

Effect of *Trypanosoma congolense* Infection on Pyruvate Concentration in Serum of Yankassa Sheep

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Abstract: The study involved the evaluation of the effect of *T. congolense* infection on pyruvate concentration in the serum of Yankassa sheep. Three groups of six sheep each were used; groups A and B were experimentally infected with *T. congolense*. Animals in group A were treated with Diminazene aceturate after first peak of parasitaemia but group B animals were left untreated. Sera of infected and uninfected control sheep were analyzed using DNS method to determine pyruvate and its concentrations. Group B animals which were not treated had mean values which were lower compared to that of group A (post treatment) and the control. Mean values of 72.4-25.7 g L⁻¹, 79.6-5.2 g L⁻¹ for groups A and B respectively were significantly different (p<0.0001) and indicative that the presence of parasites might have enhanced depletion of the pyruvate in the plasma. However, the cause of pyruvate depletion which occurred when the trypanosome parasites were in general circulation needs further investigations.

Key words: Sheep, pyruvate, depletion, dns, *t. congolense*

INTRODUCTION

African trypanosomes are protozoan parasites that cause the disease trypanosomosis which is potentially fatal in domestic livestock. They have a life cycle that alternates between the vector and definitive hosts. In the blood stream forms anaemia is a clinical feature in the affected animals; as well as also being the main pathological feature in livestock infected with *T. congolense* (Nok and Balogun, 2003).

Trypanosomes in the blood stream; in a bid to survive use host's abundant glucose to produce their required energy in an inefficient form of glycolysis, which result in two molecules of pyruvate as end product from one molecule of glucose (Hunt, 2004).

The host gets its required energy through glycolysis, where the reduction of NAD⁺ to NADH is rapidly and easily done to keep the TCA cycle going and pyruvate is important and necessary for the continuation of the cycle for the production of energy (ATP) and subsequent survival of the animal (Hunt, 2004)

Pyruvate which is an intermediate product of carbohydrate, protein, and Lipids (triacyl glycerides) metabolism and fermentations, is also an end product of glycolysis by trypanosomes (Anonymous, 2006). A trypanosome has a large capacity to metabolize glucose, consuming the equivalent of its own dry weight in an

hour. It lacks carbohydrate stores and oxidative phosphorylation, and therefore is dependent on the continuous supply present in the blood and body fluids of host (Anonymous, 1996). Trypanosome uses one molecule of glucose to produce two molecules of pyruvate which the parasite cannot further utilize (Nyindo, 1992; Hunt, 2004) but remains in the blood circulation of the host. What happens to the large amount of pyruvate and how is it depleted may be investigated further.

This study was carried out to relate that the presence of *T. congolense* enhances the depletion of pyruvate in the plasma of infected animal host.

MATERIALS AND METHODS

Eighteen Yankassa breed of sheep were used in this study. The animals were divided into three groups (A, B and C) of six each. Animals in groups A and B were infected with 10⁵ *T. congolense* parasites through the jugular vein and monitored daily for parasitaemia. Group C animals served as the uninfected control.

Group A animals were treated with diminazene aceturate (Berenil®) at dose of 3.5 mg kg⁻¹ on day 23 post infection (pi) when the first peak of parasitaemia was detected while group B animals were left untreated up to the end of the study.

Sampling

Blood: Eight mls of blood was taken daily from each animal with 2 mLs placed in clean sterile sample bottles containing EDTA as anticoagulant and the remaining 6 mls in a clean sterile sample bottles without anticoagulant for serum production.

Monitoring of parasitaemia using the 2 mL of blood was done through wet and thin smears and Haematocrit Centrifuge Technique (HCT) as described by Lumsden *et al.*, (1973) and Paris *et al.*, (1982).

Serum: The 6 mL of blood sample collected from each animal was allowed to stand at room temperature sufficiently enough for serum separation. Samples were centrifuged at 2000 g for 15 min and the resultant serum harvested into a clean serum vial and stored at -20°C until analysed.

Analyses: The method of Dinitrosalicylic acid (DNS) (Miller, 1959) assay was used for the determination of the concentration of pyruvate. A mixture of 3 mL of serum and 1 mL of reagent (DNS) was incubated by boiling for five minutes and then allowed to cool at room temperature. Concentration of pyruvate was measured using the Jenway colorimeter (Jenway 6051 Colorimeter, UK Essex) at 540 nm.

RESULTS AND DISCUSSION

In group A, pre-infection mean concentration of pyruvate was 73.26±1.2 g L⁻¹ while the concentration at week-1 was 72.4±0.84 g L⁻¹ which dropped to 33.9±2.5 g L⁻¹ in week 2 post infection. The concentration further decreased to 25.7±1.9 g L⁻¹ in week 4 (day 23) which coincided with the peak of parasitaemia. On the same day this group of animals was treated with Berenil® (Hoechst, Germany) at 3.5 mg kg⁻¹ (intramuscularly) thereafter the concentration increased to 34.3±2.1 g L⁻¹ in week 11, by then parasites were cleared.

In group B, the mean concentration of 79.6±1.1 g L⁻¹ at week-1 decreased to 42.1±2.0 g L⁻¹ in week 2 and continued to fall to 5.2±2.3 g L⁻¹ by week 11 post infection (Fig. 1).

In the uninfected control Group C; the mean concentration of pyruvate remained relatively steady, between 53.7±0.9 week-5 and 61±1.1 g L⁻¹ week 11.

Patent parasitaemia was detected as from day 8 but by day 13, all infected animals were positive for the parasites. The first peaks of parasitaemia in groups A and B were in week 4 (day 23) p.i. with mean values of 10^{6.4} for group A and 10^{5.9} for group B, respectively. The mean parasitaemia of group A dropped to zero after 48 h of

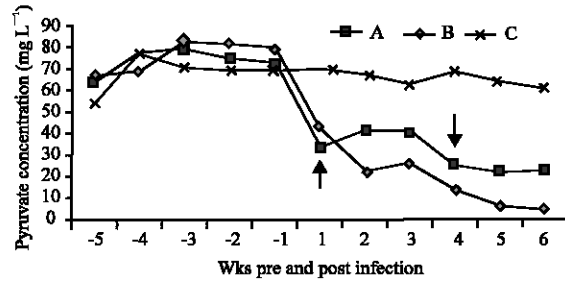


Fig. 1: Mean concentration of pyruvate in *T. congolense* infected and uninfected sheep

Note: A-group of animals that were infected and treated
 B-group of animals that were infected and not treated
 C-Uninfected control

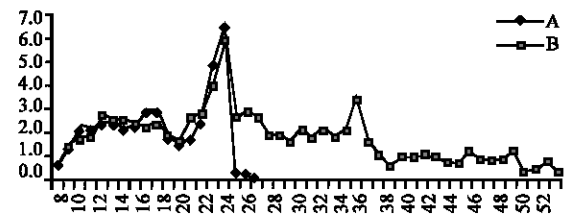


Fig. 2: Level of parasitaemia in *T. congolense* infected sheep

treatment but that of group B (untreated) continued to fluctuate until second peak in week 5 (day 35) with 10^{3.4} parasites. Thereafter, the mean level of parasitaemia continued to fluctuate gradually until it became 10^{0.4} in week 11 (Fig. 2).

The mean concentration of pyruvate of group A animals was found to be relatively constant (64.1-72.4 g L⁻¹) during the pre-infection period but depleted by 53.2% in first week post infection. The depletion continued until it was 25.7 g L⁻¹ (depleted by 64.5%) which coincided with peak parasitaemia. The depletion of pyruvate by 64.5% corroborates (Joshua *et al.*, 1986) where they reported that there was severe hypoglycaemia (25 g L⁻¹) in infected sheep with *T. simiae* compared to 65 g L⁻¹ for the control animals.

After treatment, mean pyruvate level in group A continued to deplete (from 25.7 to 22.4 g L⁻¹). This may be related to earlier observation made by Kalu *et al.* (1988) that large numbers of trypanosome parasites were seen in blood samples of infected ruminants after treatment with Berenil®. This large number of parasites from the microcirculation which further depleted the concentration of pyruvate might be responsible for the depletion of pyruvate after treatment. When the parasites were cleared, the mean pyruvate level rose from 22.4 to 34.3 g L⁻¹.

When groups A and B were compared, it was obvious that the presence of parasites was required in the depletion of pyruvate as seen in group B while in group A, the pyruvate concentrations depletion continued only after treatment but the values increased when the parasites were cleared.

Although the level of parasitaemia was low in group B, the continued presence of parasites depleted the concentration of pyruvate to 5.2 g L⁻¹ by week 11. Animals in this group became very weak, emaciated and with depraved appetite; observations confirming earlier report by Joshua *et al.* (1985) that hypoglycaemia contributes to weakness and death of animals infected with trypanosomes.

It is apparent from this study that the levels of pyruvate fluctuated along side the level of parasitaemia. Although the pyruvate is produced in large quantity by the parasites, it also means that the depletion of the pyruvate is dependant on the presence of these parasites in the blood stream.

CONCLUSION

The depletion of pyruvate concentrations in both groups A and B, depended on the presence of parasites. When group A animals were treated, there was increase in the concentration of pyruvate but in the untreated infected group, the depletion continued. The fate of pyruvate in the blood stream of the infected animal host needs to be further investigated.

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