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Full Length Research Article

Clinicopathological Parameters and Sialyltransferase Activity in *Trypanosoma Congolense*-Infected Sheep

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ABSTRACT

Cleavage of erythrocyte surface sialic acid (SA) by the sialidase produced by trypanosomes is implicated in the pathogenesis of anaemia in African animal trypanosomiasis (AAT). Sialyltransferase (ST) mediates the attachment of SA to cell surface glycoproteins and glycolipids of desialylated erythrocytes. ST activities in *Trypanosoma congolense*-infected sheep and control groups were investigated and variations in their physio-biochemical properties were evaluated. Six (6) apparently healthy Nigerian *Yankassa* breed of sheep comprising of *T. congolense*-infected (n=3) and non-infected (n=3) groups were used for the experiment. Parasitaemia and packed cell volume (PCV) were determined daily over a 5-week period. Enzyme kinetics of partially purified ST from the thyroid gland were also evaluated. Anaemia (mean PCV= 18.83±0.71%) was observed in the *T. congolense*-infected sheep when compared with the non-infected control group (mean PCV 39.75±0.35%) and the observed differences were significant ($p < 0.05$) after five weeks post infection. Variations were also observed in the physio-biochemical properties (pH, temperature, activation energy) of the ST isolated from the *T. congolense*-infected and control sheep. This finding is believed to have been an induced response by the host to parasite's activity and could be exploited further as a possible target in the control of AAT.

Keywords: Anaemia, Sialic acid, Sialyltransferase, Sheep, Thyroid gland, *Trypanosoma congolense*

INTRODUCTION

African animal trypanosomosis (AAT) is a very complex and debilitating vector-borne disease of domestic and wild animals in sub-Saharan Africa (CFSPH, 2018). The disease is a major challenge to food security and public health and is widely known as sleeping sickness and 'nagana' in man and animals, respectively (Sulaiman and Adeyemi, 2010; WHO, 2021). In Nigeria, the most important trypanosome species are *Trypanosoma brucei, T. congolense, T. vivax*, and *T. evansi* in livestock, although *T. brucei gambiense* infect human beings (Abubakar *et al*., 2010). Tsetse flies are the most important vectors of transmission in AAT, although some *Trypanosoma* species have been reported to be mechanically transmitted by other biting flies (WHO, 2021). Trypanosomal sialidase (SD) has been implicated in the pathogenesis of anaemia in AAT (Esievo, 1979). The enzyme is responsible for the cleavage of sialic acid (SA) from a wide range of sialic acid-containing cells including erythrocytes (Esievo, 1979; Pereira, 1983). Sialyltransferase (ST) is known to be involved in re-sialylation of erythrocyte surface in the course of trypanosomiasis; hence, playing important role in the recovery phase of anaemia in AAT (Esievo *et al*., 1986). Sialyltransferase activities have been reported in various tissues including liver and mammary glands (Alhadeff and Holzinger, 1979; Ellies *et al*., 2002; Paltrinier *et al*., 2012) and on cell surfaces (Porter and Bernacki, 1975; Wang *et al*., 2001). However, there is still paucity of information on the ST

activity in trypanosomes infected and non-infected animals. Thus, in this study we compared the physio-biochemical properties of ST isolated from the thyroid glands of *T. congolense*-infected sheep and apparently healthy group.

MATERIALS AND METHODS

Experimental Animals and Sample Collection

Six (6) apparently healthy Nigerian Yankassa sheep aged between 1 and 2 years were used for the study. The animals were kept in a fly-proof pen to acclimatize for 2 months and carefully screened for internal and external parasites before the commencement of the study. These animals were later randomly grouped into *T. congolense*-infected and non-infected groups, each group containing 3 animals (n = 3). Experimental animals were fed with wheat offal, groundnut and cowpea hays and water was given *ad libitum*. *Trypanosoma congolense* (savannah strain) was obtained from the National Institute for Trypanosomiasis Research Vom, Nigeria. The *T. congolense*-infected group were inoculated via the jugular vein with 1×10^2 trypanosomes/mL. One (1) mL of blood was collected via the jugular vein on a daily basis for 30 days from *T. congolense*-infected and non-infected groups and transferred into vacutainers containing ethylene diamine tetra acetic acid (EDTA) for the detection and monitoring of parasitaemia, and determination of packed cell volume (PCV).

After 30 days post infection, 5 mL of whole blood was collected from the experimental animals into screw-capped test tubes without anticoagulant to obtain serum which was centrifuged at 5,000 rpm for 5 minutes and the supernatant transferred into serum vials and stored at -20 ºC until used for ST assay. The experimental procedure followed the ethical guidelines for animal care in research of the Ahmadu Bello University Zaria, Nigeria. Experimental animals were subsequently humanely sacrificed and selected organs harvested for sialyltransferase enzyme isolation

Partial Purification of Sialyltransferase

Sialyltransferase from the thyroid, salivary glands and liver were isolated using Triton X-100 from *T. congolense*-infected and non-infected groups as described by Sticher *et al*. (1991). Thyroid gland which had highest enzyme (ST) activity was subjected to further purification steps.

Ammonium Sulphate Precipitation

Crude enzyme extract from the thyroid gland of *T. congolense*-infected and non-infected groups were precipitated by addition of ammonium sulphate up to 70% (w/v) saturation. The precipitate recovered after centrifugation was reconstituted in 20 mL phosphate buffered saline and dialyzed over night against three changes of the same buffer at 4 ºC (Hidari *et al*., 2005).

Sialyltransferase Assay

Sialyltransferase activity was determined using the method described by Křen and Thiem, (1997) with some minor modifications such as the use of pestle and mortar, Triton X-100 and Dowex column 1 × 8, (3cm length). Each assay mixture contained the following components: buffer system, bovine serum albumin, asialofetuin (acceptor substrate), cytidine monophoshate neuraminic acid (CMP-Neu5Ac) (donor substrate) and enzyme solution. The control tubes had no acceptor substrate.

Ion Exchange Chromatography

This was carried out using DEAE-cellulose (DE-52) column (1.6 x 30 cm). Two columns were set for the *T. congolense*-infected and non-infected groups. The flow rate of the columns was adjusted to 0.75 mL/min and 5 mL fractions were collected in each tube. Tubes with highest enzyme activity were pooled.

Gel Filtration

This was carried out using Sephadex G-100. Two columns were similarly prepared as described for experimental groups for further enzyme fractions purification. Both columns had a flow rate of 0.4 mL/min. The eluates were collected in 5 mL fractions for sialyltransferase assay.

Optimum pH

The activity profile of the partially purified ST from harvested thyroid tissue of *T. congolense*-infected and non-infected groups in terms of pH of maximum activity was determined. The enzymes activities were determined as described at pH range 4 – 8 viz 5.0 mM sodium acetate buffer pH 4-4.5; phosphate buffer pH 6-7 and Tris-HCl buffer pH 7.5-8. The sialyltransferase enzyme activities versus pH was plotted to determine optimum pH.

Optimum Temperature

Equal volumes (25µl) of the enzymes and substrates were incubated at intervals of 5 ºC for 30 minutes, at 25°C, 30°C, 35°C, 40°C, 45°C and 60°C. The various tubes were cooled to room temperature and assayed for ST activities as described. A plot of ST activities against temperature was used to determine the optimum temperature.

Activation Energy

Activation energy (E_a) was deduced using the relationship $E_a/R = \ln K$, where R and ln K are the molar gas constant and rate constant for the chemical reaction, respectively. Arrhenius plot for E_a determination was obtained by plotting natural logarithm of the enzyme activity (ln K) against the reciprocal of temperature (1/T) in Kelvin (^oK) for the various temperature intervals.

Kinetic Studies

The substrate (asialofetuin) concentration was varied over the range of 1-10 mg/ml. The effect of substrate concentration on ST activities were carried out as outlined in the standard assay procedure. The kinetic constants, Km and V_{max} for both enzymes were determined from Lineweaver-Burk reciprocal plot.

Statistical Analysis

Data from the study was computed as mean ± standard deviation (SD) and analyzed using independent *T* test for comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Parasitaemia

Parasitaemia was observed 4-5 days' post infection in the *T. congolense*-infected group as shown in Figure 1. Peak parasitaemia was noticed on day 16, with a sharp decline on days 17 and 18, and thereafter fluctuated moderately for the rest of the experimental period. *T. congolense*-infected animals had lower mean PCV values when compared with the non-infected group (Figure 2). The lowest mean PCV value (18.3±2.1%, day 30 of the experiment) was recorded in the *T. congolense*-infected group, while the highest mean PCV value (48.5%, day 1 of the experiment) was recorded in the non-infected group. The mean weekly PCV values in the two experimental groups were statistically significant. ($P<0.05$) (Table 1).

Table 2 shows the summary of purification of sialyltransferase from the thyroid glands of *T. congolense*-infected and the non-infected groups. The most effective of the purification steps was the use of Sephadex G-100 chromatography. With this purification step, enzyme yield was 13.7% and 16.6% for the non-infected and *T. congolense*-infected groups, respectively. The specific activity was 756.3 U/mg and purification fold of 4528 for thyroid gland samples of non-infected group. Similarly, the enzyme specific activity for the infected group was 566.67 U/mg and purification fold of 1045.50. On SDS-PAGE, the enzyme migrated either as a 49 KDa or 56 KDa band for the *T. congolense* infected and non-infected groups, respectively (Plate1).

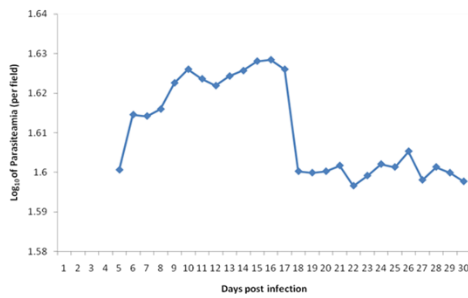

Figure1: Parasitaemia in *T. congolense* infected sheep 30 days' post infection

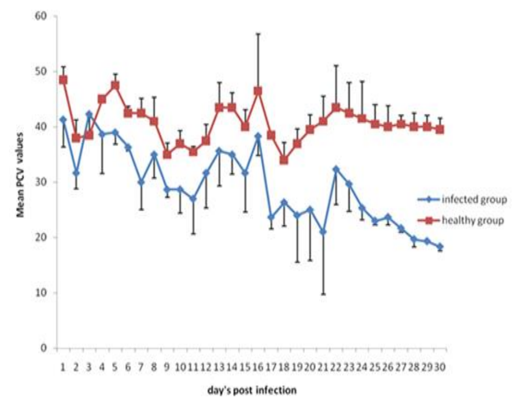

Figure 2: Daily mean PCV of *T. congolense*-infected and non-infected sheep

Table 1: Mean PCV of *T. congolense* infected and non-infected sheep.

	GROUPS	
Weeks post-infection	Infected	Non-infected
1	37.05±4.69$^{a(a)}$	43.21±4.08$^{a(b)}$
2	31.67±3.61$^{b(a)}$	39.00±3.63$^{a,b(b)}$
3	27.14±5.93$^{b,c(a)}$	39.50±3.85$^{a,b(b)}$
4	25.05±5.49$^{c(a)}$	41.21±1.35$^{a,b(b)}$
5	18.83±0.71$^{d(a)}$	39.75±0.35$^{b(b)}$

The data are expressed as mean ± SD (n = 3). Values with different superscripts within a column (Post hoc analysis) and between columns (in bracket) are significant different ($p < 0.05$)

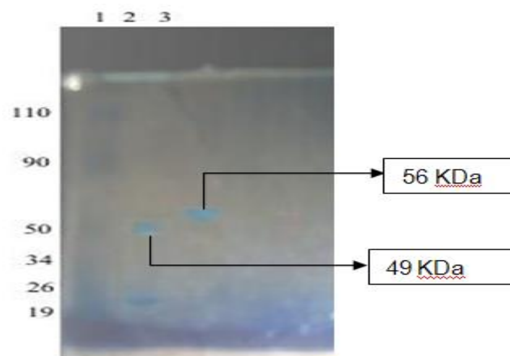

Plate1: SDS-PAGE to determine molecular weight of partially purified ST isolated from thyroid glands of sheep in the experimental groups.
Key: well 1 = Protein molecular weight marker; well 2 = partially purified ST isolated from *T. congolense* infected sheep; well 3 = partially purified ST from non-infected sheep.

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Table 2: Purification scheme of ST Enzyme isolated from thyroid tissue of *T. congolense*-infected and non-infected sheep

PURIFICATION STEPS	VOLUME (ML)		PROTEIN (MG)		ACTIVITY (U)		YIELD (%)		SPECIFIC ACTIVITY (U/MG)		PURIFICATION FOLD	
	IG	NG	IG	NG	IG	NG	IG	NG	IG	NG	IG	NG
Triton extraction	14.70	13.4	1700	11800	922	1966	100.0	100	0.542	0.167	1.0	1.0
$(NH_4)_2SO4$	6.80	4.47	218	1073	670	1311	72.2	66.7	3.073	1.22	5.7	7.3
DEAE-Cellulose	0.95	0.75	19	25.03	211	430	22.9	21.1	11.11	17.20	20.5	103.0
Sephadex G-100	0.14	0.24	0.26	0.357	153	270	16.6	13.7	566.67	756.3	1045.5	4528.0

Key: IG = Infected group; NG = Non-infected gro

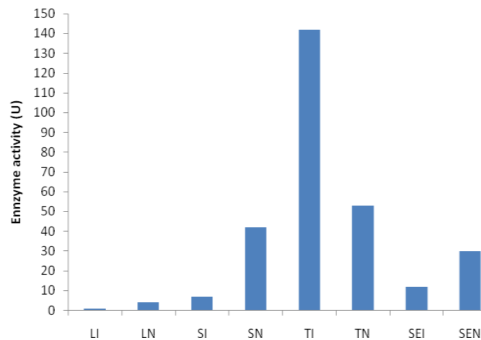


Figure 3: ST enzyme activities in crude extracts from selected organs and serum samples obtained from *T. congolense* infected and non-infected sheep.ST activity was obtained in duplicates.

Key: LI = Liver infected group; LN = Liver non-infected group; SI = Salivary gland infected group; SN = Salivary gland non-infected group; TI = Thyroid gland infected group; TN = Thyroid gland non-infected group; SEI = Serum infected group; SEN = Serum non-infected group.

Crude Enzyme Activity in the Thyroid and Salivary Glands, Liver and Serum

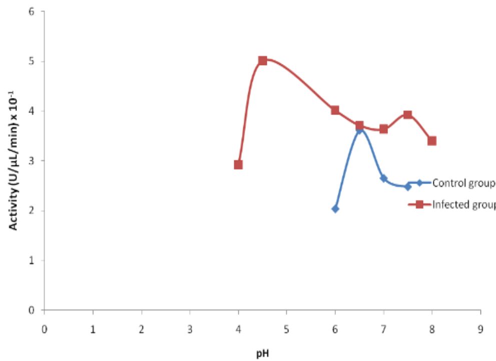
ST activities were detected in the thyroid and salivary glands, liver and serum (Figure 3). However, the thyroid gland of *T. congolense*-infected group had the highest ST activity of 142 U/ml.

pH and Optimum Temperature

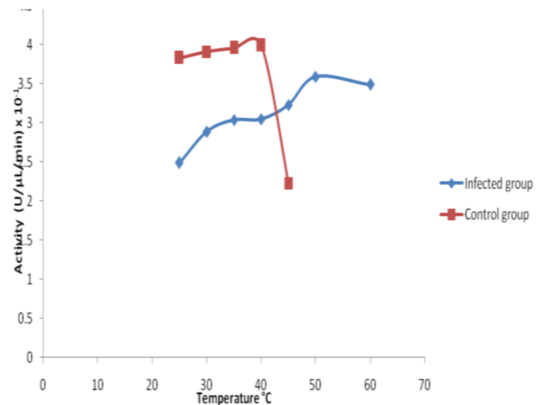
The optimal pH of ST isolated from thyroid gland of *T. congolense*-infected and non-infected groups were 4.5 and 6.5, respectively (Figure 4a). The optimum temperature of ST isolated from *T. congolense*-infected and non-infected groups were 51˚C and 39˚C, respectively (Figure 4b).

Activation Energy (E_a)

The activation energies of ST from *T. congolense*-infected and non-infected groups were 9.10 kJ/mol (Figure 5a) and 1.84 kJ/mol (Figure 5b), respectively.

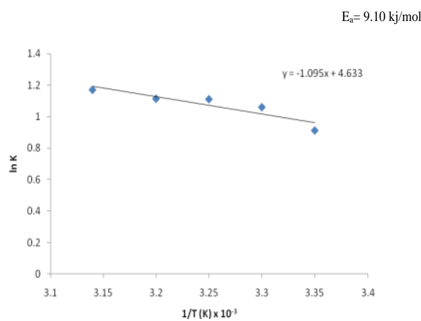


(a)

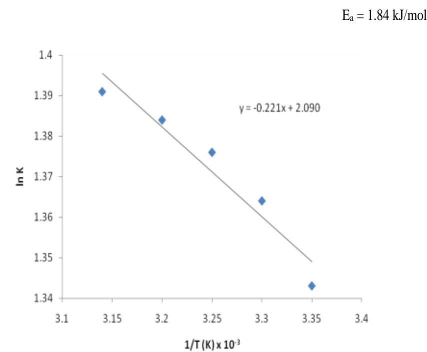


(b)

Figure 4: Optimum pH (a) and temperature (b) for ST catalytic stability isolated from thyroid gland of *T. congolense*-infected and non-infected sheep



(a) Infected

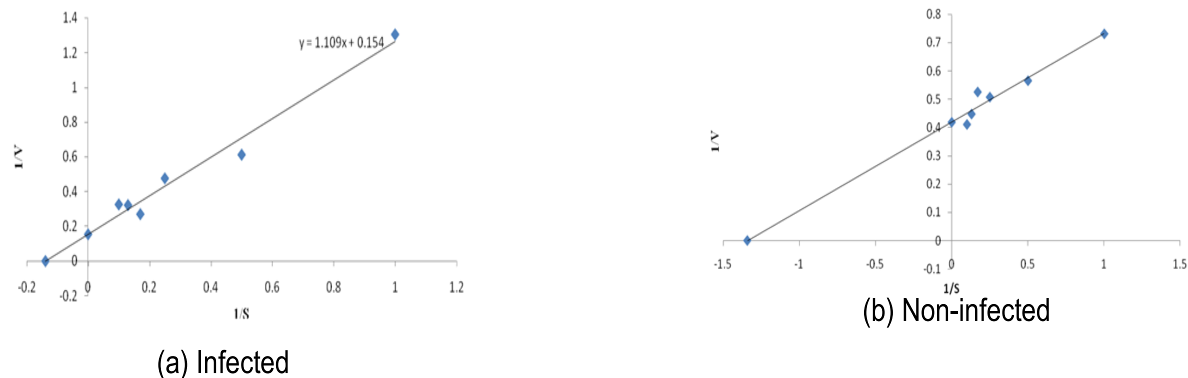


(b) Non-infected

Figure 5: Activation energy of partially purified sialyltransferase from thyroid gland of *T. congolense* infected group and non-infected sheep.

Kinetic Studies

The K_M and V_{max} values of the partially purified sialyltransferases from thyroid gland of *T. congolense*-infected and non-infected groups are presented in Figure 6a and 6b, respectively. For the *T. congolense*-infected group the K_M and V_{max} were 7.19 µM and 6.494 mM/min, respectively. While K_M and V_{max} of the non-infected group were 0.769 µM and 2.39 mM/min, respectively.



(b) Non-infected

(a) Infected

Figure 6: Line-Weaver Burk plot for K_M and V_{max} of ST isolated from thyroid gland of *T. congolense*-infected sheep and non-infected sheep.

DISCUSSION

The parasitaemia observed on day 5 post-infection with *T. congolense* in sheep was in agreement with the reports of Taylor and Authie (2004) and CSFPH (2018). In this study, there was continuous presence of trypanosomes at detectable levels until peak parasitaemia developed on day 16. The reduction in PCV was statistically significant ($p < 0.05$) between *T. congolense*-infected and non-infected sheep, suggesting haemolytic characteristics of *T. congolense*. Hence, in this study progressive anaemia followed increase in parasitaemia as reported earlier (Esievo *et al*., 1982; Sekoni *et al*., 1990; Nok and Balogun, 2003), where more erythrocytes were lysed in the acute phase of the infection. Anaemia has remained an important feature of clinical trypanosomosis (Luckins, 1992; Ogunsanmi and Taiwo, 2004) and also the cause of most tissue and organ degenerations (Ekanem and Yusuf, 2008; Akanji *et al*., 2009). The lowest PCV observed in *T. congolense*-infected sheep in the present study, could be due to the activity of SD produced by the trypanosomes (Esievo, 1979; Pereira, 1983) that cleaved erythrocytes surface SA (Esievo *et al*.,1982; Umar *et al*., 2003; Useh *et al*., 2006) from the non-reducing end of galactose residues (Angata and Varki, 2002).

Sialyltransferase activity of the thyroid gland of *T. congolense*-infected animals was much higher than observed in the other selected organs harvested from the experimental animals. The findings were in close association with the report of Joseph and Sherry, (2000) who found highest activities of some sialyltransferases in mucin rich submandibular gland. In the present study, ST in the thyroid gland was probably activated during trypanosome infection, while STs in the liver, salivary gland and serum were deactivated or destroyed during infection.

The influence of pH as observed in this study showed distinct differences in catalysis of sialic acid. Sialyltransferase isolated from the *T. congolense*-infected sheep showed optimum activity at pH as low as 4.5. It is possible the physiological response to low pH induced by the trypanosome infection through the cleavage of available SA for the parasite's metabolism is the increase synthesis of ST isoforms (enzyme yield 16.6%) from thyroid gland of *T. congolense*-infected sheep unlike ST isolated from thyroid gland of non-infected sheep (yield 13.7%) that showed activity around neutrality (pH 6.8-7.1). Sticher *et al*. (1988) reported that pH studies in enzymes generally are functions of the ionization effects of the various amino acids interacting to assume a tertiary globular structure of the enzyme protein. This in turn plays a role in defining the final ionic charges in the binding as well as active sites of the enzyme, a major requirement in enzyme catalysis. A sub-cellular build-up of sialic acid in trypanosome infection may cause pH changes which in turn affects the

catalytic stability of STs. Interestingly, The K_M and V_{max} as determined in this study suggests STs isolated from infected animals to be less active or dormant at low substrate (sialic acid) concentrations in non-disease state and more efficient during disease condition where there is high concentration of the substrate (accompanied by a low blood pH) resulting from activity of trypanosomal sialidase. In speculatory terms, the non-infected group may have no immediate subcellular requirement for enzymes that catalyze at higher K_M (0.769) and V_{max} (2.39) since the more sensitive STs may quite sufficiently play the role of sialic acid sialylation of terminal glycans of glycoconjugates (Kono *et al*., 1997; Joseph and Sherry, 2000). This finding is in agreement with various studies that have shown the K_M and V_{max} of sialyltransferases in non-diseased conditions fall within the range of 10^{-1} to 10^{-9}. The release of SA into lysosome of vertebrate cell is transported back into the cytosol allowing the SAs to be neutralized or degraded (Harduin-Lepers *et al*., 1995; Angata and Varki, 2002). SA could also be re-cycled to generate cytidine monophosphate-sialic acid (CMP-SA) by cytidine triphosphate activation pathway (Kean, 1991).

Increase in body temperature has been shown to be a trend in trypanosomosis (Taylor and Authie, 2004), a physiological state that further stabilizes sialyltransferase isoforms as observed in *T. congolense*-infected animals which were most active at temperatures between 49 ºC – 51 ºC *in vitro*. It is of interest to note that the activation energies as calculated for the enzymes suggest sialyltransferase isolated from *T. congolense*-infected group had higher E_a (9.10 kJ/mol) which probably explains the corresponding high Km which is a function of substrate concentration necessary for enzyme activation. The ST in the non-infected group requires less E_a (1.84 kJ/mol) and thus favored under normal conditions. The resialylation phenomenon mediated by predominance of ST isoforms stable at low pH and high SA concentrations probably explains the increase in survival rates of some trypano-susceptible breeds of animals that survive anaemia in trypanosomiasis (Esievo *et al*., 1986).

Molecular weights of the enzymes isolated from the thyroid gland of *T. congolense*-infected and non-infected groups as estimated were 49 KDa and 56 KDa, respectively. This is in agreement with the findings of Sticher *et al*. (1991) and Takeshi *et al*. (2007). Sialyltransferases have been shown to exist as isoforms (Kitagawa *et al*., 1996; Taniguchi, *et al*., 2002), catalyzing similar reaction (Groux-Degroote *et al*., 2008), and possessing the ability to exist in oligomeric forms. Oligomeric enzymes form close associations that increase catalytic stability and efficiency. The enzyme isolated from thyroid gland of *T. congolense*-infected group may exist as dimer, requiring a subunit which may possibly increase the stability and efficiency of this enzyme in disease conditions.

CONCLUSION

In this study, sialyltransferase from thyroid gland of *Trypanosoma congolense*-infected and non-infected control sheep was isolated, partially purified and characterized. The enzymes from both groups showed marked variability in pH, temperature, activation energy, kinetic constants and molecular weights. In general, the high sialyltransferase activities observed in the thyroid gland of *T. congolense*-infected sheep coupled with changes in kinetic properties could be the result of a physiological response to parasite's activity in the challenged group. Based on these findings it could be speculated that sialyltransferase exist in different forms, i.e., in disease and normal physiological states. This difference is a powerful tool which could be investigated further for the control of AAT.

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