

# Serum biochemical changes in West African Dwarf sheep experimentally infected with *Trypanosoma brucei*

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OGUNSANMI (A.O.), AKPAVIE (S.O.), ANOSA (V.O.). Modifications biochimiques chez des moutons de la race naine de l'Afrique de l'Ouest infectés expérimentalement par *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1994, 47 (2) : 195-200

Les valeurs biochimiques du sérum et du plasma ont été déterminées chez des brebis de la race naine de l'Afrique de l'Ouest expérimentalement infectées par *Trypanosoma brucei*. Les résultats montrent une élévation du fer sérique, des chlorures, du bicarbonate, des phosphates inorganiques, de la créatinine, de l'urée, des protéines totales, des globulines et du fibrinogène plasmatique. L'albumine sérique, le rapport albumine/globuline ainsi que les valeurs du potassium, du cuivre et du magnésium ont baissé. Les résultats semblent montrer un mauvais réemploi du fer au niveau de l'érythropoïèse et un dysfonctionnement probable au niveau de la parathyroïde, du foie et/ou du rein.

Mots clés : Ovin - Brebis - Mouton Djallonké - *Trypanosoma brucei* - Infection expérimentale - Sérum - Plasma - Biochimie - Nigeria.

## INTRODUCTION

African trypanosomiasis is one of the important vector-borne diseases of human and livestock in tropical Africa. It is known that pathophysiological alterations occur in the cellular and plasma components of blood during infection (19, 30) and that the pathogenic mechanism is not the same for different species of trypanosomes. *Trypanosoma brucei* invades host tissues and the pathology induced is related to the site of localization (17). OTESILE *et al.* (22) suggested that serum biochemistry might give an indication of the degree of damage to host tissues as well as the severity of infection.

Small ruminants are fully susceptible to *T. brucei* infection and the economic impact of trypanosomiasis on these animals has been shown to be substantial (18). However, the effects of the disease on serum biochemical constituents have not been fully investigated in small ruminants. Biochemical changes in bovine trypanosomiasis have been reported 47 years ago by FIENNES *et al.* (11) in *T. congolense* infection of cattle. Few reports exist on normal levels of protein fractions, mineral components (20) and plasma volumes (3). This investigation was therefore carried out to examine the serum biochemical changes that occur in acute or chronic experimental *T. brucei* infection of West African Dwarf sheep.

## MATERIALS AND METHODS

Eight female West African Dwarf sheep, about two years old, were used for the experiment. They were purchased from a local market in Ibadan, Nigeria, housed in fly-proof pens on concrete floors and conditioned for a minimum of 4 weeks before infection. Grass, water and salt lick were provided *ad libitum* and sheep ration given at 0.5 kg per sheep/day.

The animals were treated intramuscularly with diminazene aceturate at 7 mg/kg body weight and oxytetracycline hydrochloride at 50 mg/kg. They were also treated orally with thiophanate at 50 mg/kg and given a tick bath using coumaphos.

Five of the eight conditioned animals were infected intraperitoneally with  $2.27 \times 10^6$  trypanosomes of *T. brucei* stock MKAR/84/NITR/6 isolated during an outbreak of fatal *T. brucei* infection of pigs in Mkar, Benue State, Nigeria. The other three animals served as controls.

## Parasitology

Jugular vein blood samples were collected twice every week before and after infection to obtain serum and plasma and examined for blood parasites. Serum and plasma samples were kept at -20°C until analysed.

## Biochemistry

The serum iron, copper and magnesium were measured using an atomic absorption spectrophotometer (Perkin-Elmer, USA). Sodium and potassium concentrations were determined with the flame photometer (Corning model 400, Corning Scientific Limited, England) and albumin values were determined by the bromocresol green method described by DOUMAS *et al.* (10). The total plasma protein was estimated by the Biuret method (26) while the globulin and albumin/globulin ratio were calculated according to COLES (7). Serum calcium was measured by the cresolphthalein complexone technique (31), while phosphate, chloride and serum bicarbonate were measured according to TORO and ACKERMAN (13, 28, 31), respectively. The serum urea and creatinine were measured according to HARRISON (15) and plasma fibrinogen was determined according to the method of COLES (7).

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The data obtained were subjected to Student's test using the Statistical Analysis Systems (29) computer programme. Tests were carried out at a 95 % level of confidence ( $p \leq 0.05$ ) or higher ( $p \leq 0.01$ ) and  $p \leq 0.001$ ).

## RESULTS

All infected sheep became parasitaemic within 7 to 10 days. The first peak of parasitaemia occurred within 9 days and was associated with a pyrexia ( $40.0^{\circ}\text{C}$ ) which peaked on day 14. The parasitaemia was later intermittent but usually scanty throughout the course of infection, especially during the chronic stage, that is, after 30 days of infection. All control sheep survived and none became infected with trypanosomes. Infected animals showed clinical signs as reported by OGUNSANMI *et al.* (21).

### Biochemical changes

#### Serum minerals

The pre- and post-infection biochemical values of sheep experimentally infected with *T. brucei* are shown in figures 1 to 9. The post-infection iron values were higher than the pre-infection values. Two prominent peaks were observed on days 4 and 84 after infection (fig. 1). The mean copper values of infected sheep decreased sharply from day 28 onwards (fig. 1).

The mean serum calcium values first dropped slightly and became significantly depressed on day 42 after infection (fig. 2). Similarly, the mean magnesium values showed an initial drop and become permanently low on day 56 post-infection.

The mean serum inorganic phosphate values of the infected sheep increased progressively, while the increase in serum chloride became prominent from day 70 post-infection (fig. 3). The mean serum sodium values increased progressively, becoming prominent on day 42, and attained a peak on day 70, while the serum potassium values decreased progressively and attained the lowest values on day 42 after infection (fig. 4).

Serum bicarbonate values were higher than the pre-infection values except for the sharp drop observed on day 56 post-infection (fig. 5). The mean post-infection values for urea were also increased throughout infection and peaked on day 70. The mean post-infection creatinine values were significantly increased from day 42 onwards and peaked on day 70 (fig. 6).

#### Serum proteins

The mean total serum protein values were high between days 28 and 56 after infection and thereafter returned to normal levels (fig. 7). The serum albumin values decreased progressively from day 42, attaining the lowest values on day 70 post-infection (fig. 8). The globulin

values started increasing from day 28, peaked on day 42 and remained high until day 98 post-infection (fig. 7). The albumin/globulin ratio increased slightly on day 14 but thereafter dropped sharply until day 98 and became normal again on day 105 post-infection (fig. 8).

The mean plasma fibrinogen values fluctuated throughout infection with peaks on 21, 56 and 91 days post-infection. Considerable fluctuations were also observed for plasma fibrinogen levels in the control animals (fig. 9). Apart from the fluctuations in plasma parameters were within the normal range for West African Dwarf sheep as shown in the various figures.

## DISCUSSION

The values of serum iron in sheep experimentally infected with *T. brucei* increased throughout infection, while that of copper decreased during the later stages. Elevated iron values have been previously reported in *T. brucei* and *T. congolense* infections of cattle by DARGIE *et al.* (8, 9). The hyperferraemia observed in this study could partly be attributed to the defective iron re-utilization as a result of bone marrow hypofunction, as suggested by WELLDE *et al.* (32), and dyshaemopoiesis (8, 9).

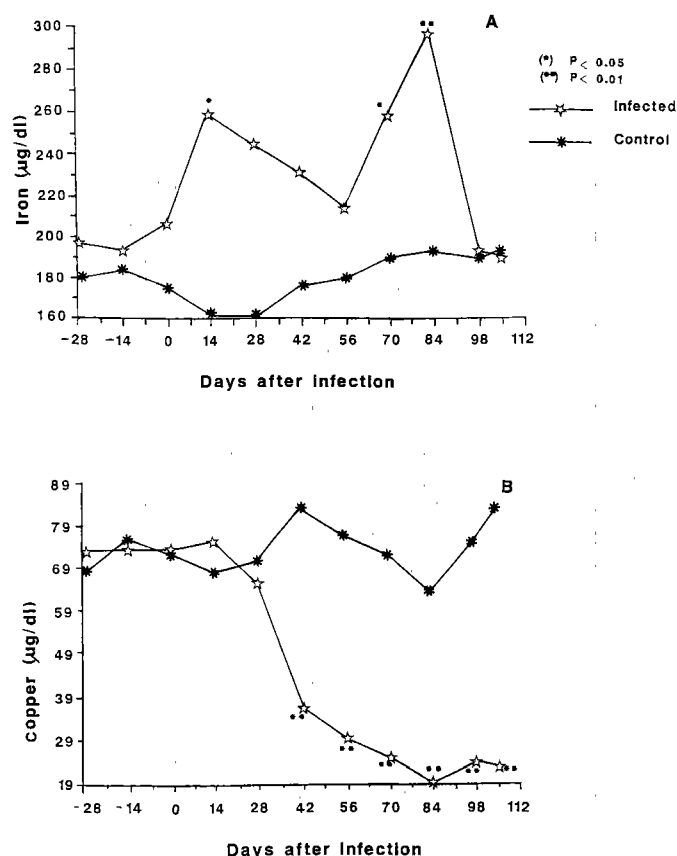


Figure 1 : Changes in min serum iron ( $\mu\text{g/dl}$ ) and copper ( $\mu\text{g/dl}$ ) values in *T. brucei* infected and control sheep.

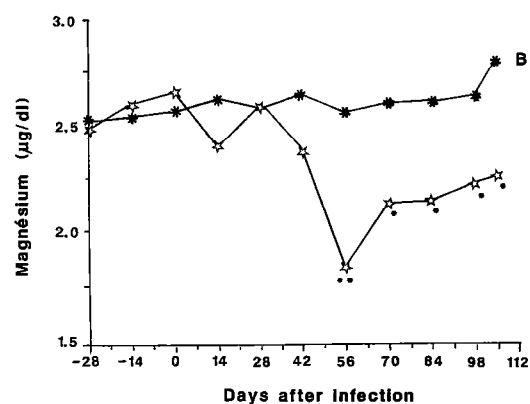
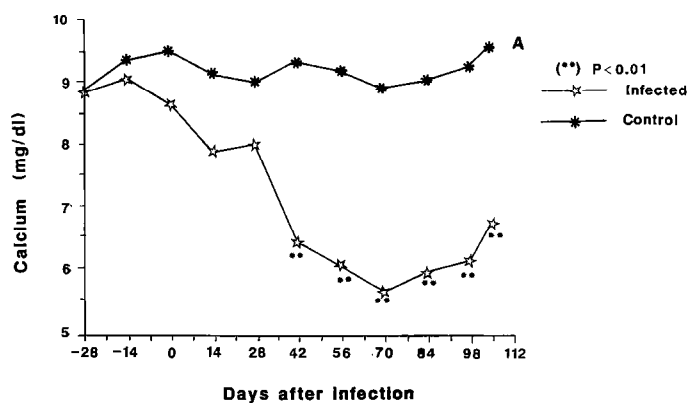


Figure 2 : Mean serum calcium (mg/dl) and magnesium ( $\mu\text{g/dl}$ ) levels in *T. brucei* infected and control sheep.

The observed decrease in copper values in this study does not agree with the findings of SAROR (27) who reported fluctuating copper values in *T. vivax* infected cattle. The effects of copper deficiency, as observed by previous workers (23), could induce depressed erythropoiesis by contributing to the defective re-utilization of iron and haemoglobin synthesis with the resultant anaemia during the later stages of infection.

The serum calcium were rapidly and permanently depressed while those of inorganic phosphate were initially normal but elevated during the later stages of infection. Serum magnesium values were initially normal and then decreased. The fall in calcium and the rise in phosphate values match the findings of FIENNES *et al.* (11) in cattle infected with *T. congolense* and GOODWIN and GUY (14), in rabbits infected with *T. brucei*. The observed hypocalcaemia and hyperphosphataemia may be attributed to a deficiency of parathyroid hormone (PTH), probably due to damage to the parathyroid glands (5). Hyperphosphataemia may also be due to haemolysis (4).

Since hypocalcaemia often occurs concurrently with hypomagnesaemia in hypoparathyroidism (34), the hypomagnesaemia observed in this study could also be attributed to damage to the parathyroid glands. The hypernatraemia observed in this study is in agreement with the

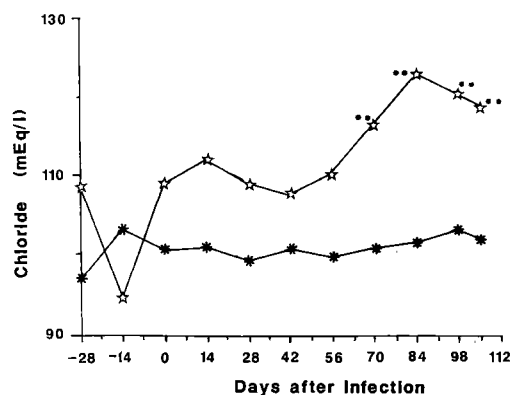
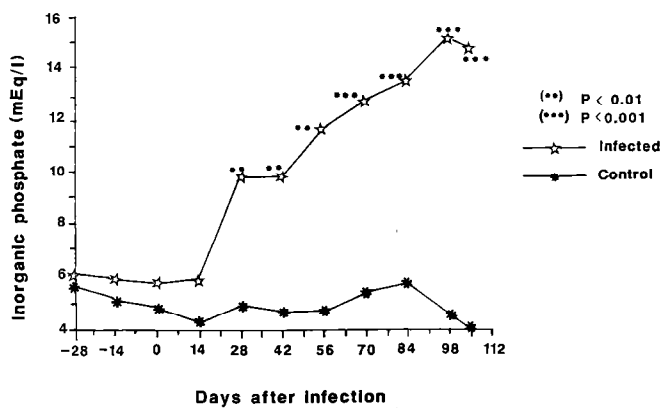


Figure 3 : Mean serum inorganic phosphate (mEq/l) and chloride (mEq/l) levels in *T. brucei* infected and control sheep.

findings of FIENNES *et al.* (11) in *T. congolense* infected cattle and this may be related to the anaemia resulting in haemodilution.

The mean serum bicarbonate levels were observed to have been elevated although a sharp drop was observed on day 56. This observation does not agree with the report of GOODWIN and GUY (14), who reported a decrease in serum bicarbonate in *T. brucei* infected rabbits. The observed increase in serum bicarbonate may be attributed to the generation of bicarbonate ions but the kidney (6), which may be increased during trypanosome infection. The sharp drop in the serum bicarbonate levels during the later stages of infection may be due to acidosis associated with anaemia, renal malfunction and release of toxic metabolites such as free fatty acids by trypanosomes (1).

Serum potassium was observed to have decreased while chloride increased. These observations have been previously reported by RAISINGHANI *et al.* (24) and GOODWIN and GUY (14) in *T. evansi* and *T. brucei* infected camels and rabbits, respectively. The hyperchloraemia associated with hypokalaemia and hypernatraemia may be related to renal dysfunction as suggested by ZILVA and PANNALL (34) and CARLSON (6).

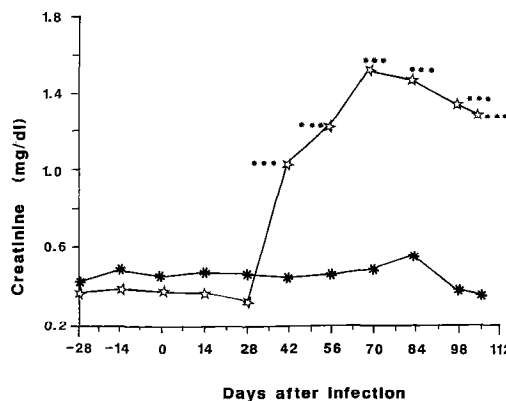
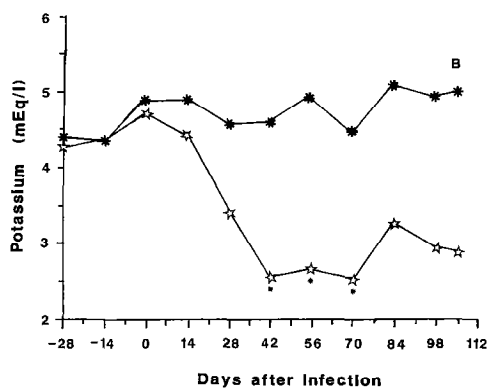
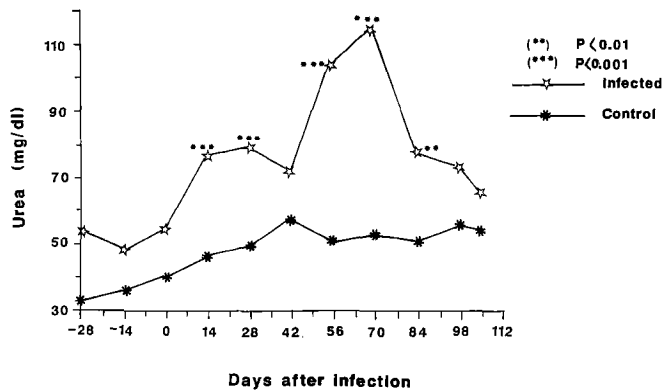
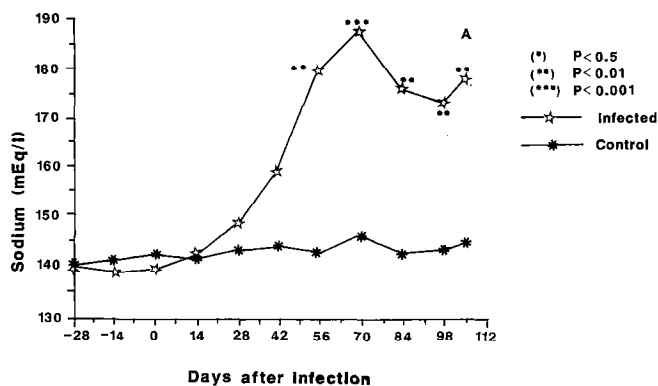


Figure 4 : Mean serum sodium (mEq/l) and potassium (mEq/l) in T. brucei infected and control sheep.

Figure 6 : Mean serum urea (mg/dl) and creatinine (mg/dl) levels in T. brucei infected and control sheep.

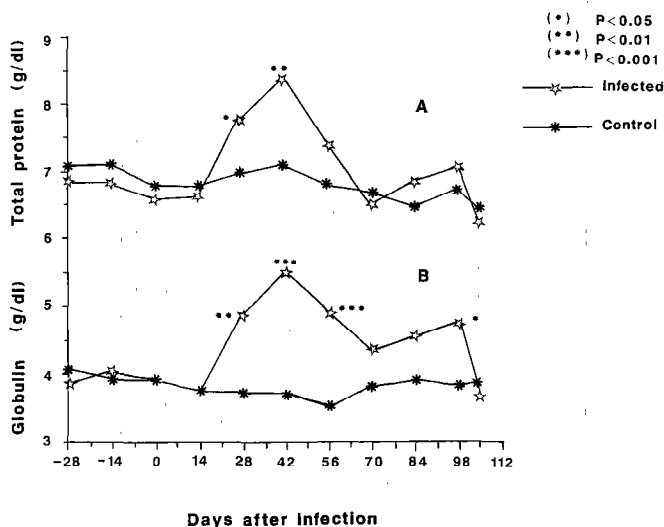
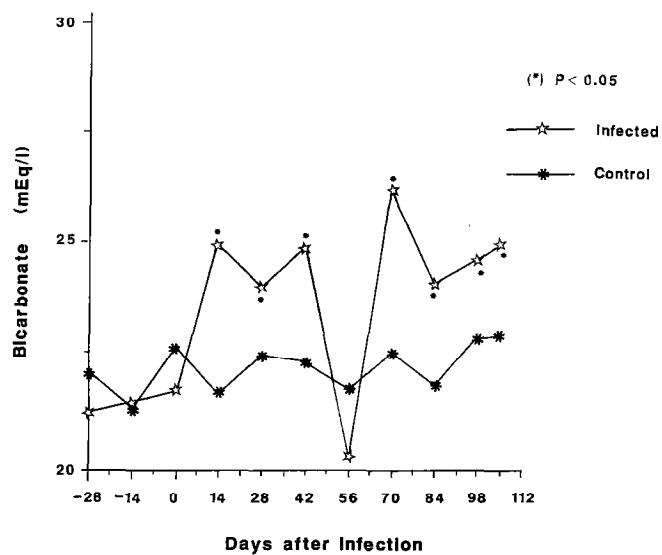


Figure 5 : Mean serum bicarbonate (mEq/l) levels in T. brucei infected and control sheep.

Figure 7 : Mean total serum protein (g/dl) and globulin (g/dl) levels in T. brucei infected and control sheep.

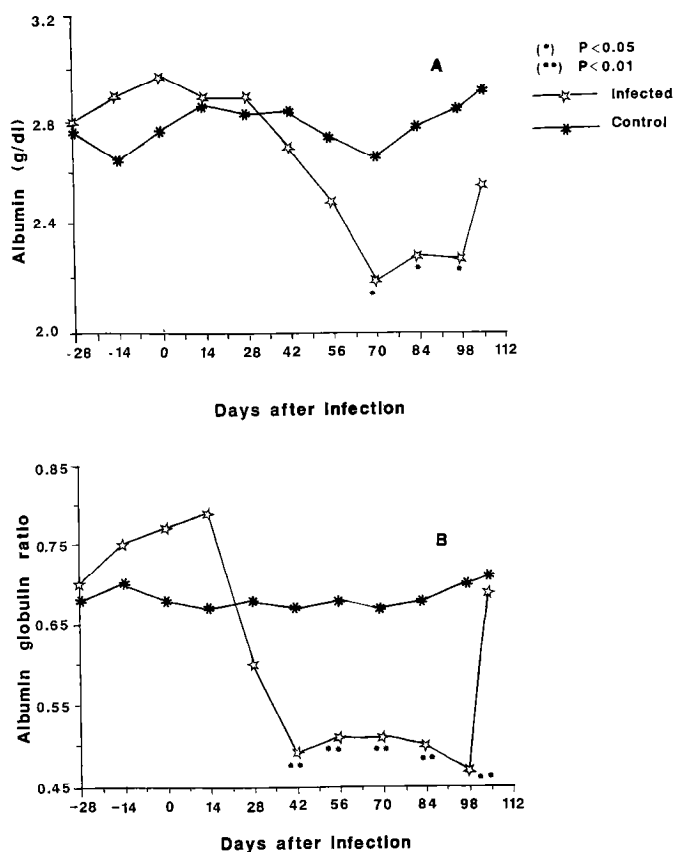


Figure 8 : Mean serum albumin (g/dl), albumin globulin ratio in *T. brucei* infected and control sheep.

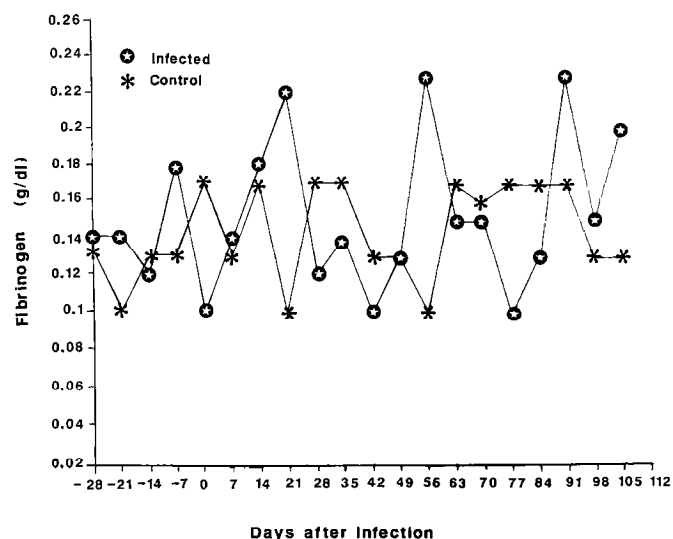


Figure 9 : Mean plasma fibrinogen (g/dl) in *T. brucei* infected and control sheep.

The high blood urea and creatinine values observed in this study are in agreement with the reports of GOODWIN and GUY (14) and ISOUN *et al.* (16) concerning *T. brucei* and *T. vivax* infections of rabbits and cattle, respectively. The increases in blood urea and creatinine levels could be attributed to fever, tissue damage and renal dysfunction, as suggested by FINCO (12).

The observed increases in total serum protein levels, although not significant, could be attributed to hypergammaglobulinaemia, which is a prominent feature of trypanosomiasis, primarily due to increase in IgM levels (2). The decrease in albumin/globulin ratio observed in this study coincides with the report by REES and CLARKSON (25) concerning *T. vivax* infected sheep. The decrease obviously resulted from a decrease in albumin and an increase in globulin values during infection.

The plasma fibrinogen levels fluctuated widely but the occasional increase was not significant. This is similar to the findings of WELLDE *et al.* (33) in *T. vivax* infected cattle. Hyperfibrinogenaemia is mainly seen in dehydration and inflammatory conditions (7).

It is therefore concluded that *T. brucei* infection in sheep causes marked biochemical changes which may be associated with the pathology induced by the parasite. It will therefore be necessary to determine whether differences occur in infections with other trypanosome species.

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Serum and plasma biochemical values were determined in female West African Dwarf sheep experimentally infected with *Trypanosoma brucei*. The results show an increase in the values of serum iron, chloride, bicarbonate, inorganic phosphate, creatinine, urea, total protein, globulin and plasma fibrinogen. The serum albumin, albumin/globulin ratio, potassium, copper and magnesium values are depressed. These findings suggest defective re-utilization of iron in erythropoiesis and probable parathyroid gland, hepatic and/or renal malfunction.

Key words : Ewe - Djallonké sheep - *Trypanosoma brucei* - Experimental infection - Serum - Plasma - Biochemistry - Nigeria.

OGUNSANMI (A.O.), AKPAVIE (S.O.), ANOSA (V.O.). Cambios en la bioquímica sérica de ovejas enanas de Africa del Oeste, infectadas experimentalmente con *Trypanosoma brucei*. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (2) : 195-200

Se determinaron los valores bioquímicos, séricos y plasmáticos en ovejas enanas de Africa del oeste, infectadas experimentalmente con *Trypanosoma brucei*. Los resultados muestran un aumento en los valores séricos de hierro, cloruro, bicarbonatos, fosfatos inorgánicos, creatinina, urea, proteína total, globulinas y fibronógeno plasmático. Las tasas de albúmina sérica, la proporción albúmina:globulina, de potasio, de cobre y de magnesio se encuentran reducidas. Los hallazgos sugieren una reutilización defectuosa del hierro en la eritropoyesis y probablemente una disfunción de la glándula paratiroides, hepática y/o renal.

Palabras clave : Oveja - Ovino Djallonké - *Trypanosomoma brucei* - Infección experimental - Suero - Plasma - Bioquímica - Nigeria.