

1 **Original Research Article**
2 **Biochemical changes induced by natural**
3 **infection of trypanosomosis in Burkinabese**
4 **local breeds of small ruminants**

5
6 **ABSTRACT**
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Aims: This study aimed **establishing** standard values of some biochemical parameters in burkinabese small ruminants' breeds and their changes due to the infection of trypanosomes. This work **helps determine** the prevalence of trypanosomosis in small ruminants in the study area.

Material and methods: A total of 317 small ruminants (173 sheep and 144 goats) were sampled for cross sectional survey of trypanosomosis from 10 villages of the agro pastoral zone of Sideradougou in the southern of Burkina Faso in July 2010. The parasitological analysis was carried out by microscopy according the Buffy Coat Technique (BCT). The biochemical parameters were analyzed using commercial kits (BIOSYSTEMS® SA).

Results: The overall parasitological prevalence was 5.99%. Infections were due to *T. congolense* and *T. vivax* with predominance of the latter (68.42% of cases). The values of biochemical parameters found in sheep and goats were within the ranges of physiological values. However, these values varied with age and trypanosomosis infection status. In young animals for both species, cholesterol and magnesium values were significantly lower than the adult subjects. Various biochemical parameters related to nutritional status were lower in young and trypanosome infected animals than in adults and healthy animals, respectively. The values of total proteins were significantly higher in the infected goats than healthy ones ($p=0.001$), while the contrary was observed in sheep. The gamma globulin values were significantly increased in the trypanosome infected subjects ($p<0.001$ in goats).

Conclusion: Trypanosomosis infection induced changes in the values of some biochemical parameters in small ruminants. The results of this study could help clinicians to improve the diagnosis of diseases in local small ruminants' breeds by good interpretation of biochemical analyses.

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9 **Keywords:** Biochemical parameters, Trypanosomosis, Goats, Sheep, Burkina Faso

1. INTRODUCTION

Small ruminants are very important in the economy of Burkina Faso. The country hosts nearly 20 million small ruminants^[1]. Almost all the families in rural area in Burkina Faso possess some small ruminants which represent the main investment occupation for the rural farmer and serves as cash flow in case of urgent need. More than one million small ruminants are exported annually from Burkina Faso to the costal countries in West Africa; mainly to Côte d'Ivoire and Ghana^[1]. Despite the huge socio economic importance of small ruminants, husbandry practices are still traditionally managed with little access to veterinary care and good management support in terms of housing and nutrition. The traditional livestock keeping is hampered by diseases and feed availability, the most important of these diseases being trypanosomoses^[2].

Animal trypanosomosis hinders livestock development in tsetse infested areas of Burkina Faso. Indeed, one third of the country, the sub humid area is infested by tsetse flies and therefore livestock reared in this zone are at risk of trypanosomosis infection. The annual economic losses due to trypanosomosis in Burkina Faso are under estimated. It has been established that 2.6 million doses of trypanocidal drugs are used yearly to treat or to prevent the disease in susceptible livestock^[1]. Amongst ruminants, cattle are most susceptible to trypanosomosis followed by sheep and goats^[3].

In Burkina Faso, animal trypanosomosis is caused by *Trypanosoma vivax* and *T. congolense* transmitted cyclically by tsetse flies or *Glossina spp* and, to some extent, *T. evansi* and *T. vivax*, mechanically transmitted by various hematophagous insects (tabanids, stomoxines, hippoboscids, etc.). *Trypanosoma spp.* are protozoan parasites that live in blood stream and body fluids. During their development in the host, the parasites escape the host immune system by changing their surface antigenic coat, the variable surface glycoproteins (VSG). Throughout the trypanosome infection, the mammalian host physiology can be dramatically altered with changes in blood pH, hormones, metabolites and nutrient concentration^[4].

Although, the mechanism of pathogenicity of trypanosomosis is not well known, trypanosome infection induces biochemical changes in blood parameters^[5]. Previous research have shown that trypanosomosis induces changes in the concentration of biochemical constituents within the ill subjects^[6,7,8,9,10,11].

Most of these studies report infection in experimental conditions. The objectives of this study are to determine the prevalence of trypanosomosis infection in small ruminants in the agro-pastoral zone of Sideradougou in Burkina Faso; to establish the standard values of clinicopathologic variables for local goat and sheep breeds and finally, to determine the possible influence due to trypanosomosis infection.

2. MATERIAL AND METHODS

2.1. Study area

The study was carried out in July 2010 in the agro-pastoral zone of Sideradougou located between 10°40' - 11°10' N and 3°55' - 4°30' W, in the southern part of Burkina Faso. The study area is located south of Bobo-Dioulasso and covers a surface of 3500 km². The zone lies within the River Koba basin. The agro-pastoral zone of Sideradougou is a sub-humid with an annual rainfall of 1200 mm. The agro-pastoral zone was targeted to alleviate poverty through the improvement of livestock and crop production and the settlement of transhumant livestock keepers in order to improve local animal breeds by good management of natural pasture. The study area, like all other areas in the sub-humid zone of Burkina Faso, is tsetse and trypanosomosis infested^[12].

2.2. Sampling

For the purpose of this study, 10 villages were randomly selected from the entire list of villages in the agro-pastoral zone of Sideradougou. From these 10 villages a total of 317 small ruminants (173 sheep and 144 goats) were sampled in 29 herds.

Two blood samples were taken from each animal into plain glass tubes and EDTA coated glass tubes by the jugular vein puncture. Blood samples collected into the plain tubes, were allowed to clot and serum separated by centrifugation at 1500 g for 10 min and stored at -20°C for subsequent biochemical analyses. EDTA blood samples were used for PCV determination and trypanosome detection. For each animal, we determined the age based on specific dental features^[13]. Then animal were divided in two age groups (young < 2 years and adults).

Sixty-five Djallonke small ruminants breed were sampled for the biochemical parameters analysis, of which 20 sheep and 45 goats. The sample included 10 sheep and 9 goats infected with trypanosomosis. The average ages of the small ruminants were 2.45±1.47 years and 2.84±1.5 years for sheep and goats respectively. The trypanosome-infected subjects were detected by microscopy (BCT).

2.3. Parasitological analysis

The BCT was used for the detection of the trypanosomes as described by Murray *et al.*^[14] At least 40 fields were observed before declaring a slide as negative. The positive blood samples were further processed as blood smears for trypanosome species identification by Giemsa-stained thin blood smears examined under oil immersion objective lens (x100 magnification). All positive animals were treated with diminazene aceturate (Trypadim[®] MERIAL) at 3.5 mg/kg body weight.

2.4. Biochemical analysis

Biochemical analyses determined levels for metabolites (total serum proteins, albumin, cholesterol, creatinine and Urea), enzymes (aspartate transaminase (AST) and Alanine transaminase (ALT)), and ions (Calcium, Magnesium and Phosphorus). Biochemical analyses were carried out using commercial kits (BIOSYSTEMS[®] S.A., Barcelona, Spain). The colometric reactions were measured using spectrophotometer (BIOSYSTEMS[®] S.A., Barcelona, Spain).

Agarose gel electrophoresis was performed on each sample using Hydragel Protein K20 SEBIA[®] Electrophoresis kits (SEBIA, France), according to manufacturers' suggested procedures.

2.5. Statistical analysis

Data were computed on Excel[®] and analyzed using STATA SE 9.2[®] software. The prevalence of trypanosomosis was calculated for each species of small ruminants. The mean and standard deviation were calculated for each biochemical parameter. Student's t-test was used to compare values of biochemical parameters in the different groups of small ruminants (trypanosome-infected versus healthy small ruminants). Differences were considered to be statistically significant with values of $p < 0.05$.

3. RESULTS

3.1. Prevalence of small ruminants' trypanosomosis

Parasitological prevalence of trypanosomosis in small ruminants was 5.99%. In total 10 (5.78%) and 9 (6.25%) cases of trypanosomosis infections were found in sheep and goats respectively. Table 1 shows the details of trypanosome infection according to *Trypanosoma* species and small ruminant species. The infections were mainly due to *T. vivax* (13 cases) and 4 cases of *T. congolense* and 2 mixed infections (*T. vivax* + *T. congolense*).

Table 1. Prevalence of trypanosomosis in small ruminants in the Agro-pastoral zone of Sideradougou, Burkina Faso.

Trypanosoma species	Sheep (n=173)				Goats (n=144)			
	T.v	T.c	T.v + T.c	Total	T. v	T. c	T. v + T. c	Total
Number of positive cases	8	2	0	10	5	2	2	9
Prevalence (%)	4.62	1.16	-	5.78	3.47	1.38	1.38	6.25

T.v = *Trypanosoma vivax* ; T.c = *Trypanosoma congolense*

The mean PCV values were 30.70±7.5% and 30.75±7.2% in sheep and goats respectively. There was a significant difference between PCV values of trypanosome-infected and healthy small ruminants ($p < 0.001$). The PCV mean values in infected sheep and goats (25.7±4.7% in sheep and 23.62±4.9% in goats) were much lower than the values in healthy animals (35.7±6.5% in sheep and 32.29±6.7% in goats).

3.2. Biochemical parameters

Biochemical parameter values of the Burkinabese small ruminants' breeds were within the range of the standard values found in sheep and goats, elsewhere [22, 23, 27]. However, we observed some little difference in the value of few parameters. Table 2 summarizes the clinical biochemical parameters for the Burkinabese sheep and goat breeds.

Table 2. Biochemical parameters for Burkinabese' local breeds of small ruminants compared with the reference standards

Parameters	Sheep (n=20)		Goats (n=45)	
	Mean	Ref. val.	Mean	Ref. val.
Total proteins (g/l)	60.63±15.4	72.0±5.2 [†]	72.83±11.1	69.0±4.8 [†]
Albumin (g/l)	28.91±8.1	24.0–30.0 [†]	32.94±4.9	27.0–39.0 [†]
Globulins (g/l)	31.72±10.7	36±5 [†]	39.88±11.7	22.4±3.9 [†]
alpha1 globulins (g/l)	4.53±3.6		4.48±0.6	6.47±1.6 [†]
alpha2 globulins (g/l)	7.69±2.8		8.86±2.5	11.45±4.8 [†]
Beta globulins (g/l)	8.81±11.4		4.63±2.8	10.3±0.5 [†]
Gamma globulins (g/l)	12.70±8.4	17±4.4 [†]	23.56±11.9	7±2.2 [†]
Albumin/globulins	0.97±0.4		1.06±0.4	0.8±0.3 [§]
ALT (U/l)	8.19± 5.1	30±4 [†]	10.85± 7.1	6–19 [†]
AST (U/l)	35.54±15.1	(77–88) [†]	35.58±14.9	27.13±9.5 [§]
Cholesterol (mmol/l)	0.49±0.5	1.66±0.31 [†]	1.20±0.5	2.07–3.37 [†]
Urea (mmol/l)	6.58±3.3	2.86–7.14 [†]	3.57–7.1	3.57–7.14 [†]
Creatinine (μmol/l)	104.10±56.3	106–168 [†]	174.82±45.8	88.4–159 [†]
Magnesium (mmol/l)	0.91±0.6	1.03 ±0.12 [†]	1.07±0.6	1.32±0.14 [†]
Calcium (mmol/l)	2.48±1.0	3.04 ±0.07 [†]	3.09±0.9	2.58±0.18 [†]
Phosphorus (mmol/l)	1.69±0.4	1.62–2.36 [†]	1.47±0.4	1.29–2.42 [§]

Ref. val.: Reference values; [†]= Kaneko *et al.* [22], [†]=Dimauro *et al.* [23], [§] Bhooshan *et al.* [27]

3.3. Variation of biochemical parameters according to age groups

Some biochemical parameters varied according to age groups in both small ruminants species (Table 3). In adult sheep, the concentrations of globulins, cholesterol, urea and magnesium were significantly higher than those in young subjects ($p < 0.05$). In goats, concentrations of ions like phosphorus, calcium and magnesium varied in the two age groups ($p < 0.05$). Like in sheep, oldest goats had a higher concentration of cholesterol than young ones ($p = 0.047$). Even though no difference was noticed in globulins concentration in age groups in goats, gamma globulins concentration in young goats was much higher than in adult subjects ($p = 0.039$).

3.4. Variation of biochemical parameters according to trypanosome infection status

Almost all biochemical parameters values found in infected and healthy subjects remained within normal physiological ranges except the gamma globulin value which was pathological in trypanosomosis-infected goats (Table 4). The values of total proteins, albumin, alpha2 globulins, creatinine and calcium were significantly lower in healthy sheep than the infected group ($p < 0.02$). In goats, the values of total proteins, globulins and gamma globulins were higher in the infected

160 group than the healthy one ($p<0.004$). The study found that the values of creatinine and
161 magnesium were significantly lower in infected goats than in the healthy ones.

162 **Table 3. Biochemical parameters in Burkinabese small ruminants breeds according to age groups (young < 2 years vs adult)**

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Parameters	Sheep				Goats			
	Young (n=13)	Adult (n=7)	t	p	Young (n=21)	Adult (n=24)	t	p
Total proteins (g/l)	57.20±12.1	67.01±19.7	1.392	0.909	73.63±14.9	72.13±6.5	0.448	0.328
Albumin (g/l)	29.05±6.0	28.67±11.6	0.096	0.462	33.48±4.7	32.48±5.3	0.66	0.256
Globulins (g/l)	28.15±8.7	38.34±11.5	2.242	0.018*	40.16±15.8	39.64±6.9	0.145	0.442
alpha1 globulins (g/l)	4.5±3.2	4.58±4.5	0.049	0.519	4.50±0.7	4.47±0.6	0.085	0.466
alpha2 globulins (g/l)	6.65±2.1	9.48±3.0	2.424	0.026*	8.68±2.5	8.96±2.6	0.231	0.590
Beta globulins (g/l)	8.98±10.7	8.51±13.3	0.084	0.467	3.42±1.3	5.34±3.3	1.471	0.920
Gamma globulins (g/l)	10.92±6.9	15.75±10.3	1.233	0.882	32.58±12.8	18.30±7.8	3.034	0.003*
ALT (UI/l)	8.99±5.1	9.55±7.2	0.203	0.579	10.61±8.9	12.91±10.9	0.768	0.776
AST (UI/l)	34.9±17.4	29.61±18.3	0.636	0.266	39.47±15.2	31.28±15.3	1.795	0.039*
Cholesterol (mmol/l)	0.41±0.2	0.86±0.6	2.094	0.026*	1.08±0.5	1.31±0.4	1.711	0.047*
Urea (mmol/l)	5.51±1.7	8.54±4.7	1.957	0.034*	5.29±3.1	5.45±2.3	0.199	0.578
Creatinine (μmol/l)	99.79±53.4	112.02±65.8	0.416	0.658	172.72±49.8	176.47±43.4	0.257	0.600
Magnesium (mmol/l)	0.69±0.5	1.31±0.8	2.127	0.024*	1.60±0.6	1.92±0.6	1.763	0.042*
Calcium (mmol/l)	2.30±0.9	2.77±1.2	0.921	0.815	3.46±0.9	2.77±0.9	2.561	0.007*
Phosphorus (mmol/l)	1.62±0.4	1.81±0.2	1.242	0.230	2.00±0.4	1.82±0.4	1.779	0.041*

164 *p<0.05: significant difference

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Table 4. Mean values $\pm$ SD of biochemical parameters in Trypanosome infected small ruminants compared with clinically healthy ones

Parameters	Sheep				Goats			
	Positive (n=10)	Negative (n=10)	t	p	Positive (n=9)	Negative (n=36)	t	p
Total proteins (g/l)	54.04 $\pm$ 12.2	67.23 $\pm$ 15.9	2.074	0.026*	84.02 $\pm$ 10.68	70.42 $\pm$ 9.8	3.513	0.001*
Albumin (g/l)	23.71 $\pm$ 7.2	34.12 $\pm$ 5.2	3.72	0.000**	33.73 $\pm$ 3.84	32.78 $\pm$ 5.2	0.481	0.683
Globulins (g/l)	30.33 $\pm$ 7.9	33.11 $\pm$ 13.1	0.572	0.287	50.29 $\pm$ 10.06	37.64 $\pm$ 10.9	3.006	0.004*
alpha1 globulins (g/l)	3.92 $\pm$ 2.0	5.21 $\pm$ 4.8	0.77	0.223	4.66 $\pm$ 0.69	4.35 $\pm$ 0.5	1.112	0.859
alpha2 globulins (g/l)	6.40 $\pm$ 1.3	9.13 $\pm$ 3.3	2.41	0.013*	8.80 $\pm$ 2.30	8.91 $\pm$ 2.7	0.092	0.463
Beta globulins (g/l)	8.45 $\pm$ 10.9	9.21 $\pm$ 12.6	0.141	0.447	3.60 $\pm$ 1.2	5.39 $\pm$ 3.4	1.402	0.089
Gamma globulins (g/l)	13.76 $\pm$ 7.8	11.52 $\pm$ 9.3	0.570	0.712	33.18 $\pm$ 10.1	16.58 $\pm$ 7.6	4.100	0.000**
Albumin/globulins	0.82 $\pm$ 0.3	1.16 $\pm$ 0.4	2.108	0.024*	0.69 $\pm$ 0.2	0.94 $\pm$ 0.3	2.108	0.020*
ALT (U/l)	11.22 $\pm$ 7.3	7.17 $\pm$ 2.7 $\pm$	1.128	0.863	7.49 $\pm$ 3.39	17.50 $\pm$ 20.5	1.361	0.090
AST (U/l)	35.02 $\pm$ 17.9	31.00 $\pm$ 17.6	0.495	0.686	34.29 $\pm$ 12.58	35.28 $\pm$ 16.4	0.161	0.436
Cholesterol (mmol/l)	0.61 $\pm$ 0.6	0.54 $\pm$ 0.3	0.290	0.612	0.99 $\pm$ 0.46	1.25 $\pm$ 0.5	1.448	0.077
Urea (mmol/l)	6.94 $\pm$ 4.3	6.58 $\pm$ 1.9	0.249	0.597	5.39 $\pm$ 2.6	5.39 $\pm$ 2.6	0.078	0.468
Creatinine (μ mol/l)	63.58 $\pm$ 22.4	162.00 $\pm$ 32.7	7.40	0.000**	146.30 $\pm$ 75.6	179.71 $\pm$ 38.2	1.690	0.049*
Magnesium (mmol/l)	0.72 $\pm$ 0.3	1.10 $\pm$ 0.9	1.251	0.113	1.30 $\pm$ 0.21	1.86 $\pm$ 0.7	2.458	0.009*
Calcium (mmol/l)	1.75 $\pm$ 0.5	3.13 $\pm$ 1.0	3.78	0.000**	3.19 $\pm$ 0.58	3.07 $\pm$ 1.0	0.322	0.625
Phosphorus (mmol/l)	1.56 $\pm$ 0.1	1.82 $\pm$ 0.4	1.820	0.957	1.78 $\pm$ 0.46	1.93 $\pm$ 0.3	1.080	0.143

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* p <0.05: significant difference, ** p <0.001: very significant difference

4. DISCUSSION

This study showed that the most prevalent trypanosome strain infecting small ruminants was *T. vivax* with 68.42% (13/19) of the detected cases by microscopy. The predominance of *T. vivax* might be explained by the exclusive presence in the agro-pastoral zone of tsetse flies belonging to the *palpalis* group which is known as the effective vectors of this trypanosome species^[15] and by the abundance of mechanical vectors (biting flies) also known to be effective transmitters of *T. vivax*^[16,17]. Indeed, the study area is infested by two riverine species of tsetse flies, namely, *Glossina palpalis gambiensis* and *G. tachinoides*^[18,19].

Usually the prevalence of trypanosomosis is lower in small ruminants than cattle and equines. Indeed, in the same area, the prevalences of trypanosomosis were 16.7% and 18.12% in donkeys and cattle, respectively^[11,20]. In the western part of Burkina Faso, the region of the Boucle du Mouhoun, Sow *et al.*^[3] reported a much lower prevalence of trypanosomosis in small ruminants (< 2%) than the agro-pastoral zone of Sideradougou. Small ruminants undergo for mass treatment in the rainy season with diminazene aceturate. Individual treatment is rarely applied in rural area in Burkina Faso. Although the mass treatment is not always successful because of misuse of the trypanocidal drug and the occurrence of chemoresistance in trypanosome strains^[21].

The mean values of the common biochemical parameters analyzed were close to the reference and physiological values established elsewhere worldwide^[22,23,24,25,26,27]. However the biochemical variables varied according to the age groups of small ruminants. In both species, the concentration of cholesterol was higher in adults than young animals. That might be explained by the feeding status and aging^[28]. Small ruminants are managed according to the traditional system, where animals are fed on natural pasture without additional feed supplements. The young animals (< 2 years) are weaker than the adults and therefore have less feed intake on the pasture. In experimental conditions, Mbassa and Poulsen^[29] reported that the concentration of cholesterol were higher in young goats (< 6 months) than adults subjects.

The concentration of magnesium was significantly lower in young small ruminants than the adult ones. Magnesium is an essential component of animal diet, and green plants contain great amount of magnesium because of its abundance in chlorophylls and in the seeds^[30,31]. The variation of magnesium concentration in blood might be due to dietary intake.

In young goats, the serum calcium and phosphorus concentrations were significantly different from adults' group ($p < 0.05$). This finding was documented by many studies in various species of goats^[32,33,34]. The absorption efficiency of calcium decreased with age owing to the decline in vitamins D storage^[35]. However, significant variation was not recorded in the values of calcium and phosphorus in the two age groups in sheep.

In goats, the AST activity was higher in the young than adult animals ($p = 0.039$). All values were within the range of the standard values. However, differences associated with age have been reported in sheep^[36]. Many studies reported that there was no significant difference in the values of AST in goats according to age^[32,34,37].

In both species, there was significant variation of serum proteins according to age groups, namely globulins and alpha globulins in sheep and gamma globulins in goats. However, all values were in the range of physiological values. Such findings were reported by Piccione *et al.*^[37] in goats. Variations in serum proteins are affected by numerous factors.

Total proteins and albumin can be lowered in the case of infection by blood-spoiling parasites such as trypanosome, *Babesia*, and gastro intestinal parasites in various livestock species^[11,38,39,40]. In our study, there were significant differences in total proteins and the fractions of globulins concentrations in the infected and healthy small ruminants. In sheep, there was a decrease in the total proteins ($p = 0.026$) and albumin ($p = 0.000$) values in infected subjects. This notwithstanding, in diseased sheep, the low feed intake induced by trypanosomosis might explain the low total proteins in serum. Even though difference was noted in globulins concentration in the infected and healthy sheep, the ratio albumin/globulins in infected group was lower than the healthy ones ($p = 0.024$). The disproportion of the ratio in the infected sheep showed the immune response might have been induced by the trypanosomes.

In goats, the total proteins and globulins concentrations were significantly higher in infected animals than the healthy ones. The ratio of albumin/globulins was significantly lower in infected group than healthy one ($p < 0.020$) because of the high concentration of gamma globulins in infected group. This concentration was even out of range of physiological values in goats. The high concentration of gamma globulins was a response of chronic infection as suggested by Lassen^[41] and Mouiche *et al.*^[42]. In fact, the recurrence of trypanosome in the host blood and body fluids provokes permanent antigenic stimulation which induced the increased concentration of immunoglobulins secreted as immunological response against trypanosomes in blood stream^[43, 44].

Many studies supported that animals infected by trypanosomosis, whatever the responsible pathogenic species of the parasite, showed plasmatic total proteins concentration higher than healthy subjects. Indeed, horses, donkeys, dromedaries, cattle, and even cats infected by trypanosome had proteinemia significantly higher than healthy animals^[10,11,43,44,45, 46, 47]. The values of AST and ALT were in the normal range in both species, thus there was no obvious damage in the liver. The increase of total proteins in infected goats was due to the increase of gamma globulins portion as immune response of trypanosome infection^[48].

In the current work, plasmatic values of creatinine in trypanosomosis-infected animals were significantly lower than the values obtained in healthy animals ($p = 0.000$ for sheep and $p = 0.049$ for goats). These results were different from those reported in small ruminants infected with *Babesia*^[40]. Similarly, the lower concentrations of calcium and magnesium in infected sheep and goats, respectively, than the healthy animal might be due to low feed consumption of infected animals.

5. CONCLUSION

This work highlighted some biochemical changes in the small ruminants infected with pathogenic trypanosome species, *T. vivax* and/or *T. congolense*. Indeed, trypanosomosis induced changes mainly in parameters associated to nutritional status like calcium and phosphorus, and increased total plasmatic proteins concentration due to the increase of globulins levels, especially the increased level of gamma globulins in trypanosomosis-infected goats. The results of this study would help veterinarians to improve the diagnosis of the diseases of small ruminants by good interpretation of biochemical parameters.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: A Sow and GJ Sawadogo. Performed the experiments: A Sow, AG Ouédraogo and GJ Sawadogo. Analyzed the data: A Sow, MZ Zabré, M Kalandi, J Kouamo and MMM Mouiche. Contributed reagents/materials/analysis tools: A Sow, MZ Zabré, A Bathily and MMM Mouiche. Wrote the paper: A Sow, MMM Mouiche, J Kouamo, GJ Sawadogo, AG Ouédraogo and MZ Zabré.

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