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Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in Small East Africa goats

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ABSTRACT

Serum biochemical changes in goats challenged with either *Trypanosoma congolense* or *Trypanosoma brucei* and uninfected controls were investigated. Experimental goats received a primary trypanosome challenge on day 0, treated with diminazene aceturate on day 49 and received a secondary trypanosome challenge on day 77 of the 136-day experiment. Infection was associated with development of anaemia, hypoproteinaemia, hypoalbuminaemia, hypocholesteraemia, low density and high density hypolipidaemia. In both the primary and secondary challenges, however, serum free fatty acid concentrations were significantly higher than those of the controls. These changes suggest that the growing numbers of trypanosomes post-infection in goats require some lipids and proteins to support their growth, while the higher free fatty acid concentration observed may directly contribute to the development of anaemia as free fatty acids are known to be potentially cytotoxic and haemolytic *in vitro*.

Key words: goats, serum proteins, lipids, trypanosomosis

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Introduction

Animals infected with African trypanosomes have been shown to differ physiologically from their uninfected counterparts. Abnormalities of lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosomes. In a study conducted by DIEHL and RISBY (1974) in rabbits infected with *Trypanosoma gambiense*, serum lipids and cholesterol values increased progressively with time, attaining a three- or four-fold increase over control values nearing terminal stage of infection. In a separate study ROUZER and CERAMI (1980) infected rabbits with *Trypanosoma brucei* and also observed an increase of plasma cholesterol and total lipids. In contrast, *Trypanosoma rhodesiense* infection in cattle (WELLDE et al., 1989) and *Trypanosoma congolense* infection in sheep (KATUNGUKA-RWAKISHAYA et al., 1992) were associated with a drop in plasma cholesterol concentration. Serum proteins have also shown considerable changes in trypanosomosis infections. ANOSA and ISOUN (1976) working with sheep and goats infected with *Trypanosoma vivax* reported that there were increases in serum total proteins and gamma globulins.

The present experiment was designed to study serum biochemical changes in *Trypanosoma congolense* and *Trypanosoma brucei* infection in goats, and how these changes progressed in primary and secondary trypanosome challenges. The possible association with some observed clinocopathological effects, especially anaemia, was also investigated.

Materials and methods

Experimental goats. Twenty-five male goats aged 9-12 months were purchased from a local pastoralist community in Bbale County, Mukono District. They were housed in a fly-proof animal unit on wooden slats at the Faculty of Veterinary Medicine, Makerere University. In order to avoid occurrence of pneumonia associated with transport stress and change of environment, (as had been noted in the trials) each goat on arrival was immediately treated with 250,000 International Units of Procaine Penicillin G and 250 mg of Dihydrostreptomycin Sulphate Forte (Bremer Pharma, GBBH, 27540, Bremerhaven, Germany). The treatment was repeated for two more days. All goats were dosed with albendazole, Vermaprazol®

(Hipra Laboratories, Spain) and sprayed with Taktic® to control ectoparasites.

Feeding. Basal diet consisted of cut *Brachiaria* forage and Elephant grass supplemented with banana peels mixed with approximately 90 g. of cotton seed cake per goat per day. Water and a mineral lick were available *ad libitum.*

Formation of experimental groups. Four weeks following purchase the animals were divided into three groups according to their packed cell volume and body weight. Group one of 10 goats was challenged with *Trypanosoma congolense*. Group two, also of 10 goats, was inoculated with *Trypanosoma brucei* while group three of 5 goats were the controls.

Parasites. The parasites were obtained from Livestock Research Institute (LIRI), Tororo, in Eastern Uganda. *Trypanosoma brucei* stabilate number LIRI 040898C was originally isolated from cattle at Kayindi, Najja sub-county, Mukono District, in July 1998. The strain of *Trypanosoma congolense* was stabilate number LIRI 171296A also isolated from cattle in Masaba sub-county, Busia District in December 1996. Initially, the parasites were inoculated into mice for multiplication and maintained in goats until required. Parasitaemia of the reservoir goats was estimated by the Dark ground/buffy coat method (MURRAY et al., 1983). Each experimental goat received 2 ml containing approximately 10ł trypanosomes per ml by the subcutaneous route.

Collection and handling of blood samples. Three ml of non-anticoagulated blood were collected from the jugular vein of each experimental goat once a week. The samples were left on a bench overnight. They were then centrifuged in a centrifuge at 358 x g for 5 minutes. The serum was carefully decanted into 1.5 ml serum caps and frozen at -20 °C until required for biochemical analysis.

Treatment of animals during the experiment. All goats which developed a PCV of 15% and below were immediately treated with Berenil® at a dose rate of 3.5 mg/kg^{-1}. Their measurements would subsequently be excluded from calculating the means. All goats had developed a Packed Cell volume (PCV) of 15% on day 49 and were treated. When PCV values had recovered close to pre-infection values, the goats were re-infected on day 77.

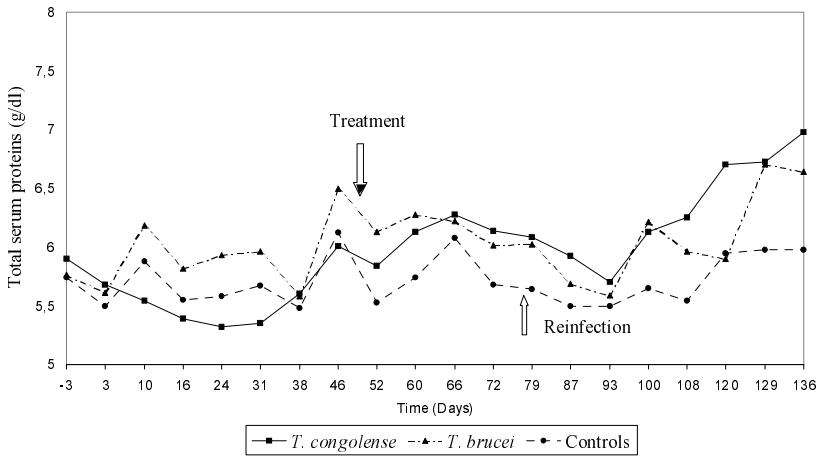
Determination of blood biochemical parameters. Total serum proteins, albumin, total cholesterol, triglycerides, low and high density lipoproteins were determined using a Ciba-Corning 560 blood chemistry autoanalyser. Bitech Laboratories' standard commercial test kits were used according to manufacturer's instructions. Total cholesterol was determined by the calorimetric enzymatic end point. Triglycerides were also determined after hydrolysis with lipases, while total lipids and free fatty acids were determined carolimetrically with a Ceril Spectrophotometer, model CE 2040, Cambridge, England.

Statistics. Data summaries were performed with the Excel computer programme; means and standard errors of the means were computed. Analysis by paired t-tests was done with the Statistical Programme for Social Science (SPSS) computer programme.

Results

Serum total proteins. In the primary trypanosome challenge (Fig. 1) serum total proteins of *Trypanosoma congolense*-infected goats were lower than those of the control goats, while the measurements of *Trypanosoma*

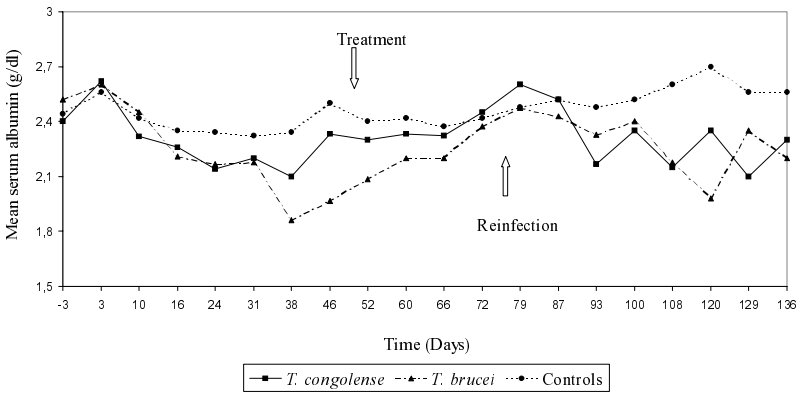
Fig. 1. Mean total serum proteins of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls



brucei-infected goats were higher than those of the controls. In *Trypanosoma congolense*-infected goats, serum total proteins dropped from 5.75 ± 0.12 g dl^{-1} three days before infection to 5.32 ± 0.20 g dl^{-1} on day 24. In the same period, values in *Trypanosoma brucei*-infected goats increased from 5.76 ± 0.14 g dl^{-1} to 5.93 ± 0.19 g dl^{-1}. After treatment with diminazene aceturate on day 49, the values in *Trypanosoma congolense* infection slightly recovered from 5.84 ± 0.41 g dl^{-1} on day 52 to 6.08 ± 0.20g dl^{-1} on day 79, while the corresponding measurements in *Trypanosoma brucei*-infected goats showed slight fluctuation from 6.13 ± 0.21 g dl^{-1} to 6.03 ± 0.21 g dl^{-1}. Serum total proteins for the controls fluctuated between 5.74 ± 0.25 g dl^{-1} and 6.12 ± 0.25 g dl^{-1}.

Serum albumin. Mean serum albumin concentration of *Trypanosoma congolense* and *Trypanosoma brucei*-infected goats were lower than those of the controls, although not statistically significant (P>0.05), in both primary and secondary challenges (Fig. 2). The mean serum albumin

Fig. 2. Mean serum albumin concentration of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls

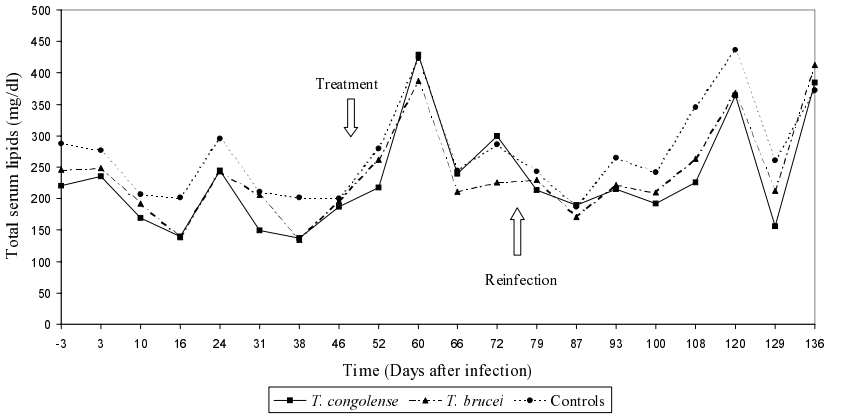


concentration of both infection groups decreased steadily between infection and treatment at day 49. After treatment, there was a slight increment of the values. After re-infection on day 77, mean serum albumin values of *Trypanosoma congolense*-infected goats fell sharply from 2.60 ± 0.04 g

dl^{-1} at day 79 to 2.17 ± 0.12 g dl^{-1} on day 97. The lowest value of 1.98 ± 0.21g dl^{-1} in *Trypanosoma brucei*-infected goats was recorded on day 120. Minor variations were observed in the control goats.

Serum total lipids. In both primary and secondary *Trypanosoma congolense* and *Trypanosoma brucei* infections, (Fig. 3), concentrations of serum total lipids were significantly lower than those of the controls

Fig. 3. Mean serum total lipids of goats exposed to both primary and secondary challeges with either *T. congolense* or *T. brucei* and the uninfected controls

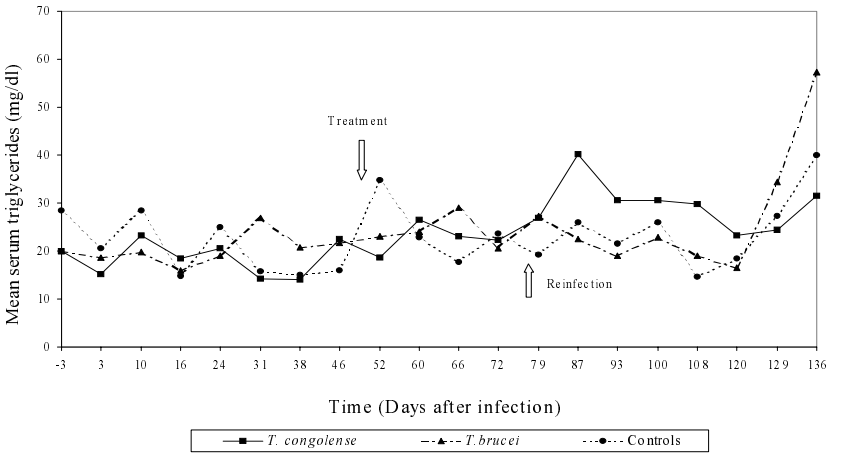


(P<0.05). There was a rapid reduction in total lipids after goats were exposed to a primary trypanosome challenge up to day 16. By day 24 total lipid concentrations rose and again decreased, reaching 137.0 ± 4.4 mg dl^{-1} at day 38 to 186.1 ± 10.1mg dl^{-1} at day 46 in *Trypanosoma congolense*-infected goats. The *Trypanosoma brucei* group showed an increment from 134.8 ± 7.9 mg dl^{-1} to 195.7 ± 11.5 mg dl^{-1} in the same period. After treatment, lipid concentrations increased in both infection groups. After re-infection with trypanosomes, mean serum total lipids in the infected group remained lower than in the control group up to the end of the experiment.

Serum triglycerides. The mean triglyceride concentrations of *Trypanosoma congolense*-infected goats (Fig. 4) reduced from 19.30 ±

2.52 mg dl^{-1} three days before infection to 15.10 ± 1.49 mg dl^{-1} three days post-infection. Thereafter, values increased and fluctuated. In the *T. brucei* group, values varied between 20.00 ± 4.75 mg dl^{-1} and 16.0 ± 2.15 mg dl^{-1}

Fig. 4. Mean serum triglyceride concentration of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls

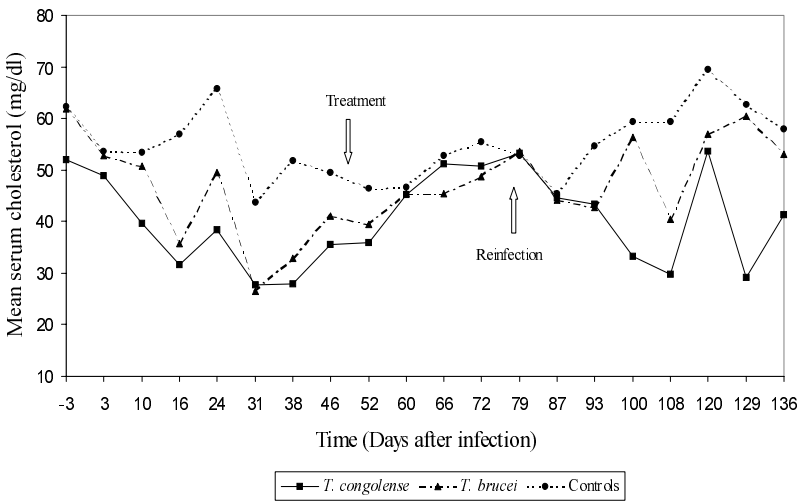


between day 16 and infection. Until day 31 there was an increase to 27.0 ± 5.30 mg dl^{-1}. By day 46 this value had reduced to 21.67 ± 4.19 mg dl^{-1}. After treatment, recovery of the values was recorded in both infected groups. Immediately after re-infection there was an increase in triglycerides in the *Trypanosoma congolense* group, reaching 40.20 ± 8.75 mg dl^{-1} on day 87. Until the end of the experiment the values dropped steadily to 31.50 ± 6.30 mg dl^{-1} on day 136. *Trypanosoma brucei*-infected goats, however, showed a different pattern: their values fluctuated between 34.50 ± 2.72 mg dl^{-1}on day 79 and 16.60 ± 3.60 mg dl^{-1} on day 120. The values in the control goats varied between 15.0 ± 2.91 mg dl^{-1} and 37.75 ± 6.4 mg dl^{-1} throughout the experiment.

Serum cholesterol. In both primary and secondary *Trypanosoma congolense* and *Trypanosoma brucei* infections (Fig. 5), the mean

cholesterol concentration was significantly lower than those of the controls ($P<0.05$). Values decreased rapidly in both infected groups after trypanosome challenge, dropping from 52.0 ± 5.74 mg dl^{-1} to 31.57 ± 3.42 mg dl^{-1} in *Trypanosoma congolense* and from 61.80 ± 5.52 mg dl^{-1} to and 35.8 ± 2.93 mg dl^{-1} in *Trypanosoma brucei*-infected goats respectively on day 16 after challenge. During the experiment, values of the control group remained higher than those of the infected goats and varied between 62.20 ± 3.97 mg dl^{-1} and 43.75 ± 8.84 mg dl^{-1} throughout the experiment.

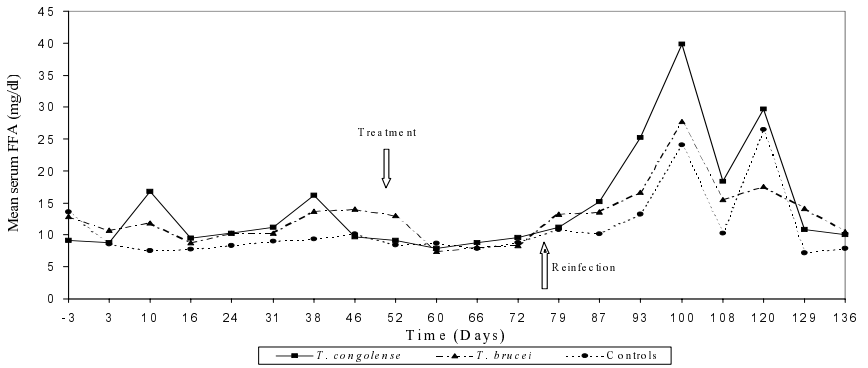
Fig. 5. Mean serum cholesterol concentration of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls



After treatment, serum cholesterol concentration in both infected groups increased gradually, reaching 53.30 ± 2.64 mg dl^{-1} in *Trypanosoma congolense* and 53.63 ± 3.74 mg dl^{-1} in *Trypanosoma brucei*-infected goats on day 79 (2 days post-re-infection). There was a more rapid decrease in *Trypanosoma congolense*-infected goats than in those infected with *Trypanosoma brucei*. The lowest concentrations were reached on day 108 in both infected groups, with values of 29.75 ± 4.17 mg dl^{-1} and 40.50 ± 5.79 mg dl^{-1} in *Trypanosoma congolense* and *Trypanosoma brucei*-infected goats, respectively.

Serum free fatty acids (FFA). In both primary and secondary *Trypanosoma congolense* and *Trypanosoma brucei* infections, FFA concentration were significantly higher than those of the control goats, and those of *Trypanosoma congolense*-infected goats were higher than those in goats infected with *Trypanosoma brucei*. Concentrations in *Trypanosoma congolense*-infected goats increased and varied from 9.1 ± 1.09 mg dl^{-1} pre-infection to 16.76 ± 0.68 mg dl^{-1} on day 10 post-infection. There was a decrease to 9.46 ± 0.85 mg dl^{-1}on day 16, after which it increased steadily to 16.2 ± 0.10 mg dl^{-1} on day 38. There was a slight reduction to 9.72 ± 1.30 mg dl^{-1} on day 46 post-infection.

Fig. 6. Mean serum Free Fatty Acids of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls



FFA in *Trypanosoma brucei*-infected goats decreased from 12.88 ± 0.05 mg dl^{-1} three days before infection to 8.84 ± 2.6 mg dl^{-1} on day 16. Thereafter, until day 46 FFA concentrations moderately increased.

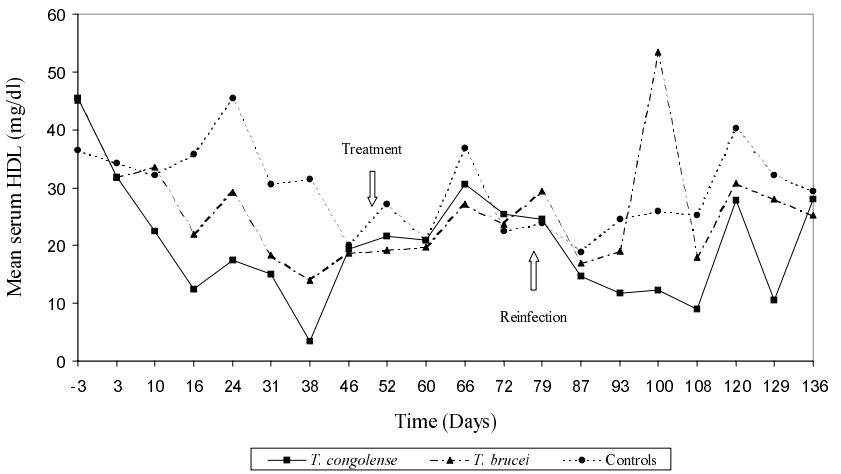
In the secondary trypanosome infection, FFA concentrations in both infected groups increased and varied between 11.2 ± 1.67 mg dl^{-1} and 39.82 ± 2.66 mg dl^{-1} in *Trypanosoma congolense*-infected goats. The corresponding values were between 13.29 ±2.36 mg dl^{-1} and 27.70 ± 3.30

mg dl^{-1} in the *Trypanosoma brucei*-infected group. In the same period, however, peaks of 24.14 ± 1.71 mg dl^{-1} and 26.50 were recorded in the control group.

Serum high density lipoproteins (HDL). Infection with *Trypanosoma congolense* and *Trypanosoma brucei* (Fig. 7) caused a significant reduction ($P<0.05$) in the primary challenge. There was no significant reduction in the secondary challenge in both groups. Immediately after challenge there were rapid reductions in HDL of *Trypanosoma congolense*-infected goats from 46.65 ± 2.62 mg dl^{-1} three days before challenge to 3.50 ± 0.43 mg dl^{-1} on day 38. After treatment, values increased to 24.50 ± 1.56 mg dl^{-1} for day 79 after which there was a rapid decrease to 9.0 ± 1.67 mg dl^{-1} on day 108. Another low value of 10.50 ± 2.02 mg dl^{-1} was recorded on day 129; this increased to 28.0 ± 7.0 ± mg dl^{-1}on day 136.

Trypanosoma brucei-infected goats showed a slight decline in serum HDL between pre-infection and day 10 and thereafter tended to show moderate decline to 14.0 ± 2.93 mg dl^{-1} on day 38. After treatment a slight increment was noted. After re-infection, values declined, although a peak

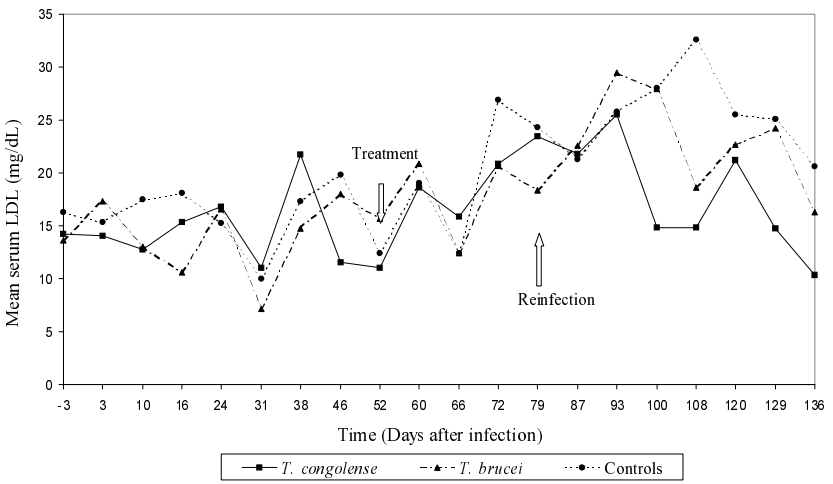
Fig. 7. Mean serum High Density Lipoproteins (HDL) of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls



value on day 100 of 53.50 ± 30.18 mg dl^{-1} thereafter, fluctuations in the values were recorded up to the end of the experiment.

Low density lipoproteins (LDL). The effect of *Trypanosoma congolense* and *Trypanosoma brucei* infection on LDL in goats is shown in Fig. 8. Concentrations of LDL in trypanosome-infected goats were lower than control values in both primary and secondary challenges. Only *Trypanosoma brucei*-infected goats showed significantly lower LDL concentrations in both primary and secondary challenges. Concentrations in the secondary challenge in both infected groups were higher than those in the primary challenge.

Fig. 8. Mean serum Low Density Lipoproteins (LDL) of goats exposed to both primary and secondary challenges with either *T. congolense* or *T. brucei* and the uninfected controls



Discussion

Trypanosome infection in the primary challenge was associated with decreases of total serum proteins in *Trypanosoma congolense*-infected goats, while increases were detected in *Trypanosoma brucei*-infected animals. In the secondary challenge, increases in total proteins in both

groups were observed. The observation in *Trypanosoma congolense*-infected goats concurs with that of KATUNGUKA-RWAKISHAYA (1996) in *Trypanosoma congolense*-infected Scottish Blackhead sheep. The increases observed, however, are in agreement with observations by ANOSA and ISOUN (1976). In this experiment, hyperproteinaemia was observed in sheep and goats infected with *Trypanosoma vivax*. Although only the albumin sub-fraction was measured in this experiment, the total protein increases could be due to increased demand of the sub-fractions involved in the immune response, like IgM for the control of the infection. The hypoproteinaemia observed could be a result of trypanosomal uptake of albumin-bound fatty acids and lipoproteins, or increased catabolism by the host. It may therefore be concluded that the high parasitaemia at the beginning of the experiment might have led to the increased utilization of albumin. Since this experiment showed an increase in plasma volume, this change, together with increased utilization, could have led to a drop in the levels of albumin.

Abnormalities of lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosomes. When rabbits were infected with various species of trypanosomes (ROUZER and CERAMI, 1980), an increase in plasma cholesterol and total lipids occurred. In contrast, *Trypanosoma rhodesiense* infection in cattle (WELLDE et al., 1989) and *Trypanosoma congolense* infection in sheep (KATUNGUKA-RWAKISHAYA et al., 1992) were associated with a drop in plasma cholesterol concentration. This finding is in agreement with observations in this experiment, where there was a rapid decrease in the days after infection in the primary trypanosome challenge in both infection groups. Another low level was noted on day 129.

Since trypanosomes have been reported to require cholesterol for membrane synthesis and growth (BLACK and VANDERWEED, 1989), and since blood-stream form organisms are known not to synthesize their own cholesterol for their growth, they must obtain it from the host. This is why reductions in cholesterol were observed in *Trypanosoma congolense* and *Trypanosoma brucei*-infected goats. The increase reported in rabbits could be from possible immobilization of the adipose tissue.

Results from the present experiment show significant reductions in HDL and LDL in both *Trypanosoma congolense* and *Trypanosoma brucei*

infected-goats. This finding is in agreement with earlier investigations (BLACK and VANDERWEED, 1989) which have shown that serum lipoprotein requirements of *Trypanosoma brucei* IL3201 and IL3202 were dependent on serum LDL and HDL to multiply under axenic culture conditions. Thus, trypanosomes require HDL and LDL for their own growth. This report concurs with the findings of GILLET and OWEN (1987) who observed that when *Trypanosoma brucei* was incubated with 400μg of LDL, per ml, the protein was degraded by the parasite after a time lag of about 30 minutes. The degradation of LDL was associated with accelerated esterification of cholesterol by the parasite, suggesting that cholesterol taken up with LDL is made available to *Trypanosoma brucei* for metabolism.

Free fatty acids (FFA), both saturated and unsaturated, are generated by autolising trypanosomes (ASSOKU et al., 1977). Mostly, stearic, linoleic, palmitic and oleic acids are generated. Similar observations were made in the present experiment: serum FFA of *Trypanosoma congolense* and *Trypanosoma brucei*-infected goats in both primary and secondary challenges were higher than those of the controls. These FFA are potentially cytotoxic and haemolytic *in vitro*. Their increase in infected animals may confirm their cytotoxic properties of erythrocytes in goats and hence their contribution to anaemia development.

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BIRYOMUMAISHO, S., E. KATUNGUKA-RWAKISHAYA, C. M. RUBAIRE-AKIIKI: Biokemijske promjene u serumu pokusno invadiranih malih istočnoafričkih koza protozoima *Trypanosoma congolense* i *Trypanosoma brucei.* Vet. arhiv 73, 167-180, 2003.

SAŽETAK

Istražene su promjene u vrijednostima serumskih biokemijskih odrednica koza invadiranih protozoima *Trypanosoma congolense* ili *Trypanosoma brucei* u odnosu na kontrolnu skupinu. Koze su bile invadirane tripanosomama nultog dana te liječene 49. dana diminazenovim aceturatom. Reinvazije su uslijedile 77. dana i 136. dana pokusa. U invadiranih koza zabilježena je anemija, hipoproteinemija, hipoalbuminemija, hipolipidemija i hipokolesteremija. Nakon prve i druge invazije u invadiranih koza zabilježene su i više vrijednosti serumskih slobodnih masnih kiselina u odnosu na kontrolu. Dobivene promjene ukazuju da tripanosome za proliferaciju trebaju lipide i proteine. Visoka koncentracija slobodnih masnih kiselina može se dovesti u vezu s razvojem anemije s obzirom da je dobro poznat njihov citotoksični i citolitički učinak *in vitro*.

Ključne riječi: koze, serumski proteini, lipidi, tripanosomoza