dl to 4.00 ± 0.04g/dl at week 4 and then declined to 3.95 ± 0.03g/dl at week 5 p.i, while the uninfected corresponding controls A increased from 3.33 ± 0.04g/dl to 3.65 ± 0.25g/dl at week 4 p.i and then declined to 3.40 ± 0.25g/dl at week 5.The group mean serum globulin in group B declined from pre-infective of 3.78 ± 0.05g/dl to 3.33 ± 0.03g/dl at week 1 p.i, then increased to 4.13 ± 0.02g/dl at week 2 p.i, then declined to 3.94 ± 0.18g/dl at week 4 p.i and then rose to 4.16 ± 0.03g/dl at week 5 p.i, while the uninfected corresponding controls increased from 3.70 ± 0.01g/dl to 4.00 ± 0.10g/dl then declined to 3.87± 0.03g/dl. The group mean serum globulin in group C declined from pre-infective value of 3.83 ± 0.05g/dl to 3.56 ± 0.05g/dl at week 1 p.i, and then rose to 4.33 ± 0.03g/dl at week 5 p.i, while the uninfected corresponding controls declined from 4.00 ± 0.01g/dl to 3.80 ± 0.01g/dl at week 3 p,i, and then rose to 4.05 ± 0.25g/dl and then declined to 3.80 ± 0.01g/dl. When groups A, B and C were statistically compared with their corresponding controls,there were very highly significant differences ($p < 0.001$) but when infected animals in groups A, B and C were compared with one another there were statistically significant differences ($p < 0.05$). At post-treatment phase (week 6-10), the group mean serum globulin in group A ranged from 3.65 ± 0.18 to 3.90 ± 0.21 g/dl at week 10, while the uninfected corresponding controls increased from 3.40 ± 0.01g/dl to 3.89 ± 0.25 g/dl. The group mean serum globulin in group B increased from 4.08 ± 0.01g/dl to 4.43 ± 0.11g/dl at week 8, then decreased to 4.11 ± 0.12 g/dl at week 10, while the uninfected corresponding controls increased from 3.85 ± 1.00 to 4.30 ± 0. 01 g/dl.The group mean serum globulin in group C declined from 4.28 ± 0.03g/dl to 3.81 ± 0.06 g/dl at week 9 and then rose to 4.04 ± 0.05 g/dl at week 10, while the uninfected corresponding controls declined from 3.95 ± 0.02g/dl to 3.65 ± 0.05g/dl at week 9 and then rose again to 4.00 ± 0.01g/dl at week 10. When groups A, B and C were statistically compared with their corresponding controls, there were no significant differences ($p < 0.05$) but when infected animals in groups A, B and C were compared with one another there were statistically significant differences ($p < 0.05$) as shown in Table 5.8.

5.3.9 The Group Mean Serum Albumin Concentration (g/dl):

At post-infection phase (week 0-5 p.i),the group mean serum albumin in group A decreased from pre-infected value of 3.41 ± 0.01 to 2.93 ± 0.02 g/dl at week 1 p.i, and then undulated with range between 2.85 ± 0.03g/dl to 3.88 ± 0.03g/dl,while the uninfected corresponding controls undulated with a range between 3.28 ± 0.13g/dl to 3.45 ± 0.01g/dl.The group mean serum albumin concentration in group B declined from 3.80 ± 0.01g/dl to 3.16 ± 0.01g/dl, while the uninfected corresponding controls decreased from 3.80 ± 0.01g/dl to 3.45 ± 0.25g/dl at week 4 and then rose to 3.63 ± 0.03g/dl at the week 5. The group mean serum albumin concentration in group C declined from pre-infective value of 3.88 ± 0.03g/dl to 3.20 ± 0.01g/dl, while the uninfected corresponding controls increased from 3.75 ± 0.05 to 3.80 ± 0.04 g/dl.When groups A, B and C were statistically compared with their corresponding controls, there were very high significant differences ($p < 0.001$) but when infected animals in groups A, B and C were compared with one another there were statistically significant differences ($p < 0.05$). At post-treatment phase (week 6-10),the group mean serum albumin in group A declined from 3.15 ± 0.01g/dl to 2.93 ± 0.01g/dl at week 9 and then rose to 3.24 ± 0.07g/dl at week 10 while the uninfected corresponding controls undulated with a range from 3.40 ± 0.01 to 3.41 ± 0.05 g/dl. The group mean serum albumin in group B increased from 3.14 ± 0.04g/dl to 3.53 ± 0.04g/dl, while the uninfected corresponding controls increased from 3.65 ± 1.00g/dl to 4.20 ± 0.01g/dl.The group mean serum albumin in group C increased from 3.20 ± 0.01g/dl to 3.95 ± 0.05g/dl, while the uninfected corresponding controls increased from 3.75 ± 0.02 to 3.95 ± 0.08g/dl . When groups A, B and C were statistically compared with their corresponding controls,there were very high significant differences ($p < 0.001$) but when compared with one another there were statistically significant differences ($p < 0.05$) as indicated in Table 5.9.

5.3.10 Group Mean Serum Albumin-Globulin Ratio (A:G)

The mean serum A:G of infected goats in group A placed on low dietary energy ration declined from pre-infective value of 1.03 ± 0.06 to 0.77 ± 0.01 at week 1 p.i., then rose to 0.97 ± 0.01, then decreased to 0.68 ± 0.14 at week 3 p.i., then rose again to 0.80 ± 0.02 at week 5 p.i., while the uninfected control A increased from 1.03 ± 0.04 to 1.06 ± 0.03 at week 2 p.i., then declined to 0.90 ± 0.03 at week 4, and then rose again to 1.00 ± 0.01. The mean serum A.G of infected goats in group B placed on medium energy ration increased from 1.01 ± 0.01 to 1.07 ± 0.03 at week 1 p.i., and then gradually declined to 0.7 ± 0.01 while the uninfected control B declined from 1.03 ± 0.04 to 0.86 ± 0.03 at week 4 p.i., and then rose to 0.94 ± 0.01 at week 5 p.i. The mean serum A:G of infected goats in group C placed on high energy ration declined from pre-infective values of 1.01 ± 0.01 to 0.74 ± 0.03 at the week 5 p.i., while the uninfected control C increased from 0.94 ± 0.01 and then decreased to 0.94 ± 0.01 at week 4, and then rose again to 1.00 ± 0.01 at week 5.

Following treatment with diminazene aceturate, the mean serum A: G kept on undulating from 0.78 ± 0.01 to 0.83 ± 0.03, while the uninfected control A declined from 1.00 ± 1.00 to 0.72 ± 0.08. The mean serum A:G of infected animals in group B following treatment declined from 0.77 ± 0.01 to 0.74 ± 0.02 at week 3, then rose again to 0.86 ± 0.03 at week 10 and the uninfected control declined from 0.95 ± 0.06 to 0.81 ± 0.05 at week 9 and then rose again to 0.98 ± 0.05. The mean serum A:G of infected animals in group C, following treatment increased from 0.75 ± 0.03 to 1.04 ± 0.01 at week 9 and then declined to 0.98 ± 0.01 at week 10, and the uninfected control increased from 0.95 ± 0.01 to 1.05 ± 0.01 at week 9 and then declined to 0.99 ± 0.02 as shown in Table 5.10 .

5.3.11 Group mean serum alanine amino transferase (transaminase ALT, I.U/L);

At post-infection phase (week 0-5 p.i),the group mean ALT of goats infected with *T.congolense* and fed three different levels of dietary energy were shown in Table 5.11 below. The group mean ALT in group A increased from pre-infective value of 16.00± 0.20 to 23.10 ± 0.33, while the uninfected corresponding controls increased from 16.50± 0.02 to 17.75±0.25.The group mean ALT in group B increased from pre-infective value of 14.25±0.14 to 16.20±0.04, while the uninfected corresponding controls increased from 15.00± 0.01 to 16.00±0.04. The group mean ALT in group C increased from pre-infective value of 14.38±0.13 to 15.50±0.04, while the uninfected corresponding controls had no significant changes and remain undulating from 15.50±0.01 to 15.50±0.04. When infected animals in group A, B, and C were statistically compared with their corresponding controls there were very highly significant differences ($p < 0.001$) in group A while groups B and C had no significant difference ($p > 0.05$) but when infected animals in groups A, B and C were statistically compared with one another, there were significant differences ($p < 0.05$). At post-treatment phase (week 6-10), the group mean ALT concentration in group A decreased from 24.33± 0.09 to 17.25±0.28, while the uninfected corresponding controls decreased from 17.60±0.05 to 16.88±0.20. The group mean ALT concentration in group B remained relatively unchanged at 16.30±0.03 to 16.63±0.34, while the uninfected corresponding controls slightly increased from 16.00±0.01 to 16.73± 0.23. The group mean ALT concentration in group C and uninfected corresponding controls were relatively thesame. When groups A, B, and C were statistically compared with their corresponding controls there were no significant differences ($p > 0.05$) but when infected animals in groups were compared with one another,group A was significantly different from groups B and C ($p < 0.05$) as shown in Table 4.11.

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5.3.12 Group Mean Serum aspartate amino transferase (Transaminase AST I.U/L);

At post-infection phase (week 0-5 p.i) the group mean AST in group A increased from pre-infective value of 21.68±0.53 to 40.13 ±0.13, while the uninfected corresponding controls increased from 22.10±0.01 to 23.45±0.05. The group mean AST concentration in group B increased from pre-infective value of 19.83±0.16 to 31.85±0.06, while the uninfected corresponding controls increased from 20.45± 0.05 to 20.50±0.04. The group mean SGOT concentration in group C increased from pre-infective value of 20.40±0.10 to 22.00±0.00, while the uninfected corresponding controls relatively remained unchanged from pre-infective value of 20.45±0.02 to 20.50±0.01. When infected animals in groups A, B and C were statistically compared with their corresponding controls there were very highly significant difference ($p < 0.001$) but when infected animals were statistically compared with one another,there were significant differences ($p < 0.05$). At post treatment phase, the group mean AST concentration in group A decreased from 35.03±2.55 to 28.40±1.18, while the uninfected corresponding controls decreased from 25.03±2.55 to 18.40±1.18. The group mean AST concentration in group B declined from 22.05±2.46 to 28.78±0.04 while the uninfected corresponding controls slightly decreased from 21.50±0.29 to 20.85±0.08. The group mean AST concentration in group C increased from 21.88±0.31 to 28.75±0.58, while the uninfected corresponding controls slightly increased from 20.85±4.82 to 21.50± 0.00. When infected animals in groups A, B and C were statistically compared with their corresponding controls there were very highly significant differences ($p < 0.001$) but when infected animals were statistically compared with one another, group C was significantly different from groups A and B ($p < 0.05$) as shown in Table 4.12.

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5.4 DISCUSSION

Sodium is an extracellular cation. It controls the osmotic activity of the extracellular fluid (ECF), since plasma osmolality depends on its concentration. Sodium retention occurs due to the action of the mineralocorticoid hormone-aldosterone, secreted by the Zona glomerulosa of the adrenal cortex and therefore, the increase in serum Na^+might be due to failure of Zona glomerulosa to produce aldosterone hormone that regulates sodium in the extracellular fluid (Zilva and Pannall, 1984; Ogunsanmi, *et al.*, 1994).In this study, there was an increase in the mean serum sodium concentration following infection and reinfection in groups A and B which was an indication of hypernatriemia which almost always is associated with elevation of serum osmolality and haemodilution.In haemodilution,there is a fall in plasma protein fractions,haemoglobin levels and packed cell volume (Zilva and Pannall, 1984; Ogunsanmi, *et al.*, 1994).This might be the cause of anaemia in this study since haemodilution is one of the causative factors of anaemia in trypanosomosis (Anosa and Isoun,1976). Although hypernatremia occurs in dehydrated animals when water losses exceed losses of sodium and potassium and should be considered as an indication of a relative water deficit (Scribner, 1969). This situation can occur in the initial stages of diarrhea, vomiting, or renal diseases if losses of water exceeds the electrolytes loss (Saxton and Seldin, 1986). As water losses are replaced by increased water consumption or enhanced renal water retention, serum sodium concentration tends to decline into or below the normal range. Hypernatremia also develops as a result of an essentially pure water loss, such as the evaporative respiratory water loss in panting animals (Tasker, 1980). Food and water deprivation in normal individuals is associated with substantial reduction of renal and faecal output (Tasker, 1967b;Carlson *et al* ., 1979b; Brobst and Bayly, 1982 ;Genetzky *et al* ., 1987). However, continued cutaneous and respiratory insensible water loss may result in hypernatremia (Elkinton and Taffel, 1942;Rumsey and Bond, 1976; Carlson *et al* .,1979b;Genetzky *et al* .,1987). Abnormal thirst mechanisms with resultant hypernatremia have been reported in young dogs (Crawford *et al*., 1984 ; Hoskins and Rothschmidt, 1984). In human subjects, hypernatremia is reported with mineralocorticoid excess (McKeown, 1986) and this agrees with the findings of Fiennes *et al.*, (1946) and Ogunsanmi *et al.*, (1994) in *T. congolense* infected cattle.In group C there was no change in mean serum Na^+.This agrees with the report of Otesile *et al.*, (1991) in boars placed on different dietary energy levels and infected with *T. b. brucei*. At post- infection phase the serum Na^+.returned to normal

concentration and this corresponded with the aparasitaemic period when the parasites were no longer in the blood of goats after treatment with diminazene aceturate.

Progressive decrease in serum potassium levels (hypokalemia) observed in group A was more severe during this study than in groups B and C .This might suggest the depletion of the body's potassium stores or a redistribution of potassium from the ECF into the ICF space (Brobst, 1986) or it might be due to excessive renal loss of potassium which results from the action of mineralocorticoid excess or as a result of altered renal tubular function in infected animals with renal tubular acidosis or post obstructive states. It might also be due to chronic dietary potassium deficiency which eventually can lead to modest hypokalemia even in normal individuals (Aitken,1976;Dow *et al* ., 1987a). A rapidly developing and profound hypokalemia can occur in animals with reduced dietary intake as a result of anorexia when coupled with other causes of excessive potassium loss (Tasker,1980). It may develop without potassium depletion as the result of intracellular movement of potassium from the ECF space. This situation occurs with an acute alkalosis (Burnell *et al*.,1966) and in patients treated with insulin and glucose infusions (Tannen, 1986). All these agree with the observations of Raisinghani *et al.*, (1981) but differs from observations of Ikejiani, (1946a,b) who reported increase in serum potassium levels.

The mean serum bicarbonate levels in all infected goats in groups A, B and C were observed to have a sharp drop during post infection and reinfection phases and almost returned to normal during post-treatment phase. Generation of a metabolic alkalosis can be due to excessive hydrogen loss,bicarbonate retention, or as a contraction alkalosis. A contraction alkalosis occurs with reduction of ECF volume resulting from a loss or sequestration of sodium and chloride containing fluid without commensurate loss of bicarbonate (Garella *et al.*,1975). Excessive hydrogen ion losses can result in a metabolic alkalosis The most common causes of increased hydrogen loss are gastrointestinal losses of chloride-rich fluids associated with vomiting in small animals (Strombeck,1979) or sequestration of chloride-rich fluid in the abomasum and forestomach of ruminants (Gingerich and Murdick, 1975b ; McGuirk and Butler, 1980). Excessive renal hydrogen loss associated with mineralocorticoid excess, and low chloride intake may cause or contribute to the generation of a metabolic alkalosis (Rose,1984). Most of these disorders are also associated with the development of significant sodium and chloride deficits and resultant decreases in effective circulating volume. These deficits and the responses that decreased effective circulating volume induce are central

features of the processes that maintain and perpetuate a metabolic alkalosis. Hydrogen loss from the ECF can also occur with hydrogen movement into the cells in response to potassium depletion (Irvine and Dow, 1968). This observation agreed with the report of Goodwin and Guy, 1973 who reported a decreased in serum bicarbonate in *T. brucei* infected rabbits and disagree with Ogunsami *et al.,* (1994) who observed elevated bicarbonate and a sharp drop on day 56 in *T brucei* infection in sheep. The sharp drop in the serum bicarbonate levels might be due to acidosis associated with anaemia, renal malfunction and the release of toxic metabolites such as free acids by trypanosome, (Anosa,1988a,b).

The mean serum calcium mildly increased in group A (hypercalcemia) during post-infection phases This might be attributed to a deficiency of parathyroid hormone (PTH) or calcitonin, which stimulates bone resorption and accretion and this disagreed with the findings of Fiennes *et al.,* (1946) in cattle infected with *T. congolense* and Ogunsanmi *et al.,*(1994) in *T. brucei* infection in WAD sheep and Goodwin and Guy, (1973) in rabbits infected with *T. brucei.*The mean serum phosphate initially dropped after infection. The changes in values disagree with the findings of Fiennes *et al.,* (1946) in cattle infected with *T. congolense* and Goodwin and Guy, (1973) in rabbits infected with *T. brucei*. The hyperyhosphataemia may be attributed to a deficiency of parathyroid hormone and may also due to haemolysis.

In this study there was an increase in blood urea nitrogen (BUN) in all infected groups but there was no significant differences with their corresponding controls .The differences in dietary energy had no influence per se on BUN. BUN is a by-product of protein catabolism. Increased BUN levels are consistent with results from infection of monkeys infected with *T. b. rhodesiense* (Sadun *et al.,* 1973) and human infected with *T. b.gambiense* (Awobode, 2006). BUN is a product cleared from the body through the kidneys and as such their measurement during disease are good indicators of renal function (Ramakrishnan *et al.,* 1995).The causes of elevated BUN levels include kidney disease such as glomerulonephritis and excessive protein catabolism and febrile conditions. Fever and glomerulonephritis are common features of trypanosomosis and presumably act together to elevate BUN. Similar defects in renal function during trypanosomosis have been observed in man (Basson *et al.,* 1977). Indeed, gross and histological changes affecting kidneys have been demonstrated in trypanosome-infected dogs (Murray *et al.,* 1975) and humans (Anosa, 1988a, b), although these changes were not observed in the present study. Under normal circumstances excessive protein

breakdown is associated with an increase in plasma urea concentration (Ehoche *et al.*, 1990). The relative increased blood urea nitrogen levels in infected animals compared with their uninfected controls in the present study, tends to suggest that, trypanosome infection was associated with excessive protein breakdown. However, it was observed that animals on the high energy intake had higher BUN levels than those on low and medium energy intake. This is in disagreement with the report of Al-Rabbat *et al.*, (1971) and Blowey *et al.*, (1973) who suggested that high energy intake facilitates microbial protein synthesis in the rumen and thus reduces ammonia concentration and blood urea 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 the liver and excreted .The increase in BUN in this study also agrees with the findings of Coles, (1986).

The changes in total proteins and albumin concentrations in this study are consistent with observations in sleeping sickness patients who developed severe hypoalbuminaemia and/or hypoproteinaemia (Jenkin and Robertson, 1959a; Abenga and Anosa, 2005; Awobode,2006). The cause of the decrease in albumin is difficult to elucidate. Albumin is a negative acute phase protein during trypanosomosis (Karori *et al.*, 2008). Its decrease could result from reduced synthesis in the liver as part of the acute phase response, loss through the kidney and intestine or increased utilization by the trypanosomes as a nutrient, since they require it for optimal survival (Coopens *et al.*,1987). Thus since infection of goats resulted in high parasitaemia, increased utilization by parasites probably contributed to the decrease in albumin. It is unlikely that a defective hepatic failure in replacement of the albumin was a major cause. This is due to the long plasma halflife of albumin of 26 days, which could have resulted in a slower depletion of albumin in such a case. Certain trypanocidal drugs such as suramin, being polyanions, have a high binding capacity for lipoproteins and albumin (Muller and Wollert, 1976). The binding of suramin to albumin enables it to remain in plasma of patients for a number of weeks following a single intravenous admini-stration (Goodman and Gilman, 1970). Thus a decrease in albumin concentration in the blood of infected patients and monkeys could affect the pharmacokinetics of such drugs, affecting their availability to trypanosomes and leading to therapeutic failure. Infected animals in the present study developed hypoalbuminaemia, hypoproteinaemia and hyperglobinaemia, which were higher in the Low and medium energy ration groups than in the high energy ration group. This finding is in support of the report of Otesile *et al.*, (1991) in boars placed on different

dietary energy levels and infected with *T. b. brucei.* Measurement of the intravascular pool of albumin in sheep infected with *T. congolense* has revealed that hypoalbuminaemia may be largely due to haemodilution (Katunguka-Rwakishaya *et al.,* 1992a), although Wellde *et al.,* (1974) have suggested that, in cattle, it may be due to excessive protein catabolism while increased catabolism of albumin contribute to the fall in PCV and protein concentration were reported to occour in the early stage of trypanosomosis (Dargie,1980). Makinde *et al.,* (1991) observed that pigs given a low energy ration developed significant increases in plasma volume leading to an increase in blood volume, while the increase in plasma volume was not significant in animals given a high energy ration. An increase in plasma volume, as an important factor in the development of anaemia in sheep infected with *T. congolense* has been reported (Katunguka-Rwakishaya *et al.,* 1992a). Greater increases in plasma volume in animals receiving low energy intake compared to those on high energy intake (Makinde *et al.,* 1991) may contribute to development of anaemia, which is greater in animals on the low energy ration compared to those on high energy ration intake, and to a greater reduction in blood biochemical parameters than in animals receiving a high energy intake. It is therefore possible that trypanosomal uptake of albumin-bound fatty acids and lipoproteins for their metabolism and growth (Vickerman and Tetley, 1979), and haemodilution (Holmes, 1976) may account for the decrease in plasma albumin concentration. The hypoproteinaemia, hypoalbuminaemia and hyperglobinaemia observed in this study agreed with the findings of Zilva and Pannall, 1984;Ogunsanmi *et al.,* (1994).

The albumin:globulin values fluctuated in this study during post-infection although albumin level was lower than globulin in all the groups. Decreased albumin is a common form of dysproteinemia. Fundamentally, the decrease can be attributed to either albumin loss or failure of albumin synthesis. Depending on the stage of the disease, it can be associated with hypoproteinemia. Therefore, the total serum protein is not a reliable index of albumin status and albumin must be determined. Because of its small size and osmotic sensitivity to fluid movements, albumin is selectively lost in renal disease (Grauer, 2005), gastrointestinal disease, (Kaneko *et al.* , 1965 ; Meuten *et al.* , 1978), and in intestinal parasitism (Dobson, 1965). Furthermore, because of the sensitivity of albumin synthesis to protein and nitrogen loss such as that occurring in some forms of gastrointestinal disease, albumin loss impairs albumin synthesis and further compounds the hypoalbuminemia. Because of this same sensitivity of albumin synthesis to protein and nitrogen availability, decreased albumin concentration precedes the development of

generalized hypoproteinemia in dietary protein deficiencies. Classic human protein-calorie malnutrition, kwashiorkor, is characterized by hypoalbuminemia and hypoproteinemia. The liver is the only site of albumin synthesis, and hypoalbuminemia is an important feature of chronic liver disease and when accompanied by marked decrease in total protein is indicative of terminal liver cirrhosis (Sevelius and Andersson, 1995). Additionally, albumin is a negative APP and extensive inflammation accompanying any of the aforementioned conditions may compound the hypoalbuminemia.This finding agreed with the that of Ogunsanmi *et al.*, (1994). ALT and AST are normally localized within the cells of the liver, heart, kidney, muscles and other organs (Hanley *et al.*,1986). They are well known transaminases that play important roles in amino acids metabolism and providing necessary intermediates for gluconeogenesis. They have also been implicated as major enzymes in assessing and monitoring liver cytolysis. Their elevation in the serum gives information on organ dysfunction (Baron,1973; Kockmar *et al.*,1976;Hanley *et al.*,1986). The significant increase in the serum of both enzyme activities in infected animals in all the groups compared to uninfected corresponding controls gives an indication of cytotoxicity to these organs and hence the leakage of the enzymes into the serum from affected organs (Kockmar *et al.*,1976). The mean ALT and AST values were found to increase progressively during post infection phases in groups A, B and C although it was more pronounced in group A than other groups. This report agrees with the earlier finding in dog infected with *T. brucei* that penetrates tissues where enzyme is located and cause its release, Adah *et al.*, (1992) and Kagwa *et al.*, (1984) also reported an increase in AST activities following infection of goats with *T. congolense* and Otesile *et al.*,1991 in boars infected with *T.brucei* and on different planes of dietary energy. The increases in ALT and AST might be derived from haemolysed red blood cells or from the parasites themselves, although the period of long infection might allow for significant tissues damage but one wonder for this elaboration of these enzymes since *T.congolense* is restricted to the blood vessels only. These observations (Moon *et al.*, 1968;Kagwa, *et al.*,1984) have been made at day 5 (Whitelaw *et al.*,1980) and day 8 (Anosa, 1988a) respectively. The ALT of all infected goats in groups A, B and C and their corresponding controls did not returned to their preinfection levels during post treatment phase .This agrees to some extent with Adah *et al* .,(1992) although ALT returned to its normal values during post treatment phase which is at variance with Adah *et al.*,(1991) observation. The differences in serum transaminase levels recorded might be due to different levels dietary energy.

CHAPTER SIX

EXPERIMENT 3

6.0 STUDIES ON COMPLEMENT LEVELS IN *TRYPANOSOMA CONGOLENSE* INFECTED WAD GOATS FED THREE DIFFERENT LEVELS OF DIETARY ENERGY

6.1 INTRODUCTION

The complement system comprises a group of serum proteins and cell membrane receptors that function primarily to fight infection. Clinically, measurement of complement pathway activity and individual component levels is of value in cases of immunodeficiency and inflammatory conditions that involve complement activation (Jonathan and Whaley,2001).Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria and its activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes. However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells (Roitt, *et al*., 1989; Arinola, 2004). Complement can also have detrimental effects on the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis (Roitt, *et al*., 1989; Arinola, 2004) .Complement comprises over 20 different serum proteins that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells (Roitt, *et al*., 1989;Arinola,2004). Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria (Roitt, *et al*.,1989; Arinola,2004). Trypanosomal infections results in a profound hypocomplementamia in humans,(Greenwood and Whittle,1976),cattle (Kobayashi and Tizard,1976;Nielson *et al.*,1978;Tabel *et al.*,1986),and sheep (Malu and Tabel,1986).*Trypaanosoma congolense* and *T.brucei.brucei* organisms that are not coated with variant surface glycoprotein (VSG) are lysed by human serum via the alternative complement pathways (ACP)(Ferrante and Allison,1983). Blood stream forms of *T.b.gambiense* also activate the

ACP of human serum directly (Devine *et al.*,1986).The serum complement concentration C3,total and alternative haemolytic complement had been studied in goats (Kumshe,2005) in cattle (Talabi,2006)but there was relatively no information on the influence of different levels of dietary energy on these complement system, therefore,the influence of different levels of dietary energy on complement C3, total and alternative haemolytic complement was studied .

6.2 MATERIALS AND METHODS

6.2.1 The experimental site, experimental animals, infection techniques, animal grouping and feeding and feeds proximate analysis were as described for Experiment 1.

6.2.2 Determination of complement

Testing of haemolytic complement and its components was done as described by Kumshe (2005). The procedure involves preparation of erythrocytes, sensitization of erythrocytes complex formation, standardization of erythrocyte complex formation cells, testing of haemolytic antibody titre, haemolytic complement determination and interpretation of results.

6.2.3 Preparation of RBC Membranes (Stromata) for Immunization of Rabbits

Five (5ml) milllitres of blood was collected from jugular vein of each goat with equal volume of Alsever's solution. The blood samples were pooled together and disperse into plastic test-tubes (2ml into each tube). These were transferred into a cold centrifuge and spun at 1000xg for 5minutes at 4^0C after which buffy coat and supernatant solution were discarded. Five milliliter (5ml) of isotonic Nacl (*aq*) was added to each tube gently shaken to ensure even distribution of the cells. The tubes were transferred to the centrifuge and spun at 1000xg for 5 minutes at 4^0C after which supernatant solution was discarded. Counting of RBC was done according to Jain (1986) so as to determine the approx. no. of RBC, that was given in order to estimate the weight of RBC stromata. With little modification of method of Rapp and Borsos,(1963).Half milliliter (0.5ml) of washed erythrocyte containing $1.04x10^9$/ml equivalent 2mg of RBC stromata (Dodge *et al.,* 1963) was dispensed into plastic test tube and subsequently lysed with 20 volume of hypotonic PBS.The tubes were spun at 500xg for 30 min. at 4^0C. The supernatant solution was discarded while cell membrane stuck to the bottom of the tubes. Washing of RBC membrane was done several times until a clear supernatant solution was obtained. The supernatant solution was discarded and RBC membranes were dispensed into sterile Bijou

bottles containing 7ml of isotonic saline. Each bijou bottle containing approximately 2mg of RBC membrane was thoroughly stirred to ensure even distribution of the cell membranes. All the bottles were tightly capped and autoclaved for 30mins at 120^0C after which they were stored at -20^0C until use.

6.2.4 Intravenous injection of Rabbits with RBC membrane

The procedure of Meyer and Kabat (1961) and Lachman *et al*., (1973) were used with slight modification as described by Kumshe (2005) to raise haemolysin against caprine RBC.Each rabbit was placed in a restraining device. The ear was pulled out and the marginal ear vein located by pressing lightly on the base of the ear. With the use of 27 gauge needle 1ml (2mg suspension) of RBC membrane in isotonic saline solution was slowly injected into the vein. Intravenous injection of RBC stromata was repeated at 24hour intervals into two adult rabbits as scheduled below:

Days of injection	**Dose of RBC stromata**
1	2mg in 1ml isotonic saline
2	3mg in 1ml isotonic saline
3	3mg in 1ml isotonic saline
4	3mg in 1ml isotonic saline
5	3mg in 1ml isotonic saline
6	4.5mg in 2ml isotonic saline
7	4.5mg in 2ml isotonic saline
8	4.5mg in 2ml isotonic saline
9	4.5mg in 2ml isotonic saline
10	4 .5mg in 2ml isotonic saline
11	4.5mg in 2ml isotonic saline

6.2.5 Collection of rabbit anti – RBC serum (Haemolysin).

Seventy two (72) hours which after the last injection, the rabbits were bled though the marginal ear veins and later through jugular vein. The blood was collected into 10ml test tube. The blood was collected and the clot was separated from the sides of the test tubes using sterile Pasteur pipettes and kept at 4^0C overnight to allow it to contact.

The serum containing haemolysin was removed by centrifugation at 500xg for 10minutes caprine was pooled together and dispensed in 0.5ml aliquots into sterile bijou bottles and labeled. The samples were stored at $–20^0$C until use.

6.2.6 Production of anti-complement C3 serum

Zymosan C3 serum was produced as described by Hudson and Hay (1980).One sixty two milligram (162mg) of Zymosan was boiled for 30 minutes in 20ml of normal saline. The content was centrifuged (300xg) for 5 minutes and the supernatant solution was discarded after cooling, the precipitate was mixed with 12ml of fresh goats serum (FGS) and incubated at 37^0C for 30 minutes mixing at 10min interval to ensure suspension of zymosan in serum. The suspension was centrifuged at 900 x g for 5 minutes and the supernatant was discarded, The washing of zymosan – C3 complex was done 6 times in barbitone buffered saline (BBS) at PH 8.6. The zymosan- C3 precipitate was suspended in 12ml of BBS and dispensed into each of 4 centrifuge tubes, centrifuged and the supernatant discarded. The centrifuged tubes with the contents was properly capped and stored at –20^0C until required for immunization.

6.2.7 Immunization of rabbits with zymosan – C3 precipitate

Zymosan – C3 precipitate was re-suspended in 0.4ml BBS, transferred into a bijou bottle and 2.4ml of freud's incomplete adjuvant (FIA) was injected subcutaneously into each of the four sites of an adult chinchilla rabbit viz right and left shoulder regions and the right and left rump regions. This is equivalent to a total of 0.8ml zymosan – C3/FIA emulsion per rabbit each of the two rabbits was immunized 3 times at 10 days interval viz 0, 10 and 20.

6.2.8 Collection of rabbit Anti – C3 serum

Seven days after the last immunization with zymosan – C3 / FIA emulsion, rabbit was bled through the marginal ear and later through the jugular veins. Blood was collected into the test-tubes and allowed to clot for 4 hours at room temperature. All the samples were spun at 100xg at 40^0C for 10mins. Anti – C3 serum was collected and dispensed in 0.5ml aliquots and stored at – 20^0C until use.

6.2.9 Study of Haemolytic complement activity using Tris-saline buffer.

Half milliliter (0.5ml) haemolysin dilution of 1:50 and 1:100 was prepared with TSB .Half milliliter (0.5ml) of 5 x10^8 goat RBC/ml was added into each haemolysin dilution. They were incubated at 37^0C for one hour with constant shaking at 10mins interval to ensure proper mixing of anti serum with erythrocytes (for adequate erythrocyte sensitization). The sensitized goat erythrocytes were re-suspended in one of the same buffer. One milliliter (1ml) of 40% fresh goat serum (FGS) constituted with TBS as appropriate was added into the tubes containing RBC with the haemolysin (1:50 and

1:100). Positive control tubes was set up by dispending 1ml of 0.1m Na_2Co_3 solution into each tube containing sensitized RBC.

Negative control tubes were set up by the addition of 1ml 40% decomplemented goat serum with each of the tubes containing sensitized RBC. All the tubes were run induplicate. All the tubes with their contents were incubated.

6.2.10 Single Radial Immunodiffusion (SRID) Assay for Complement C3

Mancini technique, otherwise known as Single radial immunodiffusion assay was used.This method is based on the interaction of antigen and antibody to form complexes leading to formation of precipitates.When the reaction is carried out in a gel such as agarose,the precipitate remains localized (Dias da Silva, 1986;Phimister and Whaley,1990).Serum containing the component to be assayed (Antigen) is placed in wells cut in agarose containing the antibodies.As the antigen diffuses radially a ring of precipitate is formed around the well and becomes stationary at equivalent point.At this point the diameter of the ring is proportional to the antigen concentration in the well ,since the antibody concentration is constant (Phimister and Whaley 1990).Using standards of known complement concentration and plotting the square of the diameter against concentration , a calibration curve can be drawn to determine the concentration of the complement in test serum of the complement.

$$\% \text{ complement} = \frac{\text{Mean OD of test sample- Mean OD of negative}}{\text{Mean OD of positive control-Mean OD of negative}} \times 100$$

(Phimistre and Whaley,1990).

6.2.11 Determination of Serum C3 complement:

Two percent (2%)agarose was prepared by boiling 2gm of agarose in 100ml of sterilized distilled water for 30mins in a Pipex reagent bottle with 0.02% sodum azide added as a preservative.Thirteen millilitre (13ml) of 1% anti-serum was made by dispensing 6.5ml of molten agar rose solution into a universal bottle, adding 5.2ml of barbital buffer and 1.3ml of anti-C3serum. All the reactions were carried out at 56^0C. After proper mixing, the contents were gently and carefully poured on a clean sterilized glass plate (measuring 10.5cm by 8.5cm) placed in a level horizontal table. Leveling was achieved with the use of a spirit level. Afer the solidification of the agar rose anti-C3 gel, 2mm diameter wells were punched using a circular gel puncher and the gel in the wells removed carefully using a Pasteur pipette attached to a vacuum pump (Brown and Hobert).Thirty six(36) well-defined wells were punched.All the goat serum samples were

each tested in duplicate after prompt identification based on the groups and planes of nutrition , various dilution (1:2; 1:4; and 1:8) of the standards pooled goat sera (pooled fresh goat serum from apparently healthy goats) were prepared in barbitone buffer pH 8.6. The goat test sera and standards were applied into an appropriate wells in 5ml volumes using the microdispenser . All the plates were placed in a humified chamber and incubated at room temperature for 48hrs. The diameter of the precipitate was measured to the nearest 0.01mm using Hyland eye precision viewer (Traverol Lab., USA). The concentration of the standards were plotted against their corresponding square diameter on a graph paper. The concentration of the test sample were read off the standard curve C3.

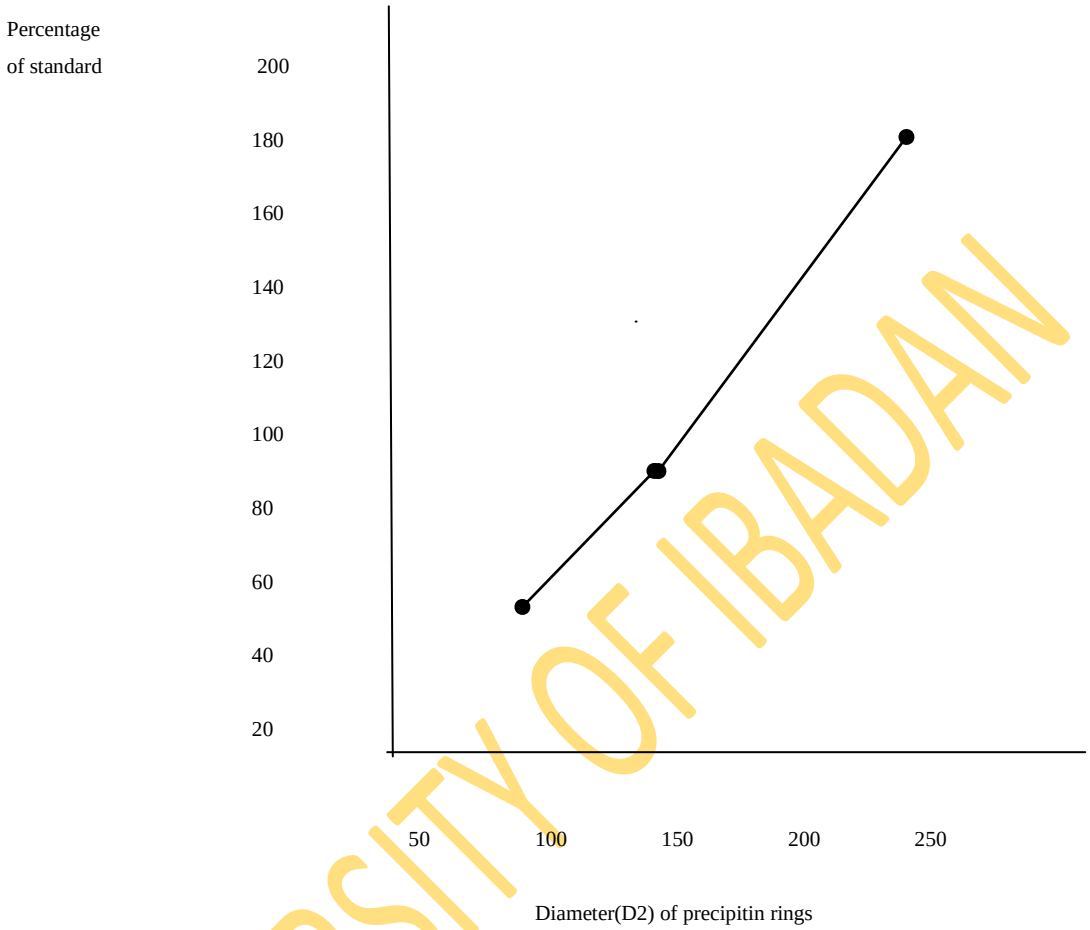

Fig. 6.1: Standard Curve used for the calculation of the Relative C3 concentration

6.2.11.1 Total Haemolytic Complement Assay

Total Haemolytic Complement assay is based on the ability of the classical complement pathway to induce haemolysis of sheep erythrocytes. Haemoglobin released by the lysis of erythrocytes can be measured spectrophometrically and related to the percentage of cells lysed (Barta and Hubbert, 1978). The relationship between the amount of complement present and the proportion of cells lysed follows a sigmoid curve. The curve is steep in the central area where the degree of lysis is sensitive to small change in the amount of complement present (Meyer and Kabert, 1961). The serum dilution which causes 50%lysis of indicator erythrocytes is determined and defined as 50%haemolytic unit of the complement or CH_{50} (Meyer and Kabert, 1961; Talwar, 1983)

6.2.11.2 Determination of total haemolytic Complement

Using evacuated tubes, blood samples were obtained from 40 apparently healthy WAD goats. After clotting, serum was decanted, spun at 800 ×g for 30mins and clear serum was stored at -20^{0}C until used. A micromethod of the standard haemolytic of the assay was used for the determination of the serum complement activity (Ogundele, 1998). Each sample was assayed in triplicate 50µl of 1:50 dilution of haemolysin was pippeted into 90 wells of U-shapebottom microplates and 50µl of 5 X 10^{8}cells/ml of washed sheep red cells was added into each well. Four wells used for each of positive and negative controls while 4 wells were left blank. The plates were covered with plate covers and incubated at 37^{0}C for 1hr with shaking at 10mins interval , after which the sensitized RBC was adjusted to 50µl with Tris saline buffer and 50µl of 40% FGS added into each test well and severally shaken to ensure even suspension of erythrocytes with FGS (giving a final reation concentration of 20% FGS). Positive control wells were made by dispensing 50µl 0f 5 X 10^{8}cells/ml of washed sheep red cells plus 50µl 0f 0.1M Na2C03, while negative control wells were set up by dispensing 100µl of 20% decomplemented FGS . All the controls tubes were run in quadruplicate. The plates were covered and incubated for 1hr at 37^{0}C with shaking at 10mins interval. The plates were sealed with a sealing tape and spined at 200 xg for 10mins. The supernatant was pippeted and transported into flat bottom microplates. The optical density reading of the released Hb was read at 541nm using multiwell plate reader. The degree of the lysis was calculated .

6.2.11.3 Altenative Haemolytic Complement (AHC) Assay

The addition of rabbit erythrocytes to human serum activates the alternative haemolytic pathway leading to lysis of target cells (Talwar, 1983; Dias da Silva, 1986). The AHC activity is defined as the serum dilution which causes 50% lysis of erythrocytes and is defined as the AP-CH_{50} unit (Talwar, 1983).

6.2.11.4 Determination of Alternative haemolytic Complement

The method of assaying AHC activity is the same as described for THC, the only exception being that the rabbit erythrocytes was used in places of sheep erythrocytes:

1) The rabbit erythrocytes need no sensitization with haemolysin
2) Ethylene glycol bis-amino tetra acetic acid EGTA replace Calcium chloride in Tris saline buffer .The addition of rabbit erythrocytes to human serum activates the alternative complement pathway leading to haemolysis (Dias da Silva, 1986). The AP-CH50 unit is depressed in the same way as the CH_{50} unit. The assay is performed in beaker containing MG-EGTA. EGTA chelates calcium and prevents C1-dependent classical pathway activation, while the added magnesium aided the formation of C3bBb complex (Phimister and Whaley, 1990).

6.2.12 Statistical Analysis : Experimental datae on C3,THC and AHC were analysed by ANOVA while parasite counts were correlated with these complement levels at $p < 0.05$.

6.3 RESULTS

6.3.1 The group mean Serum C3 complement:

The results of the influence of three different levels of dietary energy on the complement C3 of the goats infected with *T. congolense* are as shown below in Table 6.1.At post-infection phase (week 0-5 p.i),in group A, the group mean serum C3 component concentration values decreased from pre-infective values of 129.75±0.01 to 62.63±10.64 while that of corresponding controls decreased from pre-infective value of 129.50±0.87 to 117.25±19.08. In group B, the group mean serum C3 component concentration values decreased from pre-infective values of 138.25±0.41 to 72.75±1.91, while that of corresponding controls decreased from pre-infective values of 140.0±1.44 to 121.76±08.92. In group C, the group mean serum C3 component concentration values decreased from pre-infective value of 138.75±0.49 to 76.88±0.49,while that of corresponding controls decreased from pre-infective values of 141.50± to126.50±18.51.When group A, B, and C were compared with their corresponding controls there were very highly significant differences ($p < 0.001$) but when the infected groups were compared with one another, there were significant differences ($p < 0.05$). At post treatment phase (week 6-10),in group A, the group mean serum C3 component concentration values increased from 37.00±0.27 to 90.13±1.46, in group B, the mean serum C3 component concentration values increased from 44.00±0.96 to 105.50±0.42, while the mean serum C3 values of group C rose from 47.25±0.31 to 109.50±0.63 .When group A, B and C were compared with their corresponding controls ,there were very highly statistical significant differences ($p < 0.001$),but when compared with one another there were statistically significant differences ($p <0.05$) as shown in Table 6.1 and Figure 6.2.

6.3.2 The group mean serum total haemolytic complement:

The influence of three different levels of dietary energy levels on total haemolytic complement (THC) is shown in Table 6. At post infection phase (week 0 -5 p,i) in group A, the group mean total haemolytic complement declined from pre-infective values of 57.50±0.42 to 17.50±0.42, while that of corresponding controls declined from pre-infective value of 60.25±0.45 to 43.50±0.58 while group B decreased from pre-infective values of 60.00±0.60 to 25.00±0.60, while that of corresponding controls decreased from 63.00±0.51 to 52.50±0.29) and group C, decreased from 58.75±0.31 to 27.00±0.60, while that of corresponding controls decreased from 62.50±1.44 to 50.00±1.15.When infected animals in groups A, B and C were compared with their corresponding controls there were very highly significant differences ($p < 0.001$) but when the infected animals were compared with one another,there were significant differences ($p < 0.05$). At post-treatment phase, (week 6-10), in group A, the group mean total haemolytic complement (THC) increased from17.25±0.27 to 40.81±1.20, while group B rose from 25.50±0.42 to 45.00±0.2 and group C rose from 26.75±0.48 to 51.50±0.42. When infected animals in groups A, B and C were compared with their corresponding controls there were very highly significant differences ($p < 0.001$) but when compared with one another,there were significant differences ($p<0.05$) as shown inTable 6.2 and Figure 6.3)

6.3.3: The group mean serum alternative haemolytic complement:

The influence of three different levels of dietary energy on Alternative haemolytic complement (AHC) in goats experimentally infected with *T.congolense* was shown in the (Table 6.3 and Figure 6.4). At the post-infection phase (week 0-5 p.i), in group A, the group mean alternative haemolytic complement decreased from pre-infective values of 19.88±0.28 to 8.75±0.31, while that of corresponding controls slightly increased from 21.00±0.58 to 22.50±0.87. The group B decreased from pre-infective values of 20.25±0.56 to 11.00±0.27, while that of corresponding controls decreased from 21.00±0.58 to 17.50±0.87 and group C declined from pre-infective values of 21.50±0.42 to 13.00±0.27, while that of corresponding controls increased from 20.50±0.29 to 22.00±0.58. When infected animals in groups A, B and C were compared with their corresponding controls there were very highly significant differences ($p < 0.001$) but when compared with one another,there were significant differences ($p < 0.05$). At post-treatment phase (week 6-10),in group A, the group mean alternative haemolytic complement (AHC) increased from 6.50±0.42 to 16.75±0.42 while group B rose from 9.00±0.42 to 19.00±0.31 and group C rose from 10.50±0.42 to 22.75±0.31. When groups A, B and C were compared with their corresponding controls there were significant differences ($p < 0.001$) at the end of week 10 in group A and C while group B had significant difference up to week 9, but when compared with one another,there were significant differences ($p < 0.05$) as shown in Table 6.3 and Figure 6.4.

6.4 DISCUSSION

Authié and Pobel, (1990) and Talabi, (2006) investigated the kinetics of serum complement and C_3 during natural infection with *T. vivax* and *T. congolense* respectively in cattle of different susceptibility, in order to determine whether there was a relationship to trypanotolerance. They observed a significant correlation between minimum complement activity, C_3 and minimum PCV in early infection. These three parameters correlated with individual resistance and might therefore, be useful criteria for the identification of the most resistant individuals within a trypanotolerant breed.

The present results showed that the group mean serum C3 in all infected and reinfected goats in group A, B, and C decreased as the infection progressed .This may be due to consumption of this component and it is an important candidates for improve generalized defense mechanism ,control of parasitaemia and immunosuppression as described in trypanosome infected animals (Wimmers *et al.*,1999) and the decline in mean serum C3, may be due to failure of mononuclear phagocytic system and liver to synthesise this complement component and since liver is one of the vital organs that is clinically affected during trypanosomosis (Itard,1989). The fall in C3 agrees and in consistent with the finding of *T.congolense* infected cattle (Rurangiwa *et al.*,1980,Authie and Pobel,1990,Talabi,2006) sheep (Malu and Tabel,1986) *T.congolense* infected camel (Ouma *et al.*,1998) *T.congolense* infected mice (Otesile *et al.*, 1991b) and Kumshe, (2005) in both WAD and Born white (BW) goats infected with *T. congolense* .The depletion in serum C3 concentration is more severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy.This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. This suggested that different degrees of hypocomplementaemia were observed in all infected goats and may due to different levels of dietary energy and the mechanism by which this influences serum C3 component was not known. The amelioration of hypocomplementamia observed in this study may be due to the influence of improved nutrition vis-à-vis increased dietary energy in groups B and C of infected animals. There was a significant decrease in total haemolytic complement (THC) in all infected and re infected groups. The fall in THC agrees and corroborates the observation of *T.evansi* infected goats (Olaho-Mukani *et al.*, 1995) *T.brucei* infected Saneen goats (Baderha *et al.*,1990) *T.vivax* infected cattle (Rurangiwa *et al.*,1980) in both WAD and Born white (BW) goats infected with *T. congolense* (Kumshe ,2005) and Talabi,(2006) in cattle infected with *T. congolense*. The depletion in serum THC concentration is more

severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy.This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. A significant decrease was observed in alternative haemolytic complement (AHC) in all the infected groups . The fall in AHC has been observed and reported by (Malu and Tabel, 1986) of *T.congolense* infected sheep (Doko *et al.*, 1991) *T.bruceibrucei* infected WAD short horn cattle (Baderha *et al.*,1990) *T.vivax* infected cattle (Rurangiwa *et al.*,1980) in both WAD and Born white (BW) goats infected with *T. congolense* (Kumshe ,2005) and Talabi,(2006) in cattle infected with *T. congolense*. The depletion in serum AHC concentration is more severe and significantly lower in WAD goats on low levels of dietary energy than medium and high levels of dietary energy.This further confirms the influence of dietary energy in the susceptibility of WAD goats to *T.congolense* infection. The decline in mean C3,THC and AHT agrees with finding of Kumshe,(2005) in both WAD and Born white (BW) goats infected with *T. congolense* and Talabi,(2006) in cattle infected with *T. congolense*.The phenomenon of hypocomplmentamia (decline in mean serum C3,THC and AHT) observed in this study may be attributed to massive consumption of these complements as a result of persistent parasitaemia leading to formation of antigen-antibody complex.The two mechanisms by which trypanosomes can influence the immune system are complex,however,hypocomplementamia as caused directly or indirectly by the parasites is of considerable significance in the disease syndrome .The hypocomplementamia observed in this sytudy may also be due to failure of mononuclear phagocytic system and liver to synthesise these complement components and since liver is one of the vital organs that is clinically affected during trypanosomosis (Itard,1989).The exact mechanism by which dietary energy influence complement system is not known but Faye *et al.*, (2005) reported that the high energy demands of trypanosome infection may lead to severe energy shortage and this might be reflected in the changes from energy and to protein metabolism,since the complement system are made up serum protein ,catabolism of serum protein due to shortage of dietary energy might be responsible for the differences observed in this study and hence the different levels of dietary energy influence the activities of these complement system of *T.congolense* infected goats interm of depletion by trypanosomes and the rate of synthesis by mononuclear phagocytic system and liver.

CHAPTER SEVEN

7.0 THE INFLUENCE OF DIFFERENT LEVELS OF DIETARY ENERGY ON THE LYMPHOCYTE PROLIFERATION IN *Trypanosoma congolese* INFECTED GOATS

7.1 INTRODUCTION:

The immune system is crucial for the defense against organisms that cause infections and against toxic products that may be released from the infectious agents. A functional immune response requires rapid and extensive cell growth, proliferation, and production of effector proteins. A defect in any single component of the immune system can cause a breakdown in this defense and may lead to serious or fatal diseases such as infections, cancers, or autoimmune disorders. In addition, a growing body of evidence suggests that excess inflammation decreases longevity (Morgan *et al.*,2007;Vasto *et al.*,2007) therefore, fine regulation of this system is required to maintain health. Lymphocytes are part of the adaptive immune response and as such, are crucial for normal immune functions. T or B cell deficiencies are known to result in severe immunodeficiencies (Buckley,2004).

T-cells use glucose and glutamine as their primary fuel source (Bental and Deutsch,1993), although ketone bodies and fatty acids can also be used to a small degree. Of these nutrients, glucose appears to be particularly necessary for cell survival, size, activation, and cytokine production (Fox *et al.*,2005). Glucose provides much-needed energy for the lymphocyte in the following ways: Glucose can serve as a primary substrate for the generation of ATP; glucose can supply a carbon source for the synthesis of other macronutrients, such as nucleic acids and phospholipids; and glucose can be metabolized by the pentose phosphate pathway to generate NADPH. For ATP generation, glucose can be metabolized via glycolysis or oxidative phosphorylation (Fox *et al.*,2005). Glycolysis occurs in the cytosol, where one molecule of glucose is broken down into two molecules of pyruvate. The net reaction is oxygen-independent and yields two molecules of ATP for every one molecule of glucose. Pyruvate is then converted to lactate and generates electron acceptor NAD from NADH. Alternatively, oxidative phosphorylation is oxygen-dependent and occurs within the mitochondria. It is comprised of two reactions: conversion of intermediate molecules (pyruvate and fatty acids) to acetyl coenzyme A (coA) and degradation of acetyl coA to CO_2 in the tricarboxylic acid cycle, yielding free

electrons carried by NADH and flavin adenine dinucleotide ($FADH_2$), as well as transfer of electrons from NADH and $FADH_2$ to the electron transport chain, resulting in protons moving out of the mitochondrial matrix. The electrochemical potential is then used by ATP synthase to make ATP, with a total yield of 30 molecules of ATP from each molecule of glucose. Thus, oxidative phosphorylation is a seemingly more efficient way to generate ATP from glucose, although few metabolites remain for biosynthesis.

Resting lymphocytes have low-energy needs and derive most of their ATP from oxidative phosphorylation (Krauss *et al.*,2001) however, activated lymphocytes require a dramatic increase in metabolism upon activation. This is necessary to produce the energy required to stimulate growth and proliferation and produce the protein products expressed by activated immune cells (Bental and Deutsch,1993;Fox *et al.*,2005). Glucose metabolism changes by orders of magnitude in an activated T cell, and the transition from a resting to an activated T cell causes a switch from catabolic to anabolic metabolism, in which ATP is used to produce complex macromolecules from simpler intermediates (Jones and Thompson,2004).

Activated lymphocytes generate energy in large part by up-regulating aerobic glycolysis (Bental and Deutsch,1993;Fox *et al.*,2005;Frauwirth and Thompson,2004), which describes the metabolic program used when a cell continues to convert pyruvate to lactate, despite conditions of adequate oxygen; it is used by many types of transformed or cancer cells (Shaw ,2006). This shared metabolic program between activated lymphocytes and cancer cells further deepens the relevance of studying lymphocyte metabolism. It is not completely clear why an activated T cell chooses to use aerobic glycolysis for energy generation, although it may be that the rapid speed of glycolysis and the availability of biosynthetic precursors may favor glycolysis, despite the seemingly higher ATP production of oxidative metabolism. Regardless, a failure to increase glucose metabolism during lymphocyte activation prevents cell growth (Bental and Deutsch, 1993;Fox *et al.*,2005;Frauwirth and Thompson,2004).

T-cells proliferation is a standard method to evaluate cellular immune responses against pathogen,(Moreno-Lafont *et al* .,2003). Severe nutritional deficiencies reduces T-cell functions impairing cell mediated responses but sparing B- cell function and humoral immunity (Sheffy and William, 1982; Joshua *et al*., 1993) . Starving result rapidlly in thymic atrophy, a drop in T-cell numbers produced and found in the secondary lymphoid

tissues. T and B-lymphocytes are activated by different mitogens.Phytohaemaglutinin (PHA) and Concanavalin (Con.A) stimulate T lymphocyte.Lipopolysaccharide (LPS) mitogen stimulates B- cells. Pokeweed mitogen (PWM) stimulates T and B cells, (Roitt, 1989). Nutrition has been reported to affect cell mediated immunity but relatively there is dearth of information on lymphocyte proliferation during Trypanosomosis in WAD goats.

There is no information on the influence of different levels of dietary energy on the lymphocyte proliferation in *T.congolense* infected WAD goats; therefore, this study investigated the influence of different levels of dietary energy on lymphocyte proliferation in *T.congolense* infected WAD goats .

7.2 MATERIALS AND METHODS

7.2.1 The experimental site, experimental animals, infection techniques, animal grouping and feeding and feeds proximate analysis were as described for Experiment 1.

7.2.2 Collection from Infected Experimental Animals: 5mls of blood were collected through the Jugular venipuncture of 14 infected WAD goats from Groups A, B and C into BD vacutainer CPT TM tube (BD Bioscience), and properly shaken to prevent coagulation of the blood sample.

7.2.3 Separation and Isolation of trypanosomes from blood: Trypanosomes were separated and isolated from blood by the modification of method described by (Lanham 1968, Lanham and Gofrey, 1971). The blood from infected albino rats was centrifuged at 650 x g for 10 min. at 4^0C on a swinging bucket rotor to separate a large part of the components from trypanosomes before purification.. The plasma was removed by aspiration of the upper supernatant fluid. The underlying whitish trypanosomal layer was removed with a Pasteur pipette and then resuspended with an equal volume of Phosphoric sodium chloride glucose (PSG) buffer (58mM Na2HPO4,3mM NaH2PO4,43.6mMNacl,10mM glucose PH 8.0).Contaminants were then eliminated by further purification by ion-exchange chromatography on (DEAE)-cellulose which had been previously equilibrated with PSG buffer (Lanham 1968, Lanham and Gofrey,1971).The purified trypanosomes were eluted with the above buffer and collected when the eluate was milky –coloured.Contamination and counting of trypanosomes were routinely checked uhder a microscope using an improved Neubauer

haemocytometer with a silverd stage.The purified trypanosomes were then pelleted at 600g in a Sorval Rc-5B centrifuge at 4^0C using the SS-34 rotor.

7.2.4 Peripheral blood mononuclear cells (PBMCs) isolation:

Blood samples collected before infection and after infection were taken to the laboratory for analysis. The protocol used for the isolation of the Caprine PBMC was the general protocol used for the isolation of human PBMC (Ulmer *et al.*,1984) but with some modifications to suit caprine PBMCs. Briefly, for the isolation of PBMC, each blood samples was diluted with RPMI 1640 in the ratio of 1:1 and then layered gently on 3ml of Ficoll paqueTM with the aid of 10ml sterile pipette, and centrifuged at 1800 × g (2800rpm) on a Sorvall RT 6000 centrifuge) for 20minutes at room temperature.After centrifugation the CPT ™tubes were brought to a biological safety cabinet and their tops were carefully opened. The peripheral Blood Mononuclear Cells (PBMCs) were aspirated, transferred to a new 50ml sterile polypropylene conical tube (BD science) and washed 3 times with RPMI 1640. The optimal revolution per minute for washing was determined by varying the speed used between 500rpm and 2000rpm and the time spent for each wash. It was observed that the optimal revolution per minute was 1000rpm for 5minutes and this was used for the wash. The cells were then resuspended in 10ml of complete medium (RPMI 1640 medium supplemented with L-glutamine, Hepes, Penicilin, Streptomycin and Heat-inactivated fetal bovine serum). 10µl of the cell suspension was added to 10µl of trypan blue (0.4% solution Sigma) in order to determine cell viability and the cells were counted using haemocytometer. Cells were retained for proliferation assay.

7.2.5 Lymphocyte proliferation assay: Cells were diluted to a concentration of $3x10^5$cells/ml and proliferation assays were carried out in triplicate at 37^0C in a humidified atmosphere of 5% $C0_2$ in air for 24hours. Each well (total volume of 100µl) contained complete medium with PBMC added at a final concentration of 3 $x10^5$cells/ml.

7.2.6 Antigen stimulation of Lymphocytes: The *Trypanosoma congolense* extract (antigen) was allowed to thaw at 4^0C and serially diluted 1:100-1:6400 in complete medium and these various dilutions were used to stimulate the PBMC. For the positive control, Phytohaemaglutinin was used at a concentration of 3µl/ml to stimulate the cells and the negative controls were unstimulated cells (i.e. had no antigen added at all). This was incubated at 37^0C in a humid atmosphere of 5% $C0_2$ in air for 24hours. 20µl of Alamar Blue dye was added into each well to indirectly measure the proliferation of the cells after the 24hour incubation period and then incubated for another 24hours.

Absorbance was measured at 570nm and 600nm with an ELISA plate reader. Alamar Blue assay was developed as a non-radioactive lymphocyte proliferation assay that indirectly measures cell proliferation (Ahmed *et al.*, 1994). The dye is added in an oxidized form (blue colour) and is reduced (red colour) with cell proliferation.

7.2.7 Statistical Analysis: Data collected on live and dead lymphocytes ratio, Optical density of stimulated and non stimulated lymphocytes were analysed and microscopic lymphocyte counts up to 72 hours after stimulation using SPSS V. 10.and statistical method used was One way analysis of variance (ANOVA) and the means were separated using Duncan Multiple Test and significant difference. Results are presented as Means± standard error (SE).

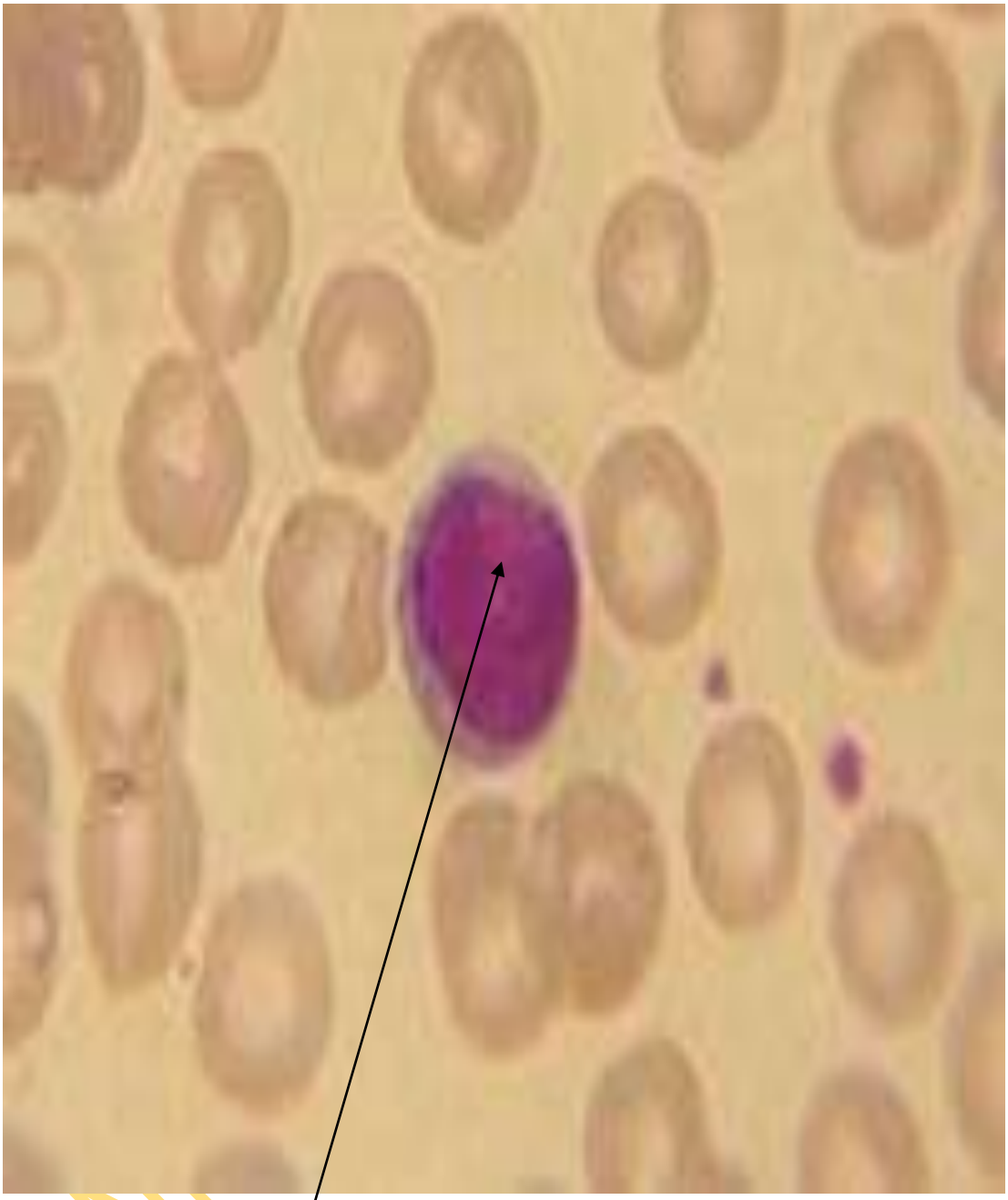

Plate 111:showing lymphocytes

7.3 RESULTS:

The ratio of live and dead lymphocytes was shown in Table 7.1.The live and dead lymphocytes decreased significantly ($p < 0.05$) as from one week post infection in all infected groups but the decline was more pronounced in goats on low level of dietary energy. The optical density (OD) of non stimulated lymphocytes before and after incubation did not shown any significant changes in their proliferation ($p > 0.05$), although there was a significant decline in lymphocytes as infection progressed from week 1 to week 5 ($p < 0.05$) between infected A, B and C (Table 7. 2).The significant difference was more pronounced in goats on low dietary energy level.

The OD of stimulated lymphocytes before and after incubation in all infected goats showed a significant changes in their proliferation ($p < 0.05$) and the rate of proliferation was more pronounced in goats on high level of dietary energy (Table 7. 3).

Week	LIVE-LYMPHOCYTES			DEAD LYMPHOCYTES		
	INFECTED-A	INFECTED-B	INFECTED-C	INFECTED-A	INFECTED-B	INFECTED –C
1	82.67 ±0.67 [a]	86.50 ±.56 [b]	89.33 ±0.42 [c]	17.33 ±0.67[a]	13.50 ±0.56 [b]	10.67 ±0.42 [c]
2	76.50 ±0.56 [a]	81.83 ±0.65 [b]	84.00 ±0.37 [c]	23.5 ±0.56 [a]	18.17 ±0.65 [b]	16.00 ±0.37 [c]
3	73.67 ±0.43 [a]	78.83 ±0.40 [b]	81.17 ±0.40 [c]	26.33 ±0.4 [a]	21.17 ±0.33 [b]	18.83 ±0.40 [c]
4	73.67 ±0.33 [a]	78.67 ±0.33 [b]	81.17 ±0.40 [c]	26.33 ±0.4 [a]	21.33 ±0.50 [b]	18.83 ±0.21 [c]
5	72.67 ±0.50 [a]	76.50 ±0.50 [b]	79.69 ±0.21 [c]	27.33 ±0.3 [a]	23.50 ±0.50 [b]	20.31 ±0.75 [c]

a, b,c means in the same row followed by different superscript differ significantly ($p < 0.05$)in infected A,B and C.

Table 7. 1: The effect of *T.congolense* infection on Live and dead lymphocytes (Mean ±S.E %) of WAD goats fed at different levels of dietary energy.

OD of non-stimulated lymphocyte before incubation			OD of non-stimulated lymphocyte after incubation		
Week	Infected A	Infected B	Infected C	Infected A	Infected B	Infected C
1	0.40±0.01	0.40±0.02	0.42±0.01	0.40±0.01	0.40±0.01	0.42±0.02
2	0.38±0.01	0.40±0.01	0.41±0.02	0.38±0.01	0.40±0.02	0.42±0.02
3	0.35±0.02	0.38±0.03	0.39±0.03	0.34±0.02	0.39±0.03	0.40±0.02
4	0.34±0.01	0.35±0.02	0.39±0.02	0.35±0.08	0.36±0.02	0.40±0.03
5	0.33±0.04	0.32±0.02	0.38±0.05	0.34±0.02	0.33±0.02	0.39±0.04

Table 7.2: The effect of *T.congolense* infection on Mean ±S.E OD of non-stimulated lymphocytes before and after incubation of WAD goats fed at different levels of dietary energy. There were no significant differences ($p > 0.05$) in infected A, B and C although the lymphocytes declined during the course of infection from week 1 to week 5.

	OD of PHA stimulated lymphocytes before incubation.			OD of PHA stimulated lymphocyte after Incubation		
Week	Infected A	Infected B	Infected C	Infected A	Infected B	Infected C
1	0.42±0.01	0.43±0.01	0.44±0.02	0.75±0.01	0.78±0.02	0.79±0.01
2	0.39±0.01	0.41±0.02	0.42±0.02	0.67±0.02	0.75±0.03	0.77±0.01
3	0.36±0.02	0.39±0.01	0.39±0.02	0.53±0.03	0.72±0.04	0.74±0.03
4	0.36±0.02	0.37±0.01	0.39±0.04	0.50±0.03	0.71±0.02	0.74±0.03
5	0.34±0.02	0.35±0.02	0.39±0.02	0.45±0.04	0.59±0.03	0.70±0.04

Table 7.3:The effect of *T.congolense* infection on WAD goats at different planes of nutritional energy: Mean ±S.E OD of PHA stimulated lymphocytes before and after incubation showed a significant difference ($p < 0.05$).

PRE-INFECTION-GRP		0HR	24HRS	48HRS	72HRS
		MLCPM $\times 10^3$	MLCPM $\times 10^3$	MLCPM $\times 10^3$	MLCPM$\times 10^3$
	A	1.0±0.02	1.4±0.03	1.6±0.01	1.7±0.02
	B	1.4±0.01	1.8±0.02	2.2±0.03	2.3±0.04
	C	1.4±0.02	2.2±0.05	2.8±0.01	3.6±0.02
POST INFECTION					
WK1	AI	0.8±0.02	1.2±0.02	1.4±0.01	1.5±0.02
	BI	0.8±0.04	1.6±0.03	2.1±0.02	2.1±0.03
	CI	0.8±0.03	2.0±0.02	2.7±0.01	3.0±0.05
WK2	AI	0.7±0.06	1.0±0.03	1.1±0.01	1.3±0.04
	BI	0.7±0.01	1.5±0.01	1.9±0.02	2.0±0.03
	CI	0.8±0.20	2.0±0.02	2.5±0.01	2.7±0.01
WK3	AI	0.6±0.01	0.9±0.03	1.0±0.03	1.1±0.02
	BI	0.7±0.02	1.4±0.04	1.8±0.03	1.9±0.02
	CI	0.8±0.03	1.9±0.02	2.4±0.03	2.6±0.01
WK4	AI	0.56±0.01	0.86±0.01	0.9±0.04	0.9±0.02
	BI	0.66±0.91	1.34±0.02	1.74±0.03	1.70±0.01
	CI	0.70±0.03	1.80±0.03	2.36±0.02	2.48±0.01
WK5	AI	0.50±0.01	0.76±0.02	0.80±0.04	0.90±0.03
	BI	0.66±0.01	1.30±0.01	1.70±.0.06	1.70±0.01
	CI	0.70±0.02	1.76±0.01	2.30±0.02	2.42±0.01

Table 7.4: The effect of *T.congolense* infection on Microscopic cell counts Mean ±S.E of PHA-P stimulated lymphocytes before and after incubation WAD goats fed at three different levels of dietary energy.

PRE -INFECTION					
WK 0	GRP	0HR	24HRS	48HRS	72HRS
		MLCPM×10^3	MLCPM×10^3	MLCPM×10^3	MLCPM×10^3
	A	1.0±0.02	1.00±0.03	1.00±0.01	1.00±0.02
	B	1.4±0.01	1.35±0.02	1.40±0.03	1.40±0.04
	C	1.4±0.02	1.40±0.05	1.40±0.01	1.40±0.02
POST INFECTION					
WK1	A	0.8±0.02	0.8±0.02	0.8±0.01	0.8±0.02
	B	0.8±0.04	0.8±0.03	0.8±0.02	0.8±0.03
	C	0.8±0.03	0.8±0.02	0.8±0.01	0.8±0.05
WK2	A	0.7±0.06	0.7±0.03	0.7±0.01	0.7±0.04
	B	0.7±0.01	0.7±0.01	0.7±0.02	0.7±0.03
	C	0.8±0.20	0.8±0.02	0.8±0.01	0.7±0.01
WK3	A	0.6±0.01	0.6±0.03	0.6±0.03	0.6±0.02
	B	0.7±0.02	0.7±0.04	0.7±0.03	0.7±0.02
	C	0.8±0.03	0.8±0.02	0.8±0.03	0.8±0.01
WK4	A	0.56±0.01	0.56±0.01	0.56±0.04	0.56±0.02
	B	0.66±0.91	0.66±0.02	0.66±0.03	0.66±0.01
	C	0.70±0.03	0.70±0.03	0.70±0.02	0.70±0.01
WK5	A	0.50±0.01	0.50±0.02	0.50±0.04	0.50±0.03
	B	0.66±0.01	0.66±0.01	0.66±.0.06	0.67±0.01
	C	0.70±0.02	0.70±0.01	0.70±0.02	0.70±0.01

Table 7.5: The effect of *T.congolense* infection on WAD goats at three different levels of dietary energy: Microscopic counts Mean ±S.E of PHA-P stimulated lymphocytes before and after incubation. Unstimulated control cells maintained a uniform response over the 3-day period between (0.4 to 0.8 mlcpm)

7.4 DISCUSSION

The results obtained in the present study indicated that addition of Alamar blue (AB) to cultured cells did not alter their viability unlike that which occurs during monitoring with trypan blue.The infection of WAD goats with *T.congolense* and on different levels of dietary energy led to a significant changes in the ratio of live-dead lymphocytes. As infection was in progress the number of live cells declined while dead cells increased but more significant in infected animals in group A than groups B and C. lymphocyte proliferation by PHA mitogen had significant changes than non stimulated lymphocytes. The significant changes was associated with different level of dietary energy, It is well known fact that PHA is a potent polyclonal cell activator (Roitt *et al.*, 1989).The magnitude of the proliferative response induced by PHA mitogen in this study could be attributed to the fact that the isolated cells contained both B and T lymphocytes as well as nutritional energy. The observation that infected WAD goats on low energy diet tended to develop lower ratio in live-dead lymphocyte level and low level of lymphocytes proliferation than their better fed counterparts suggest that cellular immunity may be modulated by nutritional energy and that adequate feeding may assist in ameliorating the deleterious effect of trypanosomosis.

T cells require glucose for proliferation and survival. In the absence of glucose, T cells will not proliferate, even if adequate glutamine is present. Protein synthesis during lymphocyte growth also depends on glucose metabolism for ATP and biosynthetic substrates, and thus, cells deprived of adequate glucose levels cannot produce the immune products required for effector function, such as IFN-g ((Krauss *et al.*,2001; Frauwirth and Thompson,2004;Greiner *et al.*, 1994; Proud,2002). Therefore, the growth, function, and survival of an activated lymphocyte depend on a dramatic increase in glucose metabolism that is not simply responsive to energy demands but is directly regulated and has a profound impact on T cell survival and function. In conclusion, it was observed from this work that if goats were given adequate feed with required energy level, this will boost cellular immunity and reduce the susceptibility to *T.congolense* infection .

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

In the present study, the chapter three investigated preliminary survey on seasonal influence on prevalence of trypanosomosis in West African Dwarf goats in five local governments in Ogbomoso area of Oyo State, Nigeria **.** Out of 675 goats examined, 24 were infected with trypanosomes, with total prevalence rate of 3.5%. *Trypanosoma congolense* accounted for all the infections. The mean PCV values for non infected goats were 27.6% while the values for dry and rainy season were 13.3% and 16.2% respectively for infected goats. Infection caused significantly decrease in mean PCV ($p < 0.05$) in blood of infected WAD goats in these areas. Chapter four was designed to study the live weight changes and haematological observations in *Trypanosoma congolense* infected WAD goats fed three different levels of dietary energy during post infection and post treatment phase.This is important because there was dearth of information on controlling caprine trypanosomosis vis-à-vis dietary energy and this will to some extent prevent trypanosome parasite resistance to trypanocidal drugs and their toxicity associated with their use. From this result it was shown that different levels of dietary energy had a marked influence on the dynamics of *T.congolense* infection in WAD goats vis-à-vis the intensity of parasitaemia,body weight gain, and haematological changes. It was concluded that the high levels of dietary energy lower the intensity of parasitaemia, increased body weight gain and lower the degree of anaemia. It was observed that dietary energy had influence on the course of parasitaemia, following the establishment of infection, animals on a different dietary energy experienced greater retardation of growth than their corresponding control groups.The retarded growth was more pronounced in infected animals on a low dietary energy ration than infected animals on medium and high levels of dietary energy ration. The present study showed that high levels of dietary energy ration ameliorates the influence of trypanosome infection on growth rate. Following infection, WAD goats of different dietary energy ration had a decline in PCV values, Hb concentration and RBC values with different degrees of anaemia but the anaemia was more pronounced in goats on low dietary energy intake.The anaemia observed was normocytic-normochromic in all infected animals in group A, B and C . It was observed in the present study that after therapeutic treatment ,all there were improvement on the

body weight gain of both treated and the corresponding controls although there were significant differences between them .The PCV,Hb concentration, and RBC values relatively returned back to normal levels in all the groups. Chapter five was designed to study changes in biochemical parameters in experimental *Trypanosoma congolense* infection on WAD goats fed three different levels of dietary energy during post infection and post treatment phases.The result showed that there was a significant increased ($p < 0.05$) in group mean serum Ca^{2+}, $PO4^{2-}$, Globulin, AST and ALT in infected animals in group A,B and C compared with their uninfected corresponding controls respectively. At post infection phase they returned back to normal values without any significant differences ($p > 0.05$). The group mean serum Na^{+} in infected animals on low and medium levels of dietary energy increased with very highly statistically significant differences ($p < 0.001$) while that of high dietary energy had no significant changes during post infection . At post treatment phase, the mean serum Na^{+} was normal. BUN of infected animals on low and high levels of dietary energy increased while that of medium levels of dietary energy was normal. After treatment, BUN returned to normal values. TP of infected animals on high level was normal ,medium plane decreased while that of low plane mildly increased, After treatment ,low plane was normal while that of medium and high plane increased . The mean serum K^{+}, $HCO^{3-,}$ and Albumin of infected animals on low, medium and high planes of nutrition decreased during post infection and returned to normal after treatment.

Chapter six was designed to study complement levels in *Trypanosoma congolense* infected WAD goats fed three different levels of dietary energy during post infection, and post treatment phases.The result indicated that the respective group mean serum C3 components concentration,total haemolytic complement (THC) and alternative haemolytic complement (ATH) of infected animals on low, medium,and high levels of dietary energy decreased ($p < 0.001$) compared with their corresponding controls.After treatment,the mean C3 , THC,and AHC relatively returned to normal. In this study, there was a different degree of hypocomplementamia with high hypocomplementamia in infected goats on low dietary energy .This indicated that dietary energy influenced the complement activities in this study. Chapter seven investigated the influence of different levels of dietary energy on the lymphocyte proliferation in *Trypanosoma congolese* infected goats. It was observed that Mean lymphocyte count per minute within stimulated infected groups from weeks 1-5 were significantly increased in the order of CI > BI > AI, while unstimulated control cells maintained uniform response over a 72–hour period.

From this study it can be said that Increased dietary energy intake in goats up to 2670.40 kcal/kg energy increased their tolerance to *Tc* infection. It is recommended that dietary energy along with other nutrients be adequately provided for goats to reduce clinical effects of trypanosomosis.

Area of Future Research.

1-The future research should be channelled to influence of different levels of dietary crude protein on the pathogenesis of trypanosomosis in other ruminant (goats , sheep and cattle).

2-The role of vitamins A, C, D and E on the pathogenesis of trypanosomosis.

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APPENDIX 1: NON-DISPOSABLE EQUIPMENT

1. Aspirator bottle. Product of Volac, England.
2. Bench centrifuge (Chilspin). Product of Measuring and Scientific equipment Ltd. England.
3. Bijou bottles (sterile). Product of Volac, England.
4. Bunsen burner. Product of Volac, England.
5. Deep freezer. Sanyo SFC 2850. Product of Sanyo Electric Company Ltd, Japan.
6. Diamond pencil. Product of Volac, England.
7. Ear tages. Product of Bovtag, England
8. Erlenmeyer flask. Product of Volac, England.
9. Glass plate (10.4cm x 8.4cm). product of Volac, England.
10. Glasswares: Conical Falsks, Beakers, Volumetric Flasks, Measuring cylinders. Reagent bottles, pipettes. Product of Volac, England.
11. Humidified chamber
12. Hyland eye precision viewer. Product of Travenol Laboratories incorporated. U.S.A
13. Levelled table
14. Magnetic stirring bar. Product of Gallen Kamp, England. Cat NO. SS615
15. Magnetic stirrer-Hot Plate. Product of Gallen Kamp, England. Cat No. SS615
16. Mettler balance. Product of Sartorius-Werke, GMBH. Germany.
17. Micro dispenser (Ziptrol). Product of Drummond Scientific Company, U.S.A
18. Microhaematocrit centrifuge. Product of Galman-Hawksley Limited, England.
19. Microhaematocrit reader. Product of Glaman-Hawksley Limited, England.
20. Micropipette (5-20ul). Product of Finnipipette Ky, Finland.
21. Microscope (binocular). HM-LUX. Product of Ernst Leitz GMBH, Germany.
22. Neubauer haemocytometer. Product of Weber Scientific International Ltd, England.
23. 0.5 tonne Avery scale. Avery, England.
24. Oven. Product of Charles Hearosn and Company Limited, London.
25. pH meter (digital). Model PW9410. Product of Philips, England.
26. Puncher (2mm)
27. Refrigerator. Product of General Electric Company, Brazil.
28. Room temperature of 20^0C obtained with the use of an air conditioner
29. Soda lime tube. Product of Volac, England.
30. Spectrophotometer. Model 6100. product of Jenway England.

31. Test tubes (sterile plain glass). Product of Volac, England.
32. Test tubes (sterile plastic). Product of Volac, England.
33. Universal bottles (sterile). Product of Volac, England.
34. Volumetric flask. Product of Volac, England. BS 1792.
35. Water bath. Product of Werke, GMBH. Germany. Type 1004

APPENDIX II: CHEMICALS AND REAGENTS

1. Acetic acid. BDH Chemical Ltd. Poole, England. Product NO 10001
2. Agarose (Ultra pure). Product of Life Technologies Inc. U.S.A. Part No 11351 C96
3. Alamar Blue dye.
4. Barbitone (diethyl barbituric acid). BDH Chemicals Ltd. Poole, England. Product No 27282
5. Barbitone sodium. BDH Chemicals Ltd. Poole, England. Product No 27283
6. Barium chloride. BDH Chemicals Ltd. Poole, England. Product No 10047
7. Calcium Chloride AR. BDH Chemical Ltd. Pool, England. Product No 10069
8. Citric acid. BDH Chemicals Ltd. Poole, England. Product No 10081
9. Copper Sulphate. BDH Ltd. Poole, England, Product No. 10091
10. Diethyl amino ethyl(DEAE)
11. D-Glucose. BDH Chemicals Ltd. Poole, England. Product No 10117
12. Disodium hydrogen orthophosphate (anhydrous). BDH Chemicals Ltd. Poole, England. Product NO 10249.
13. Disodium hydrogen orthophosphate (dodecahydrate). BDH Chemicals Ltd. Pool, England. Product No 10248
14. Ethylene diamine tetra acetic acid (disodium salt). BDH Chemicals Ltd. Pool, England. Product No 10093
15. Ethylene glycol tetra-acetic acid (Chel-De). Product of Ciba-Geigy AG Basle, Switzerland. Batch NO 03780-1574
16. Fetal bovine serum
17. Formaldehyde (Formalin) solution. BDH Chemicals Ltd. Pool, England. Product No 10113
18. Freund's incomplete adjuvant (FIA). Control No 700837. product of Difco laboratories, Detroit-Michigan, U.S.A.
19. Gelatin. Product of Oxford, London. Batch No 1513998
20. Gentian violet. BDH Chemicals Ltd. Pool, England. Product No 34033
21. Giemsa's stain. BDH Chemicals Ltd. Pool, England. Product No 34034

22. Magnesium Chloride. BDH Chemicals Ltd. Pool, England. Product No 10149
23. Phytohaemaglutinin(PHA)
24. Phosphoric sodium chloride glucose (PSG)
25. Polyethylene glycol 600. Fluke AG, Buchs SG. Switzerland. Batch no 211836-1079
26. Potassium cyanide. BDH Chemicals Ltd. Pool, England. Product No 10201
27. Potassium dihydrogen orthophosphate. BDH Chemicals Ltd. Pool, England. Product No 10203.
28. Potassium ferricyanide. BDH Chemicals Ltd. Pool, England. Product No 10204
29. Potassium iodide. BDH Chemicals Ltd. Pool, England. Product No 10212
30. RPMI-1640
31. Sodium acetate trihydrate. BDH Chemicals Ltd. Pool, England. Product No 30103
32. Sodium Azide. BDH Chemicals Ltd. Pool, England. Product No 30111
33. Sodium Carbonate. BDH Chemicals Ltd. Pool, England. Product No 30121
34. Sodium Chloride. BDH Chemicals Ltd. Pool, England. Product No 10241
35. Sodium citrate. BDH Chemicals Ltd. Pool, England. Product No 30152
36. Sodium hydroxide. BDH Chemicals Ltd. Pool, England. Product No 10252
37. Sodium potassium tartarate. BDH Chemicals Ltd. Pool, England. Product No. 10291
38. Sucrose. BDH Chemicals Ltd. Pool, England. Product No 10274
39. Sulphuric acid. BDH Chemicals Ltd. Pool, England. Product No 10276
40. Toluidine blue. BDH Chemicals Ltd. Pool, England. Product No 34077
41. Tris (hydroxymethyl) aminomethane. Trizma base. Product of SIGMA chemical Co. U.S.A. Lot 5IH5606.
42. Trisodium citrate. BDH Chemicals Ltd. Pool, England. Product No 10242
43. Xylene. BDH Chemicals Ltd. Pool, England. Product No 10293
44. Zinc sulphate. BDH Chemicals Ltd. Pool, England. Product No 30621
45. Zymosan A (yeast cell wall extract) from Saccharomyces cerevisiae (58856-93-92) Z-4250 Lot 31K1531. Product of SIGMA chemical Co., U.S.A.

APPENDIX III: EXPERIMENTAL ANIMALS

White Male Chinchilla Rabbits

Alibino Mice

West African Dwarf goats

APPENDIX IV: DISPOSABLE EQUIPMENT

1. Cotton wool
2. Coverslips (18mm^2). Manufactured by Corning Glass Works, New York.

3. Microhaematocrit capillary tubes. 75mm with internal diameter of 1.2mm. Manufactured by Hawkskey and sons Limited, England. Cat NO. 01604.
4. Microscope slides. 75x25mm single forsted, pre-cleaned. 0.96-11.06mm thick, product of Corning Glass Works, New York.
5. Neeldes 27G, 21G and 18G.
6. Scapel Blade
7. Syringes (sterile disposable plastic) 1m1, 2m1, 5m1, 10m1 and 20m1.

APPENDIX V: BUFFER AND REAGENT PREPARATIONS

1. 200mM of disodium ethylene diamine tetra acetic acid (Na_2EDTA) solution

   Na_2 EDTA (2g)

   Distilled water (make up to 26.7ml)

   Store on the bench until ready for use

2. Modified Drabkin's solution

   Potassium ferricyanide (200gm)

   Potassium cyanide (50gm)

   Potassium dihydrogen phosphate (140mg)

   Distilled water (amek up to 1 litre and pH adjusted to 7.0)

3. RBC diluting fluid

   3% aqueous solution of sodium citrate (99ml)

   40% formaldehyde (1m1)

4. WBC diluting fluid

   Aqeous solution of acetic acid (3%)

   Gentian violet (1%)

5. Biuret reagent (50ml of Biuret reagent +250ml of Biuret reagent B)

   Biuret regent A

   0.2N NaOH (400ml)

   Sodium potassium tartrate (45g)

   Copper Sulphate (15g)

   Ptassium iodide (5g)

   0.2 N NaOH (make up to 1 litre)

   Biuret reagent B

   Potassium iodide (5g0

   0.2 N NaOH (make up to 1 litre)

6. Commercially prepared standard protein (8g%)

I satchet of standard protein

Distilled water (make up to 10ml)

7. Zinc sulphate solution

Zinc sulphate (208mg0

Distilled water (make up to 1 litre)

8. Barium chloride solution

Barium chloride (1.15g)

Distilled water (make up to 100ml)

9. Barium sulphate standard

Barium chloride solution (3ml)

0.2N H_2SO_4 (make up to 100ml)

10. Barbitone buffered saline (BBS) (pH 8.6)

Sodium hydrogen diphosphate (0.181g)

Sodium chloride monophosphate (1.252g)

Sodium chloride (8.5g)

Distilled water (make up to 1 litre)

11. Barbitone (Veronal) buffer (pH 8.6)

Barbitone sodium (8.87 g)

Sodium acetate trihydrate (6.5g)

Barbitone (1.13g)

Distilled water (make up to 1oml) and pH 8.6 with IN HCI

Store at 4^0C (7iMaximum 8 weeks)

12. Hypotonic tris-buffer (pH 7.4)

Tris (1.211g)

Na_2 EDTA (0.3722g)

Distilled water (make up to 1 litre) and pH 7.4 with IN HCI

Store at 4^0C until use

13. Isotonic tris-saline (pH 7.4)

Tris (1.211g)

NaCL (8.77g)

Distilled water (make up to 1 litre) and pH 7.4 with IN HCI

Store at 4^0C until use

14. Alsever's solution

D-Glucose (290.5g)

Trisodium citrate (8.0g)

NaCl (4.2g)

Distilled water (make up to 500ml)

Store at 4^0C (Maximum 4 weeks)

15. Normal Saline (0.15M NaCl)

NaCl (8.77g)

Distilled water (make up to 1 litre)

Store at 4^0C until ready for use

16. Isotonic tris-buffered saline

Tris (1.211g)

NaCl (8.77g)

$CaCl_2$ (0.0441g)

$MgCl_2$ (0.203g)

Distilled water (make up to 1 litre) and pH 7.2 with In HCl

Store at 4^0C until use

18. 0.1M Na_2CO solution

Na_2CO_3 (1.60)

Distilled water (make up to 100ml)

19. 2% Agarose

Agarose (2.g)

Sodium Azide (0.1g)

Distilled water (make up to 100ml

In a pyrex reagent bottle

Boil at 100^0C for 30 minutes, with constant shaking

Store at 4^0C until ready for use.

20. 2.5 mM magnesium –Ethylene Glycol bis-amino tetra acetic acid (Mg-EGTA)

100 mM EGTA (50ml)

Magnesium Chloride (35ml)

100 mM EGTA

EGTA (7.6g)

Distilled water (make up to 100ml + stirring

10M Sodium hydroxide to dissolve and pH 7.4

Distilled water (make up to 200ml)

Store at 4^0C (Maximum 4 weeks)

Magnesium Chloride ($MgCl_2$) solution

$MgCl_2$ (10.g)

Distilled water (make up to 500 ml)

DGVB=

D-Glucose (50g)

Distilled water (make up to 1000ml)

GVB =

10% Gelatin (10ml)

Boil

Distilled water (100 ml)

5XVBS (200 ml)

Distilled water (make up to 100 ml)

21. Trypanosome counting fluid

3% trisodium citrate (4.5 ml)

10% formalin (4. ml)

1% toluidine blue (0.75ml)

Three articles were published from this thesis before completion.