RESEARCH ARTICLE

EVALUATION OF HEMATOBIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN NATURAL BOVINE *TRYPANOSOMA BRUCEI* INFECTION

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ABSTRACT

This study aimed to evaluate the hematobiochemical and oxidative stress levels and some antioxidant enzyme activities in cattle naturally infected with T. *brucei* compared to the healthy controls. Blood and fecal samples were obtained from 120 cattle from selected farms and grouped based on infection status, Group 1 (n=32, T. *brucei* and helminthosis co-infection), Group 2 (n=41, T. *brucei* only), Group 3 (n=20, helminthosis only), and Group 4 (n=27, healthy control) to assess the association between the disease conditions and co-infection. Among the animals examined, 20 test samples and 6 healthy controls were selected from Group 2 and Group 4, respectively. Groups 1 and 3, as well as animals with a history of infection, inflammation, stress, and serum samples showing hemolysis, were excluded from the study. Hematological indices, serum biochemistry, oxidative stress biomarkers (MDA, SOD, CAT, GSH), and trace elements (Zn, Cu, Fe) were analyzed using standard assays. Infected cattle showed significant decreases ($p<0.05$) in PCV, Hb, RBCs, TLC, and platelets compared to controls, along with a significant increase ($p<0.05$) in eosinophils. Lymphocytes and monocytes also significantly decrease ($p<0.05$), while MCV, MCH and neutrophils significantly increase ($p<0.05$). Total protein, albumin, globulin, potassium and phosphorus decreased significantly ($p<0.05$). AST, ALT, ALP, GGT and creatinine also decreased significantly ($p<0.05$). MDA increased significantly ($p<0.05$), while SOD and GSH decreased significantly ($p<0.05$), indicating elevated oxidative stress. Zn also decreased significantly ($p<0.05$) in infected cattle. T. *brucei* infection caused significant hematological and biochemical alterations, disrupted antioxidant status, and decreased Zn, reflective of pathogenic effects. This study demonstrates T. *brucei* induces oxidative stress exceeding the antioxidant capacity, contributing to disease.

Keywords: Cattle, hematology, oxidative stress biomarkers, serum biochemistry, *Trypanosoma brucei*

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INTRODUCTION

African animal trypanosomosis is a blood protozoan disease caused by various trypanosome parasites (Muhanguzi et al., 2017). Among these, *Trypanosoma brucei* (T. *brucei*), is very important because it is responsible for sleeping sickness in humans and nagana in cattle (Possart et al., 2021) with devastating impacts in sub-Saharan Africa. The tissue and body organ activities of T.*brucei* can induce marked hematological, pathological, and serum biochemical derangements in different species (Ohaeri and Eluwa, 2011). The severity of these conditions can be influenced by the virulence of the infecting trypanosome, infective dose, and the host's immune status (Dagnachew et al., 2015).

Evaluating hematological values is crucial for assessing animal health, especially in cases of bovine trypanosomosis which commonly causes anemia, reflected by decreased packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) counts, along with severe leukopenia (Silva et al., 1999), with leukopenia characterised by neutropenia, eosinopenia and lymphopenia in cattle infected with T. *congolense* (Biryomumaisho et al., 2007). Tissue damage, indicated by changes in serum enzyme levels, has been reported in animal trypanosomosis. Significant increases in AST, ALP, and ALT levels have been reported in experimental infection with T. *brucei* (Orhue et al., 2005). Other reported biochemical alterations include hypoglycemia, and increased plasma bilirubin in T. *brucei* infected animals (Omotainse et al., 1994; Gow et al., 2007). Elevated serum urea levels in rats infected with T. *brucei* have also been reported (Egbe-Nwiyi et al., 2005).

Infection with trypanosome results in the increased production of reactive oxygen species (ROS) which act as cytotoxic agents (Paiva et al., 2018), and play a significant role in the pathophysiologic mechanism of trypanosomosis. Evaluating the role of oxidative stress parameters in trypanosomosis can illuminate the cellular damage inflicted by the parasite and the host's inflammatory response, with effective treatment and management strategies that can mitigate the impact of this debilitating condition.

Since oxidative stress plays a key role in tissue damage and disease severity (Abubakar and Nado, 2023), targeting this pathway rather than only eliminating the parasite could offer additional therapeutic benefits. By identifying key biomarkers in the oxidative stress response, improved therapeutic strategies through antioxidant interventions can aim at mitigating free radical damage and improving clinical outcomes.

Microminerals, including trace elements, mediate vital biochemical reactions by acting as cofactors for many enzymes, as well as acting as centers for stabilizing structures of enzymes and proteins (Cedeño et al., 2020). Emerging research has shown the importance of trace elements in the pathogenesis of regenerative mechanisms, the body's response to oxidative stress, and the maintenance of immunity against pathogens (Wada, 2004; Bonaventura et al., 2015). Microminerals such as zinc (Zn), copper (Cu), Iron (Fe), and selenium are integral parts of the antioxidant defence system. They are utilized to synthesize antioxidant enzymes that protect tissues from free radical-induced damage (Evans and Halliwell, 2001). Cu and Zn play an important catalytic role in the enzymatic activity of superoxide dismutase (SOD). Cu–Zn-SOD enzyme complex catalyzes the conversion of superoxide anion ($O2^{-}$) into hydrogen peroxide (H2O2) and water, thereby effectively neutralizing large amounts of oxidants (Das et al., 2022). Some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. Therefore, measuring Zn and Cu concentration in serum can indicate the micromineral status as well as represent their coordinated antioxidant roles accompanied by antioxidant enzyme activities. Assessment of serum iron levels can also be beneficial in clarifying the oxidant/antioxidant status (Cavdar et al., 2003).

The relationship between oxidative stress and changes in antioxidant enzymes is well established. However, its documentation in natural T. *brucei* infection in cattle remains uncertain, with limited local data. Hence, our study aimed to evaluate the hematobiochemical parameters, oxidative stress level, and some antioxidant enzyme activities in cattle naturally infected with T. *brucei*.

MATERIALS AND METHODS

Sample population and design

Blood and fecal samples were collected from 120 White Fulani cattle of both sexes and various ages from farms in Ibadan. The cattle were categorized into four groups: Group 1 (n=32; co-infection of T. *brucei* and helminthosis), Group 2 (n=41; T. *brucei* infection without helminthosis, test samples), Group 3 (n=20; helminthosis with no parasitemia), and Group 4 (n=27; absence of parasitemia and helminth parasites, healthy control). Among the animals examined, 20 test samples, and 6 healthy controls were selected from Groups 2 and 4, respectively. Groups 1 and 3, as well as animals witha history of infection, inflammation, stress, and serum samples showing hemolysis, were excluded from the study.

Two blood samples (5 ml each) were collected via the jugular vein from each animal into Sarstedt S-monovette tubes. The samples were divided into EDTA bottles (3 ml) for hematological analysis and plain bottles (7 ml) for serum chemistry. Clotted blood samples were centrifuged at 4000 revolutions per minute for 10 minutes. Clear serum was separated and stored at -20°C until needed.

Hematological assay

These utilized standard protocols. The packed cell volume (PCV) was determined through the microhematocrit centrifugation technique (Thrall and Weiser, 2002). The hemoglobin concentration (Hb) was measured using the cyanomethemoglobin colorimetric method (Higgins et al., 2008). Red blood cells (RBCs), white blood cells (WBCs) and differential WBC counts were performed using microscopic examination and Giemsa staining. The platelet count (PC) was determined following the Rees and Ecker counting technique, as described by Ihedioha and Agina (2014).

Serum biochemical analysis

Blood serum was analyzed to obtain energy, nitrogen, liver, kidney and mineral profiles using spectrophotometric methods and commercial kits Randox® assay kit (Randox Laboratories, Ltd., UK) and Teco® assay kit (Teco diagnostics, USA) for some of the parameters of the mineral profile (sodium, potassium, phosphorus and chlorides) following the manufacturer's instructions. The low-density lipoprotein (LDL) concentration was calculated using the Friedewald equation (Mukhtar et al., 2021). Total Protein (g/dL)/TP245; Albumin (g/dL)/AB362; Albumin/globulin ratio; AST (IU/L)/AS 101; ALT (IU/L)/AL 146; ALP (IU/L)/AP 542; GGT (IU/L)/GT 2750; Creatine kinase (u/L)/CK1296; BUN (mg/dL)/UR1068; Creatinine (mg/dL)/CR510; Glucose (mg/dL)/GL 364; Cholesterol (mg/dL)/CH 200; Triglyceride (mg/dL)/TR 210; HDL (mg/dL)/CH 203; Calcium (mmol/L)/CA590; LDH (u/L); Globulin (g/dL).

Determination of parasitemia

This involved a wet mount, stained thin blood smear and buffy coat as described by Cheesbrough, (1998). Hemoparasites were identified at the species level based on structural and morphometric criteria, as described by Gibson (2023).

Fecal examination

Fecal samples were screened for helminthsusing sedimentation, flotation, and modified McMaster techniques (Jeremiah and Banwo, 2018).

Serum analysis measured: superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), malondialdehyde (MDA) levels and the trace elements Zn, Cu, and Fe.

Determination of SOD activity

The activity of the SOD enzyme was assessed using the nitrobluetetrazolium (NBT) reduction assay, as described by Amar et al. (2019). This assay leverages SOD's ability to catalyze the dismutation of superoxide radicals generated by xanthine and xanthine oxidase, thereby preventing NBT reduction to blue formazan dye. The degree of NBT reduction, quantified spectrophotometrically at 560 nm, inversely correlates with SOD activity. One unit of SOD activity is defined as the amount causing a 50% inhibition of NBT reduction.

Determination of GSH activity

GSH levels in serum were evaluated with the adapted DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) method following Amar et al., (2019) with some modifications to suit cell-free serum. Proteins lacking sulfhydryl groups were precipitated, leaving only GSH to react with DTNB, forming a yellow product. The absorbance of this product at 412 nm was directly proportional to the GSH concentration, determined by a standard curve comparison.

Determination of catalase (CAT) activity

This was evaluated using a modified version of Claiborne's method as described by Amar et al. (2019). The reaction mix contained 30% hydrogen peroxide (H2O2), phosphate buffer, and serum samples. Catalase catalyzes H2O2 decomposition, decreasing its concentration over time. This change was monitored spectrophotometrically at 240 nm, as proposed by Molina and Guillen (2017). Absorbance readings at 30-second intervals tracked the rate of H2O2decomposition, proportional to catalase activity.

Determination of MDA level

Quantification of MDA, a marker of lipid peroxidation, is achieved using the thiobarbituric acid reactive substances (TBARS) assay, following a modified protocol byVarshney and Kale (1990). Proteins were precipitated with trichloroacetic acid (TCA), and MDA reacted with thiobarbituric (TBA) under acidic conditions, forming a pink MDA-TBA complex, measured optically at 532 nm. The intensity of color, determined spectrophotometrically, was directly proportional to the MDA concentration.The standard used in this modified TBARS assay is a precursor of MDA, 1,1,3,3-tetraethoxypropane (TEP), used to generate MDA standards with known concentrations, and standard curve from which the MDA levels in the serum samples are quantified. C = F x 6.41 x A (C- Concentration, F- Dilution factor, and A- Absorbance).

Trace element analysis

Serum was prepared through an acid digestion processusing nitric acid (69%), and hydrogen peroxide (33% w/v). Initially, 0.5ml of serum was combined with 1ml of concentrated nitric acid (HNO3) and 0.5ml of H2O2 in propylene tubes. This mix was heated at 60 °C for two hours to facilitate digestion. After digestion, the mix was diluted by adding 2.5ml of ultrapure water. The resulting solutions were centrifuged at 2000 rpm for five minutes. The quantification of trace elements such as Zn, Cu, and Fe in the supernatant was performed using an ICP-MS (Perkin Elmer Ultima 8000, USA) according to the protocols outlined by Luna et al. (2019). The ICP-MS calibration standard for these elements (Zn, Cu, and Fe) is 0.01-1mg/L. The analytical masses monitored included 63Cu, 66Zn, and 56Fe. Nitric acid and hydrogen peroxide were considered blank in the analytical method. To quantify the concentrations of elements, 1.2+25 (vs+vd) dilution protocol was used,vs-volume of the sample, and vd-volume of diluent water. The calibration curves were performed with multielement solutions from Specsol Brand Standards traceable by the National Institute of Standards and Technology (NIST) to ensure the quality and accuracy of the ICP-MS analysis.

Statistical analysis

All statistical analyses of the data were completed using IBM SPSS software package version 21

and GraphPad PRISM 10.2.1 (San Diego, CA, USA). Data were expressed as mean and standard deviation (SD). A confidence level of 95% and p-values< 0.05 were considered statistically significant. The distribution pattern of data was assessed by Kolmogorov-Smirnov test (p>0.05) to confirm normal distribution. Comparisons between means were made by using unpaired t-tests. The data were summarised in tables using descriptive statistics including frequencies and percentages. (F-test) was used to test associations between the four groups based on the presence or absence of Trypanosomosis and helminthosis.

RESULTS

Association between trypanosomosis and helminth-infected animals

Table 1 shows no significant association between cases of trypanosomosis and helminth. Out of the 120 cattle sampled, 41 were infected with only trypanosomosis, while 27 served as the healthy control, free from both disease conditions and any other infections that could act as confounding factors in the study.

Table 1 Trypanosomosis and helminth co-infection

Trypanosomosis and Helminth Co-infection?	Trypanosomosis Positive	Trypanosomosis Negative	Total (N)	(F. Exact Sig)	Odd Ratio	df
Helminth Positive	32 (61.5%)	20 (38.5%)	52 (43.3%)	P>0.99	1.05	1
Helminth Negative	41 (60.3%)	27 (39.7%)	68 (56.7%)			
Total	73	47	120 (100%)			

Hematological values in bovine trypanosomosis

There wasa significant decrease in the mean PCV, RBC, and hemoglobin, and a non-significant difference in MCV, MCH and MCHC (Table 2a) between the infected group and the healthy control. There is a statistically significant decrease in total leukocyte count and a significant increase in eosinophils (Table 2b). Also, there is increased neutrophil, though not significant, showing variable differences in lymphocyte, monocyte and platelet counts.

Table 2a Erythrogram values (mean ± SD) in bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
PCV (%)	30.21±1.36[a]	19.8±1.36[b]
Hb (g/dL)	26.2±1.83[a]	6.26±0.48[b]
RBC (x 106/µL)	4.82±0.22[a]	2.92±0.29[b]
MCV (fL)	62.73±0.73	69.03±4.50
MCH (pg)	20.16±0.22	21.77±1.38
MCHC (g/dL)	32.17±0.23	31.55±0.32

SD = standard deviation, MCV= mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, MCH= Mean corpuscular hemoglobin. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Table 2b Leukocyte values (mean±SD) in bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
TLC (x103µL)	4.16±1.89[a]	2.72±1.72[b]
Lymph (%)	60.90±1.18	60.1±3.12
Neut (%)	33.2±1.23	33.3±3.17
Eos (%)	2.4±0.4[a]	4.28±0.24[b]
Mono (%)	3.67±0.29	2.46±0.55
Platelets (µL)	98.28±2.46	84.6±2.61

TLC= Total leukocyte count, lymph= lymphocyte, neut= neutrophil, mono= monocyte, and eos= eosinophil. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Influence of trypanosomosis on serum biochemical indices of Cattle

There is a significant decrease in the mean total protein, albumin, globulin, potassium and phosphorus, with a significant decrease in AST, ALT, ALP, GGT and creatinine (Table 3). The Table also reflected a non-significant decrease in BUN, glucose, cholesterol, triglyceride, HDL, LDH, sodium, chloride and calcium, with a non-significant increase in albumin/globulin ratio and creatinine kinase.

Table 3 Influence of trypanosomosis on serum biochemical indices of cattle

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
Total Protein (g/dL)	7.8 ± 2.34[a]	3.7 ± 1.35[b]
Albumin (g/dL)	3.5 ± 1.29[a]	1.5 ± 0.67[b]
Globulin (g/dL)	4.3 ± 2.33[a]	2.2 ± 1.12[b]
Albumin/globulin ratio	0.81	0.68
AST (IU/L)	128 ± 10.31[a]	297 ± 12.43[b]
ALT (IU/L)	8 ± 1.43[a]	19 ± 2.13[b]
ALP (IU/L)	108 ± 4.52[a]	197 ± 6.21[b]
GGT (IU/L)	4 ± 1.56[a]	10 ± 2.78[b]
Creatine kinase (u/L)	59 ± 4.12	61 ± 3.29
BUN (mg/dL)	15.7 ± 3.54	14.5 ± 3.65
Creatinine (mg/dL)	0.4 ± 0.41[a]	0.7 ± 0.11[b]
Glucose (mg/dL)	62 ± 6.82	50 ± 11.13
Cholesterol (mg/dL)	50 ± 14.51	42 ± 9.27
Triglyceride (mg/dL)	11 ± 2.41	8.2 ± 2.13
HDL (mg/dL)	27 ± 11.45	26 ± 6.52
LDH (u/L)	116 ± 13.47	100 ± 13.38
Sodium (mmol/L)	124± 8.45	117 ± 6.38
Potassium (mmol/L)	2.9 ± 1.17[a]	1.6 ± 1.12[b]

Chloride (mmol/L)	100 ± 2.31	94 ± 4.38
Phosphorus (mmol/L)	1.9 ± 0.65[a]	1.0 ± 0.26[b]
Calcium (mmol/L)	9.5 ± 3.63	8.7 ± 4.98

AST= aspartate transaminase, ALT= alanine transaminase, ALP= alkaline phosphatase, GGT= gamma-glutamyltranspeptidase, BUN= blood urea nitrogen, HDL= high-density lipoprotein, LDH= lactate dehydrogenase, LDL= low-density lipoprotein. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Oxidative stress parameters in the two groups

Comparative evaluation of oxidative stress parameters (Mean ± SD) between healthy control and bovine trypanosomosis shows a significant increase in MDA, a significant decrease in SOD, and GSH, and Zn with a non-significant decrease in catalase, Fe, Cu (Table 4).

Table 4 Comparative evaluation of oxidative stress parameters (Mean ± SD) between healthy control and bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
SOD (u/ml)	50.26±0.0705[a]	30.43±0.1603[b]
MDA (nmol/ml)	29.83±3.357[a]	41.54±0.2287[b]
GSH (umol/l)	38.04±1.478[a]	29.17±0.5967[b]
Catalase (u/ml)	188.0±2.169	177.70±6.602
Fe (mg/l)	0.71±0.00278	0.64±0.048
Cu (mg/l)	0.45±0.0645	0.37±0.047
Zn (mg/l)	0.70±0.5354[a]	0.25±0.06455[b]

Malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH). The means bearing different superscripts (a,b) differ significantly (P<0.05) between the groups.

DISCUSSION AND CONCLUSION

Serum hemato-biochemical changes are characteristics of trypanosome infection. The characteristic changes are often determined by the pathophysiological effect of the disease on different body organs, the strain of the infecting trypanosome and the host (Awekew et al., 2017). In the present study, we reported a decrease in Hb and PCV, which may result from direct physical damage to red blood cells due to parasite whipping movement and hemolytic activity of the hemolysins that directly rupture red blood cells, causing hemoglobin release into the bloodstream. Furthermore, the decreased mean PCV, RBC and TLC concentration in trypanosomosis-affected group agrees with the work of Sivajothi et al. (2014) and several other findings (Sulaiman and Adeyemi, 2010; Hussain et al., 2016).

As observed by Pays et al. (2006), the significant decrease in TLC in cattle infected with T.*brucei* may be due to the immunosuppressive action of trypanosomes as well as the exhaustion of the immune system, leading to a lower overall output of leukocytes. Besides this, leucopenia is always seen at the terminal stage of the infection (Sivajothi et al., 2015). The eosinophilia in infected cattle agrees with the previous report of Sivajothi et al. (2014) in *T. evansi*- infected cattle, as a feature of parasitic infections which may be associated with immediate-type hypersensitivity reactions.

In the present study, we reported a significant decrease in serum albumin, total protein and globulin compared to that of the healthy control. This agrees with the works of Megahed et al., 2012 and Dagnachew et al., 2014 that hypoalbuminemia in trypanosomosis may be due to increased hepatocellular damage arising in trypanosomosis infection.

The elevated levels of AST and ALT in the infected cattle are in line with the findings of several other studies (Yusuf et al, 2012; Hussain et al., 2016) as with increased ALP in both clinical and sub-clinical trypanosome infections (Takeet and Fagbemi, 2009; Adeyemi and Sulaiman, 2012) and GGT. This suggests a probable invasion of the vital body organs like the liver, heart and brain. The increased levels of creatinine in this study could be associated with renal malfunction, as reported by Abenga and Anosa (2005).

The significant decrease in potassium levels may be due to the rapid replication and metabolism of the parasites, which may interfere with cellular uptake and homeostasis of this electrolyte. This is in line with previous reports of reduction in the level of potassium in an experimental infection with *T. brucei* and *T. evansi* (Ogunsanmi et al., 1994; Da Silva et al., 2011).This can equally be associated with inappetence in clinical infection. Some of the clinical implications of hypokalemia and hypophosphatemia are muscle weakness and lethargy exacerbating disease severity. Awekew et al. (2017) reported trypanosomes require lipoproteins for them to multiply under axenic culture. Thus, the lowering of the serum lipids and cholesterol, as observed in the present and previous studies (Biryomumaisho et al., 2003; Adamu et al., 2008) could, partly, be the result of trypanosomal utilization of the molecules.

The non-significant decrease in serum glucose in this study might be due to excessive utilization of blood glucose by trypanosomes for their metabolism through their glycolytic pathway, as reported by Kadima et al. (2000), where serum glucose levels were significantly low on Days 3, 4, and 5 post infections of cattle with T. vivax, corresponding with the first parasitemia build up.

In this study, bovine trypanosomosis was associated with a significant increase in oxidative stress markers in infected cattle compared to the control. This is supported by several studies (Hussain et al., 2018; Eljalii et al., 2015), which reported a significant rise in MDA levels, a common marker of lipid peroxidation. SOD levels decreased significantly in the infected group compared to the control group, due to the impact of oxidative stress. This agrees with the findings of Saleh et al. (2009), where a decrease in serum SOD was observed in the *T. evansi*-infected camel compared to the healthy control. Ranjithkumar et al. (2011) also reported a decrease in horses

naturally infected with *T. evansi*. The drop in SOD activity may be due to its high demand as a free radical scavenger throughout the oxidative process in the naturally occurring *T. brucei* infection.

The catalase results in this study showed an insignificant lower mean catalase concentration in T. *bruce*-infected cattle compared to the healthy control. This is closely related to a study by Mishra et al. (2017) where trypanosomosis-infected cattle showed a significant decrease in erythrocytic catalase concentration compared to that of the healthy control. Wang et al. (1999) also reported a decline in serum catalase concentration in the trypanosome-infected Cape buffalo. The serum GSH from this study shows a significant decrease in the infected group compared to the healthy control. This result agrees with the findings of Abubakar and Dabo (2023) and Saleh et al. (2009) who reported a significant reduction in serum GSH for infected compared to the control group. The infection could be impairing the mechanisms responsible for GSH synthesis, limiting its availability.

The decrease in iron (Fe) level in this study is comparable with the work of Neils et al. (2007), which reported a significant decrease in serum iron in sheep infected with *T. congolense*. This decrease in iron could be linked to anemia, a common symptom of trypanosomosis, due to the importance of iron in red blood cell production. A significant decrease in Zn levels in infected cattle might be related to impaired immune function, as Zn is crucial for immune cell development and response against parasitic infections (Dardenne, 2002).

In conclusion, T. *brucei* infection caused significant hematological and biochemical alterations, disrupted antioxidant status, and decreased Zn, reflective of pathogenic effects. This study demonstrates the parasite induces oxidative stress exceeding antioxidant capacity, contributing to disease. Further research is warranted to better characterize oxidative alterations over time and their implications on pathogenesis.

CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conceptualization, writing – review and editing – OGB; Data collection and processing – DOP; Analysis and interpretation – JA; Materials and supervision – OTJ.

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EVALUACIJA HEMATOLOŠKIH, BIOHEMIJSKIH PARAMETARA I PARAMETARA OKSIDATIVNOG STRESA KOD PRIRODNE BOVINE TRYPANOSOMA BRUCEI INFEKCIJE

SAŽETAK

Cilj našeg istraživanja je evaluacija hematobiohemijskih parametara i razine oksidativnog stresa i aktivnosti određenih antioksidativnih enzima kod goveda prirodno inficiranih sa *T. brucei* u odnosu na zdrave kontrole. Uzeti su uzorci krvi i fecesa od 120 goveda sa selektiranih farmi i podijeljeni u grupe na osnovu statusa infekcije: Grupa 1 (n=32, koinfekcija *T. brucei* i helmintoza), Grupa 2 (n=41, samo *T. brucei*), Grupa 3 (n=20, samo helmintoza) i Grupa 4 (n=27, zdrave kontrole) kako bi procijenili vezu između stanja bolesti i koinfekcije. Od svih ispitanih životinja, selektirano je 20 testnih uzoraka i 6 zdravih koontrolnih jedinki iz Grupe 2 i Grupe 4. Grupe 1 i 3, kao i životinje sa anamnezom prethodne infekcije i inflamacije i one čiji su uzorci seruma pokazivali hemolizu su isključene iz istraživanja. Hematološki indeksi, biohemijska analiza, biomarkeri oksidativnog stresa (MDA, SOD, CAT, GSH) i elementi u tragovima (Zn, Cu, Fe) su analizirani korištenjem standardnih eseja. Inficirana goveda su pokazala signifikantan pad ($p<0.05$) PCV, Hb, RBC, TLC i trombocita u odnosu na kontrolne jedinke, kao i signifikantan porast ($p<0.05$) eozinofila. Limfociti i monociti su također pokazali signifikantan pad ($p<0.05$), dok su MCV, MCH i neutrofili signifikantno porasli ($p<0.05$). Ukupni protein, albumini, globulini, kalij i fosfor su signifikantno sniženi ($p<0.05$). AST, ALT, ALP, GGT i kreatinin su također signifikantno sniženi ($p<0.05$). MDA je signifikantno povišen ($p<0.05$), dok su SOD i GSH signifikantno sniženi ($p<0.05$) ukazujući na povišen oksidativni stress Zn je kod inficiranih goveda također signifikantno snižen ($p<0.05$). Infekcija sa *T. brucei* je uzrokovala signifikantne hematološke i biohemijske promjene, poremećen antioksidativni status i snižen Zn, kao posljedice patogenog učinka. Naše istraživanje je pokazalo da *T. brucei* inducira oksidativni stres koji prevazilazi antioksidativni kapacitet i doprinosi nastanku bolesti.

Ključne riječi: Goveda, hematologija, biohemijska analiza seruma, biomarkeri oksidativnog stresa, *Trypanosoma brucei*