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Evaluation of biochemical indices in indigenous cattle breeds and crossbred genotypes under natural trypanosome challenge

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Abstract

Breeding for trypanotolerance could be a more sustainable control strategy against trypanosomiasis in African livestock species. This study was aimed at examining the biochemical indices of adult cattle (≥ 36 months of age) under natural trypanosome challenge. The cattle herd comprised of purebred N'dama (ND), Muturu (MU), Sokoto Gudahli (GU), Red Bororo (BO), and White Fulani (WF); and various crossbred genotypes. Animals sampled were ND (n = 21), WF (n = 37), GUxND (n = 3), NDxWF (n = 3); 3/4ND1/4WF (n = 3), ND(WFxMU)) (n = 6), ND(GUxWF) (n = 3), ND(BOxWF) (n = 3), and ND(GUxWFxMU) (n = 12). Serum levels of total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine and urea were compared among cattle genotypes, anaemic and non-anaemic, and parasitaemic and aparasitaemic groups. Non-anaemic cattle had higher ($p<0.05$) total protein and albumin compared to anaemic cattle. Globulin, albumin-globulin ratio, ALT, ALP, and creatinine did not differ significantly between cattle genotypes but AST was highest in anaemic NDxWF (38.50 ± 6.19 u/L) while indirect bilirubin and urea were highest in non-anaemic 3/4ND1/4WF. Aparasitaemic cattle had higher total protein, albumin and ALP but lower creatinine than parasitaemic cattle. AST and total bilirubin were highest in ND(GUxWFxMU) (33.18 ± 1.78 u/L) and ND(GUxWF) (0.30 ± 0.03 mg/dL), respectively and least in parasitaemic WF (24.63 ± 3.57 u/L) and NDxWF (0.19 ± 0.03 mg/dL), respectively. Creatinine, indirect bilirubin and urea were highest in 3/4ND1/4WF (1.23 ± 0.26 mg/dL, 0.23 ± 0.03 mg/dL, and 39.68 ± 3.05 mg/dL, respectively). ND (0.28 ± 0.10 mg/dL), parasitaemic WF (0.11 ± 0.03mg/dL), and ND(BOxWF) (22.84 ± 3.05 mg/dL) had lowest values of creatinine, indirect bilirubin and urea, respectively. Crossbred cattle and ND had higher serum total protein, albumin, AST, total and indirect bilirubin, but lower creatinine compared to WF cattle indicating better body nutrient reserves, lower oxidative stress challenge and stronger trypanotolerance capacity.

Keywords: Biochemical indices, crossbreeding, humid rainforest zone, indigenous cattle genotypes, trypanotolerance.

Running title: Trypanotolerance and biochemical indices in indigenous cattle genotypes

Évaluation des indices biochimiques chez les races bovines indigènes et les génotypes croisés en présence d'un défi trypanosomique naturel

Résumé

La sélection d'animaux trypanotolérantes pourrait constituer une stratégie de lutte plus durable contre la trypanosomose affectant le cheptel africain. Cette étude visait à analyser les indices biochimiques chez des bovins adultes (âgés d'au moins 36 mois) exposés naturellement aux trypanosomes. L'échantillon étudié comprenait des races bovines pures N'Dama (ND), Muturu (MU), Sokoto Gudahli (GU), Red Bororo (BO) et White Fulani (WF), ainsi que divers génotypes issus de croisements. Les animaux échantillonnés étaient répartis comme suit : ND (n = 21), WF (n = 37), GUxND (n = 3), NDxWF (n = 3) ; 3/4ND1/4WF (n = 3), ND(WFxMU)) (n = 6), ND(GUxWF) (n = 3), ND(BOxWF) (n = 3) et ND(GUxWFxMU) (n = 12). Les concentrations sériques de protéines totales, d'albumine, de globuline, d'aspartate aminotransférase (AST), d'alanine aminotransférase (ALT), de phosphatase alcaline (ALP), de bilirubine, de créatinine et d'urée ont été comparées entre les génotypes bovins, les groupes anémiques et non anémiques, et les groupes parasitémiques et aparasitémiques. Les bovins non anémiques présentaient des taux de protéines totales et d'albumine significativement plus élevés (p < 0,05) que les bovins anémiques. La globuline, le rapport albumine/globuline, l'ALT, la PAL et la créatinine ne présentaient pas de différences significatives entre les génotypes bovins. L'AST était toutefois plus élevée chez les NDxWF anémiques (38,50 ± 6,19 u/L), tandis que la bilirubine indirecte et l'urée étaient plus élevées chez les 3/4ND1/4WF non anémiques. Les bovins aparasitémiques affichaient des taux plus élevés de protéines totales, d'albumine et de PAL, mais une créatinine plus faible que les bovins parasitémiques. L'AST et la bilirubine totale étaient les plus élevées chez ND(GUxWFxMU) (33,18 ± 1,78 u/L) et ND(GUxWF) (0,30 ± 0,03 mg/dL), respectivement, et les plus faibles chez les WF parasitémiques (24,63 ± 3,57 u/L) et NDxWF (0,19 ± 0,03 mg/dL), respectivement. La créatinine, la bilirubine indirecte et l'urée étaient les plus élevées chez les 3/4ND1/4WF (1,23 ± 0,26 mg/dL, 0,23 ± 0,03 mg/dL et 39,68 ± 3,05 mg/dL, respectivement). ND (0,28 ± 0,10 mg/dL), les WF parasitémiques (0,11 ± 0,03 mg/dL) et ND(BOxWF) (22,84 ± 3,05 mg/dL) présentaient quant à eux les valeurs les plus faibles de créatinine, de bilirubine indirecte et d'urée. Les bovins croisés et les ND présentaient des taux sériques plus élevés de protéines totales, d'albumine, d'AST, de bilirubine totale et indirecte, mais une créatinine plus faible par rapport aux bovins WF, indiquant de meilleures réserves en nutriments corporels, un stress oxydatif plus faible et une capacité de trypanotolérance supérieure.

Mots-clés : Indices biochimiques, croisement, zone de forêt humide, races bovines indigènes, trypanotolérance

Introduction

Trypanosomiasis is a vector-borne haemoparasitic disease of vertebrate animals caused by blood parasites belonging to the genus *Trypanosoma* (Mishra *et al.,* 2017). Trypanosomes are transmitted biologically by tsetse flies of the genus Glosina, and mechanically by biting flies of the genus *Tabanus* and *Stomoxys* (Getahun *et al.,* 2022). All species of vertebrates are believed to be susceptible to at least one species of trypanosomes (Oie 2021). The major pathogenic species in livestock are *Trypanosoma congolense*, *T. vivax*, *T. brucei*, and *T. evansi* for ruminants, *T. simiae* for pigs, *T. evansi* and *T. equipadium* for horses and donkeys (Khan *et al.,* 2018). Economic losses are due to high morbidity and mortality, abortion, and infertility in susceptible hosts (Getahun *et al.,* 2022). Clinical signs include anorexia, anaemia, emaciation, profuse salivation, organ inflammation and damage, tissue degeneration, and necrosis (Igbokwe, 2018).

The mechanisms by which trypanosomes establish, multiply, and cause heavy parasitaemia in the host involve the variable surface glycoprotein (VSG) genes (Bangs, 2018). This gene pool constitute about 10-20 % of the trypanosome genome and exhibits monoallelic expression at any given time (Pereira et al., 2022). VSG genes provide the molecular resources for antigenic variation which enables trypanosomes to evade host immune system and endogenous trypanocidal factors (Abenga *et al.,* 2017; Aresta-Blanco *et al.,* 2019). In addition, trypanosomes utilize host nutrient supplies and release metabolites some of which contribute to the pathogenesis of the disease (Mishra *et al.,* 2017). Antigens from trypanosomes stimulate host defense system with release of biomolecules (immunoglobulins, lymphokines, nitric oxide, interferons) aimed against the parasite but which over production and extensive distribution becomes detrimental to the host (Kasozi *et al.,* 2021). Trypanosomes also instigate lipid peroxidation and oxidative stress which has implications for red blood cell (RBC) stability and fluidity (Mishra *et al.,* 2017). Oxidative damage to RBCs is a major contributor to the extensive erythrophagocytosis observed in susceptible animals (Kasozi *et al.,* 2021).

The pathological effects of trypanosomiasis are hence reflected in altered haematological and biochemical variables, and in the metabolites released in the host (Lelisha and Meheranet, 2021; Getahun *et al.,* 2022). Blood dyscrasias include anaemia, leukopenia, and thrombocytopaenia (Kasozi *et al.,* 2021), while biochemical alterations include markers of protein-energy metabolism, serum enzymes, and kidney function markers (Akinseye *et al.,* 2020). The severity of the various patho-physiological and haemato-biochemical perturbations depends on trypanosome species and host susceptibility (Awekwe *et al.,* 2017).

Trypanotolerance indicates the relative capacity of animal hosts to resist the pathological effects of trypanosomiasis. This heritable resistance is reflected in the genetic potential to control parasitaemia, and dampen tissue damage (Stijlemans *et al.,* 2018). In cattle, it is indicated by improved parasite control, haematopoetic system response to limit anaemia, sustained body nutrient reserves, and normal levels of markers of critical organ functions. Biochemical indices could therefore indicate disease severity and trypanotolerance in livestock breeds.

Trypanosomiasis remains a scourge, and a threat to livelihood in sub-Saharan Africa (Joan *et al.,* 2020) despite the huge resources expended on vector control and chemotherapy. As such, these control measures have become unsustainable, necessitating a change in strategy such as the exploitation of trypanotoleance genetics (Robi *et al.,* 2021). Accordingly, crossbreeding trypanotolerant and susceptible breeds is suggested as a more sustainable long term control strategy (Maganga *et al.,* 2018). Trypanotolerant breeds of cattle such as the Sanga in Ghana, Borgou in Togo and Benin, Djokore in Senegal, and Mere in Cote d'Ivoire, Burkina Faso and Mali are stabilized crosses between trypanotolerant and trypanosusceptible breeds (Ganyo *et al.,* 2018). The objectives of the present study were to evaluate the serum biochemical indices and trypanotolerance of crossbred cattle genotypes in comparison with N'Dama and White Fulani breeds under natural trypanosome challenge.

Materials and methods

The study was carried out at the cattle breeding unit of the Teaching and Research Farm, Michael Okpara University of Agriculture Umudike Abia State, Nigeria (latitude 05^{o} 29^{I} North, longitude 07^{o} 32^{I} East; ambient temperature range: 25 to 35^{o}C, annual rainfall range: 1677.5 to 2200mm) (N.R.C.R.I. 2016). The study was undertaken during the beginning of rains (March to May) and lasted for three months.

Herd structure and experimental animals

The cattle herd comprised of purebred N'dama (ND), Muturu (MU), Sokoto Gudahli (GU), Red Bororo (BO), and White Fulani (WF) and various crossbred genotypes. Animals included in the present study (Table 1), were adult cattle (≥ 36 months of age) belonging to ND (n = 21), WF (n = 37), GUxND (n = 3), NDxWF (n = 3); 3/4ND1/4WF (n = 3), ND(WFxMU) (n = 6), ND(GUxWF) (n = 3), ND(BOxWF) (n = 3), and ND(GUxWFxMU) (n = 12). The crossbred genotypes were generated in a breeding programme to develop cattle genotypes that combine trypanotolerance with improved growth performance. The experimental animals were mostly cows with one or two bulls sampled in each genotype.

Management of experimental animals

The herd was managed semi-intensively with daily grazing of natural pasture followed by supplemental feeding of combination of hay and silage, and watering in the holding area after grazing. Routine herd health management included strategic deworming at the beginning and end of rains, treatment against bacterial infections and blood parasites following diagnosis, and ectoparasite control by periodic administration of systemic or topical acaricides. The experimental animals were treated against gastro-intestinal parasites and ecto-parasites prior to the study. Treatment against hemoparasites was withheld eight weeks to the study and during the study to allow natural infection and development of trypanosomiasis in the herd (Berthier *et al.,* 2015). Routine and daily management care aimed to ensure optimal welfare and performance of experimental and non-experimental subjects.

Parasitological diagnosis and haematological indices

As reported previously (Ogbu *et al.,* 2023), hemoparasite parasitaemia was evaluated using wet, thick, and thin blood films, and the buffy coat. The specie of trypanosome was identified by examining Giemsa stained thin smear of positive blood samples. Haematological indices were evaluated using standard laboratory procedures.

Blood sample collection and determination of biochemical indices

Blood samples for biochemical evaluation were collected once a month for three months (March through May) from the jugular vein of each study animal following aseptic and ethical procedures. Briefly, the animals were adequately fed and then moved into the cattle clutch for blood collection. Veterinary personnel were utilized for blood collection to ensure minimal discomfort to the animals. Blood was collected into plain, properly labeled sample bottles, held on ice pack and taken immediately after collection to the laboratory for further analysis. Serum was harvested after centrifugation at 3000g for 5 min into clean, sterilized and properly labeled sample bottles.

Serum total protein, albumin and globulin assay

Serum total protein was determined by the direct Biuret method (Gornall *et al*., 1948). At alkaline pH, proteins form a blue coloured complex with copper II ions. The absorbance of the complex measured spectrophotometrically is proportional to the concentration of protein in serum. An aliquot of 0.02 mL of test serum, and 5.78 g/dL bovine serum protein (standard) were each mixed with 1mL Biuret reagent in two different test tubes. They were incubated for 10 minutes at room temperature and their absorbance read spectrophotometrically at 546 nm. Total protein was calculated as:

$$\text{Total protein (g/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \text{ x } 5.78$$

Serum albumin was evaluated using the bromocresol green (BCG) method (Watanabe *et al.,* 2004). Briefly, 3.0 mL BCG reagent (3,3’,5,5’-tetrabromo-m-cresol sulphonaphthalein) was added to each of three test tubes containing 0.01 mL distilled water (blank), 0.01 mL of 4.62 g/dL of bovine serum albumin (standard), and 0.01 mL of sample serum (sample), respectively and incubated at room temperature for 5 min. The absorbance of standard and sample were determined against the blank spectrophotometrically at 578nm.

Serum globulin level was obtained by subtracting the value for albumin from that of serum total protein.

Serum AST, ALT, and ALP levels

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were estimated spectrophotometrically using commercial kits (Randox, UK). AST and ALT were determined according to Reitman and Frankel (1957) and absorbance was read at 546 nm while ALP was determined according to Kind and King (1972) at absorbance of 550 nm.

Serum urea and creatinine levels

Serum urea level was determined following the method of Bauer *et al*. (1982). The absorbance of standard and test samples were read against blank at 546 nm and urea level calculated using the expression:

$$\text{Urea}\ \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{1}$$

Estimation of serum creatinine was according to Cockcroft and Gault (1976). Absorbance of sample and standard were respectively read at 20 and 80 seconds at 546 nm and creatinine level calculated using the expression:

$$\text{Creatinine (mg/dL)} = \frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of standard}}\ \text{x}\ 2$$

Serum total, indirect and direct bilirubin levels

Serum total and direct (conjugated) bilirubin levels were determined according to the method of Jendrasik and Grof (1938). Briefly, sample blank (200 μL of sample + 200 μL of sulphanilic acid HCL + 1000 μL of Caffeine sodium benzoate) and sample test (200 μL of sample + 200 μL of sulphanilic acid HCL + 50 μL of sodium nitrite + 1000 μL of Caffeine sodium benzoate) mixtures were prepared and left to stand for 10 min at 25°C. This was followed by addition of 1000 μL of tartarate sodium hydrochloride to both mixtures and incubation at 25°C for 30 min. The absorbance of the mixtures was read spectrophotometrically at 578 nm. Total bilirubin was calculated using the expression:

$$\text{Total bilirubin mg/dL} = 10.8 \times \text{Absorbance}$$

For direct (conjugated) bilirubin, sample blank (200 μL of sample + 200 μL of sulphanilic acid HCL + 2000 μL of 0.9 % NaCl) and sample test (200 μL of sample + 200 μL of sulphanilic acid HCL + 50 μL of sodium nitrite + 2000 μL of 0.9 % NaCl) mixtures were prepared and left to stand for 10 min at 25°C. The absorbance of the mixtures was read spectrophotometrically at 546 nm and conjugated bilirubin calculated as:

$$\text{Conjugated (direct) bilirubin (mg/dL)} = 14.4 \times \text{absorbance}$$

Indirect (free) bilirubin (mg/dL) was then obtained by difference: Total bilirubin – direct bilirubin.

Statistical analysis

Data were subjected to analysis of variance to compare genotypes and genotypic groups for the various biochemical indices. Significant means were separated using the Duncan's New Multiple Range Test. Comparison between trypanosome parasitaemic and aparasitaemic, and anaemic (PCV<24.0 %) and non-anaemic groups across genotypes was performed using the independent samples t-test. All analyses were performed using SPSS version 20.0 for Windows and significant differences were accepted at $p<0.05$.

Results

Summary of previous data

Previous data (Ogbu *et al.,* 2023) reported infection by *T. brucei* in three WF cattle (breed prevalence: 8.1 %; herd prevalence: 3.3 %; among anaemic cattle: 21.4 %) with an average of 6.87 parasites (paracitaemia: 7.4×10^6 parasites/mL). No other hemoparasite was detected in the experimental animals. Prevalence of anaemia was 15.4 % in the herd, 21.6 % in WF, 9.5 % in ND, and 12.1 % in crossbred cattle

The serum protein profile of cattle of different cattle genotypes (anaemic and non-anaemic) and genotypic groups are presented in Table 2.

Across genotypes, anaemic cattle had lower serum total protein and albumin levels compared to non-anaemic cattle while serum globulin level and albumin:globulin ratio did not differ significantly between the two groups. Among genotypes, non-anaemic NDxWF had the highest serum total protein which was significantly ($p<0.014$) higher compared to anaemic ND(GUxWFxMU), WF, and ND, and non-anaemic ND(BOxWF) and WF. For serum albumin level, highest values were observed in anaemic NDxWF (4.32 ± 0.50 g/dL), and non-anaemic ND(WFxMU) (4.08 ± 0.20 g/dL), and ND(GUxWFxMU) (4.07 ± 0.17 g/dL) while anaemic WF had the least value (3.19 ± 0.18 g/dL). Serum globulin level and albumin:globulin ratio (A:G) did not differ significantly between genotypes. Serum total protein and albumin were higher in crossbred and ND cattle compared to WF while serum globulin and A:G did not differ significantly between the groups. A similar result was observed when non-anaemic animals of these groups were compared. Comparison between anaemic crossbred, WF and ND cattle showed that crossbred cattle and WF had similar serum total protein levels which were significantly ($p<0.004$) higher compared to that of ND while serum albumin was similar between crossbred cattle and ND but significantly ($p<0.023$) lower in WF. Anaemic WF cattle had significantly ($p<0.019$) higher serum globulin level compared to anaemic ND. Again, A:G did not differ significantly between the groups.

Anaemic and non-anaemic cattle did not differ significantly in levels of serum enzymes (ALT, AST, and ALP) as well as in levels of creatinine (Table 3). Significant ($p<0.011$) differences were however, observed in serum levels of AST among the genotypes which was higher in anaemic NDxWF compared to anaemic WF, and non-anaemic ND(WFxMU), ND(GUxWF), 3/4ND1/4WF), and WF. Furthermore, serum AST was higher ($p<0.000$) in crossbred and ND cattle compared to WF while creatinine was significantly ($p<0.041$) higher in WF cattle compared to ND. For non-anaemic animals, AST differed significantly ($p<0.000$) with higher values in crossbred cattle, and ND compared to WF. ALT, AST, and ALP did not differ significantly between anaemic animals of these groups.

Table1: Genotypes, number and sex of animals used for the study

			Sex distribution	
Genotype	Mating	Number per genotype	Male	Female
Purebred genotype				
N’Dama	ND x ND	21	2	19
White Fulani	WF x WF	37	2	35
Crossbred genotypes				
GUxND	GU x ND	3	1	2
NDxWF	ND x WF	3	1	2
3/4ND1/4WF	ND x (ND x WF)	3	1	2
ND(WFxMU)	ND x (WF x MU)	6	2	4
ND(BOxWF)	ND x (BO x WF)	3	1	2
ND(GUxWFxMU)	ND x [GU x (WF x MU)]	12	2	10

ND: N’Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

Table 2: Serum protein profile of cattle of different genotypes, genotypic groups and anaemic status in a trypanosome endemic zone

		Serum protein			
Genotype	[#]Anaemic status	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin:globulin
[*]Across genotypes					
	Anaemic	6.52 ± 0.15^{b}	3.44 ± 0.14^{b}	3.09 ± 0.21	1.23 ± 0.15
	Non-anaemic	7.02 ± 0.09^{a}	3.83 ± 0.06^{a}	3.20 ± 0.11	1.37 ± 0.08
p-value		0.020	0.011	0.676	0.497
Among genotypes					
ND(BOxWF)	Non-anaemic	6.66 ± 0.38^{bc}	3.82 ± 0.29^{ab}	2.85 ± 0.52	1.49 ± 0.42
ND(GUxWFxMU)	Anaemic	6.51 ± 0.38^{cd}	3.86 ± 0.29^{ab}	2.65 ± 0.52	1.71 ± 0.42
ND(GUxWFxMU)	Non-anaemic	7.06 ± 0.22^{abc}	4.07 ± 0.17^{a}	2.99 ± 0.30	1.52 ± 0.24
ND(WFxMU)	Non-anaemic	7.70 ± 0.22^{ab}	4.08 ± 0.20^{a}	3.62 ± 0.37	1.20 ± 0.30
GUxND	Non-anaemic	7.12 ± 0.38^{abc}	3.89 ± 0.29^{ab}	3.23 ± 0.52	1.27 ± 0.42
ND(GUxWF)	Non-anaemic	7.52 ± 0.38^{abc}	3.85 ± 0.29^{ab}	3.92 ± 0.52	1.11 ± 0.42
NDxWF	Anaemic	7.20 ± 0.66^{abc}	4.32 ± 0.50^{a}	2.89 ± 0.90	1.50 ± 0.73
NDxWF	Non-anaemic	7.98 ± 0.47^{a}	3.37 ± 0.35^{ab}	4.61 ± 0.63	0.73 ± 0.52
3/4ND1/4WF	Non-anaemic	7.48 ± 0.38^{abc}	3.58 ± 0.29^{ab}	3.94 ± 0.52	0.91 ± 0.42
WF	Anaemic	6.68 ± 0.24^{bc}	3.19 ± 0.18^{b}	3.49 ± 0.32	0.96 ± 0.26
WF	Non-anaemic	6.68 ± 0.12^{bc}	3.63 ± 0.09^{ab}	3.05 ± 0.17	1.42 ± 0.14
ND	Anaemic	5.59 ± 0.47^{d}	3.35 ± 0.35^{ab}	2.24 ± 0.63	1.50 ± 0.52
ND	Non-anaemic	7.10 ± 0.15^{abc}	4.03 ± 0.12^{ab}	3.08 ± 0.21	1.48 ± 0.17
p-value		0.014	0.021	0.329	0.858
Between crossbred and purebred cattle					
Crossbreds		7.24 ± 0.12^{a}	3.92 ± 0.09^{a}	3.35 ± 0.16	1.31 ± 0.13
WF		6.68 ± 0.12^{b}	3.53 ± 0.08^{b}	3.14 ± 0.15	1.32 ± 0.12
ND		6.96 ± 0.16^{ab}	3.96 ± 0.11^{a}	3.00 ± 0.20	1.48 ± 0.16
p-value		0.006	0.002	0.371	0.661
Between non-anaemic crossbred and purebred cattle					
Crossbreds		7.31 ± 0.13^{a}	3.91 ± 0.10^{ab}	3.44 ± 0.17	1.26 ± 0.14
WF		6.68 ± 0.13^{b}	3.63 ± 0.10^{b}	3.05 ± 0.17	1.42 ± 0.14
ND		7.10 ± 0.16^{a}	4.03 ± 0.12^{a}	3.08 ± 0.22	1.48 ± 0.17
p-value		0.004	0.023	0.238	0.581
Between anaemic crossbred and purebred cattle					
Crossbreds		6.69 ± 0.21^{a}	3.98 ± 0.20^{a}	2.71 ± 0.32^{ab}	1.65 ± 0.25
WF		6.68 ± 0.15^{a}	3.19 ± 0.14^{b}	3.49 ± 0.23^{a}	0.96 ± 0.18
ND		5.59 ± 0.30^{b}	3.35 ± 0.28^{ab}	2.24 ± 0.46^{b}	1.50 ± 0.36
p-value		0.019	0.025	0.050	0.101

[*]comparison by independent samples T-test; [#]anaemic: PCV < 24 %; a,b,c,d: column means with different superscripts are significantly different ($p < 0.05$); ND: N'Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

Table 3: Profile of liver enzymes and creatinine in cattle of different genotypes and anaemic status in a trypanosome endemic zone

Genotype	#Anaemic status	ALT (u/L)	AST (u/L)	ALP (u/L)	Creatinine (mg/dL)
*Across genotypes					
	Anaemic	7.03 ± 0.64	29.05 ± 2.55	21.87 ± 3.64	0.55 ± 0.13
	Non-anaemic	7.57 ± 0.33	27.95 ± 0.71	26.68 ± 2.03	0.47 ± 0.06
p-value		0.509	0.581	0.339	0.586
Among genotypes					
ND(BOxWF)	Non-anaemic	5.26 ± 1.64	32.57 ± 3.58^{ab}	31.18 ± 9.61	0.45 ± 0.27
ND(GUxWFxMU)	Anaemic	7.09 ± 1.64	33.93 ± 3.58^{ab}	40.47 ± 9.61	0.40 ± 0.27
ND(GUxWFxMU)	Non-anaemic	9.20 ± 0.95	32.92 ± 2.06^{ab}	21.32 ± 5.55	0.37 ± 0.16
ND(WFxMU)	Non-anaemic	8.02 ± 1.16	27.03 ± 2.53^{b}	27.32 ± 6.79	0.49 ± 0.19
GUxND	Non-anaemic	8.11 ± 1.64	28.80 ± 3.58^{ab}	39.76 ± 9.61	0.41 ± 0.27
ND(GUxWF)	Non-anaemic	7.15 ± 1.64	25.00 ± 3.58^{b}	34.16 ± 9.61	0.39 ± 0.27
NDxWF	Anaemic	7.68 ± 2.84	38.50 ± 6.19^{a}	13.75 ± 16.64	0.11 ± 0.46
NDxWF	Non-anaemic	8.08 ± 2.00	28.75 ± 4.38^{ab}	23.44 ± 11.77	0.52 ± 0.33
3/4ND1/4WF	Non-anaemic	10.60 ± 1.64	25.20 ± 3.58^{b}	5.25 ± 9.61	1.23 ± 0.27
WF	Anaemic	7.22 ± 1.00	26.15 ± 2.19^{b}	17.08 ± 5.88	0.78 ± 0.16
WF	Non-anaemic	6.75 ± 0.53	24.39 ± 1.15^{b}	31.45 ± 3.09	0.56 ± 0.09
ND	Anaemic	5.84 ± 2.00	28.60 ± 4.38^{ab}	17.17 ± 11.77	0.09 ± 0.33
ND	Non-anaemic	7.75 ± 0.65	31.28 ± 1.42^{ab}	21.51 ± 3.82	0.30 ± 0.11
p-value		0.260	0.011	0.184	0.118
Between crossbred and purebred cattle					
Crossbreds		8.16 ± 0.49	30.03 ± 1.09^{a}	26.33 ± 3.00	0.49 ± 0.08^{ab}
WF		6.85 ± 0.46	24.77 ± 1.03^{b}	28.35 ± 2.83	0.61± 0.08^{a}
ND		7.57 ± 0.61	31.02 ± 1.37^{a}	21.10 ± 3.76	0.28 ± 0.10^{b}
p-value		0.152	0.000	0.306	0.041
Between non-anaemic crossbred and purebred cattle					
Crossbreds		8.29 ± 0.53	29.33 ± 1.04^{a}	25.30 ± 3.26	0.51 ± 0.09
WF		6.75 ± 0.53	24.39 ± 1.04^{b}	31.45 ± 3.26	0.56 ± 0.09
ND		7.75 ± 0.66	31.28 ± 1.29^{a}	21.51 ± 4.03	0.30 ± 0.11
p-value		0.125	0.000	0.146	0.165
Between anaemic crossbred and purebred cattle					
Crossbreds		7.24 ± 1.28	35.08 ± 4.69	33.79 ± 6.05	0.33 ± 0.21
WF		7.22 ± 0.90	26.15 ± 3.32	17.08 ± 4.28	0.78 ± 0.15
ND		5.84 ± 1.81	28.60 ± 6.63	17.17 ± 8.56	0.09 ± 0.29
p-value		0.782	0.335	0.110	0.089

*comparison by independent samples t-test; #anaemic: PCV < 24 %; a,b: column means with different superscripts are significantly different ($p < 0.05$), ALT: alanine aminotransferase; AST: aspartate aminotransferase ; ALP: alkaline phosphatase; ND: N'Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

Serum levels of total, indirect (free), and direct (conjugated) bilirubin, and urea did not differ significantly between anaemic and non-anaemic cattle across genotypes (Table 4). However, indirect bilirubin and urea differed significantly among genotypes with non-anaemic 3/4ND1/4WF having higher indirect bilirubin level (0.23 ± 0.03 mg/dL) than non-anaemic ND(WFxMU) and WF, and anaemic ND(GUxWFxMU), WF, and ND.

Table 4: Bilirubin and urea profile of cattle of different genotypes, genotypic groups and anaemic status in a trypanosome endemic zone

		Serum bilirubin			
Genotype	#Anaemic status	Total (mg/dL)	Indirect (mg/dL)	Direct (mg/dL)	Urea (mg/dL)
*Across genotypes					
	Anaemic	0.22 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	30.67 ± 1.89
	Non-anaemic	0.24 ± 0.01	0.17 ± 0.01	0.08 ± 0.01	27.88 ± 0.63
p-value		0.174	0.120	0.984	0.100
Among genotypes					
ND(BOxWF)	Non-anaemic	0.21 ± 0.03	0.17 ± 0.03^{ab}	0.04 ± 0.03	22.84 ± 3.04^{d}
ND(GUxWFxMU)	Anaemic	0.25 ± 0.03	0.14 ± 0.03^{b}	0.11 ± 0.04	35.33 ± 3.04^{ab}
ND(GUxWFxMU)	Non-anaemic	0.26 ± 0.02	0.16 ± 0.02^{ab}	0.10 ± 0.02	28.88 ± 1.76^{bcd}
ND(WFxMU)	Non-anaemic	0.25 ± 0.02	0.14 ± 0.02^{b}	0.11 ± 0.02	24.71 ± 2.15^{cd}
GUxND	Non-anaemic	0.27 ± 0.03	0.17 ± 0.03^{ab}	0.10 ± 0.03	28.52 ± 3.04^{bcd}
ND(GUxWF)	Non-anaemic	0.30 ± 0.03	0.21 ± 0.03^{ab}	0.09 ± 0.03	32.50 ± 3.04^{abc}
NDxWF	Anaemic	0.18 ± 0.04	0.16 ± 0.05^{ab}	0.05 ± 0.03	29.84 ± 5.26^{bcd}
NDxWF	Non-anaemic	0.18 ± 0.04	0.17 ± 0.04^{ab}	0.01 ± 0.04	25.88 ± 3.72^{cd}
3/4ND1/4WF	Non-anaemic	0.26 ± 0.03	0.23 ± 0.03^{a}	0.05 ± 0.03	39.68 ± 3.04^{a}
WF	Anaemic	0.22 ± 0.02	0.15 ± 0.02^{b}	0.08 ± 0.02	30.24 ± 1.86^{bcd}
WF	Non-anaemic	0.23 ± 0.01	0.14 ± 0.01^{b}	0.09 ± 0.01	28.30 ± 0.98^{bcd}
ND	Anaemic	0.22 ± 0.04	0.15 ± 0.04^{b}	0.07 ± 0.04	25.84 ± 3.72^{cd}
ND	Non-anaemic	0.26 ± 0.01	0.20 ± 0.01^{ab}	0.06 ± 0.01	26.08 ± 1.21^{bcd}
p-value		0.131	0.025	0.432	0.014
Between crossbred and purebred cattle					
Crossbreds		0.25 ± 0.01^{ab}	0.17 ± 0.01^{ab}	0.08 ± 0.01	29.29 ± 1.00
WF		0.22 ± 0.01^{b}	0.14 ± 0.01^{b}	0.08 ± 0.01	28.72 ± 0.95
ND		0.26 ± 0.01^{a}	0.19 ± 0.01^{a}	0.06 ± 0.01	26.06 ± 1.26
p-value		0.029	0.004	0.371	0.121
Between non-anaemic crossbred and purebred cattle					
Crossbreds		0.25 ± 0.01^{ab}	0.17 ± 0.01^{ab}	0.08 ± 0.01	28.64 ± 1.02
WF		0.22 ± 0.01^{b}	0.14 ± 0.01^{b}	0.09 ± 0.01	28.30 ± 1.02
ND		0.26 ± 0.01^{a}	0.20 ± 0.01^{a}	0.06 ± 0.01	26.08 ± 1.26
p-value		0.048	0.005	0.407	0.260
Between anaemic crossbred and purebred cattle					
Crossbreds		0.24 ± 0.02	0.15 ± 0.02	0.09 ± 0.03	33.96 ± 3.56
WF		0.22 ± 0.02	0.15 ± 0.02	0.08 ± 0.02	30.24 ± 2.52
ND		0.22 ± 0.03	0.15 ± 0.03	0.07 ± 0.04	25.84 ± 5.03
p-value		0.659	0.995	0.828	0.434

*comparison by independent samples t-test; #anaemic: PCV < 24 %; a,b,c,d: column means with different superscripts are significantly different ($p \leq 0.05$); N: N'Dama, W: White Fulani, G: Sokoto Gudali, B: Red Bororo.

Non-anaemic 3/4ND1/4WF, and ND(GUxWF), and anaemic ND(GUxWFxMU) had similar values of serum urea level which were higher ($p<0.014$) compared to other genotypes. The least value for serum urea was observed in non-anaemic ND(BOxWF). There were significantly higher values of serum total, and indirect bilirubin in ND cattle compared to WF which together with ND had similar values with crossbred cattle. A similar result was observed when non-anaemic animals of these groups were compared while the parameters did not differ significantly between anaemic groups of these genotypes.

Parasitaemic and aparasitaemic (non-parasitaemic) cattle differed significantly ($p<0.045$) in serum albumin level with aparasitaemic cattle having higher values across genotypes (Table 5).

Table 5: Serum protein profile of parasitaemic and aparasitaemic cattle genotypes in a trypanosome endemic zone

		Serum protein			
	[#]Parasitaemia	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin:globulin
[*]Across genotypes					
	Parasitaemic	6.87 ± 0.21	3.15 ± 0.16^{b}	3.72 ± 0.29	0.86 ± 0.11
	Aparasitaemic	6.95 ± 0.08	3.79 ± 0.06^{a}	3.17 ± 0.10	1.37 ± 0.08
p-value		0.865	0.045	0.307	0.231
[*]Across anaemic genotypes					
	Parasitaemic	6.87 ± 0.21	3.15 ± 0.16	3.72 ± 0.29	0.86 ± 0.11^{b}
	Aparasitaemic	6.43 ± 0.17	3.51 ± 0.16	2.92 ± 0.23	1.33 ± 0.18^{a}
p-value		0.230	0.300	0.116	0.224
Among genotypes					
ND(BOxWF)	Aparasitaemic	6.66 ± 0.40^{b}	3.82 ± 0.30^{ab}	2.85 ± 0.52	1.49 ± 0.42
ND(GUxWFxMU)	Aparasitaemic	6.92 ± 0.20^{ab}	4.02 ± 0.15^{a}	2.91 ± 0.26	1.57 ± 0.21
ND(WFxMU)	Aparasitaemic	7.70 ± 0.29^{ab}	4.08 ± 0.21^{a}	3.62 ± 0.37	1.20 ± 0.30
GUxND	Aparasitaemic	7.12 ± 0.40^{ab}	3.89 ± 0.30^{ab}	3.23 ± 0.52	1.27 ± 0.42
ND(GUxWF)	Aparasitaemic	7.52 ± 0.40^{ab}	3.85 ± 0.30^{ab}	3.92 ± 0.52	1.11 ± 0.42
NDxWF	Aparasitaemic	7.72 ± 0.40^{a}	3.69 ± 0.30^{ab}	4.03 ± 0.52	0.99 ± 0.42
3/4ND1/4WF	Aparasitaemic	7.48 ± 0.40^{ab}	3.58 ± 0.30^{ab}	3.94 ± 0.52	0.91 ± 0.42
WF	Aparasitaemic	6.66 ± 0.12^{b}	3.57 ± 0.09^{ab}	3.09 ± 0.16	1.36 ± 0.13
WF	Parasitaemic	6.87 ± 0.40^{ab}	3.15 ± 0.30^{b}	3.72 ± 0.52	0.86 ± 0.42
ND	Aparasitaemic	6.96 ± 0.15^{ab}	3.96 ± 0.11^{ab}	3.00 ± 0.20	1.48 ± 0.16
p-value		0.022	0.051	0.232	0.774
Among aparasitaemic crossbred and purebred cattle					
Crossbreds		7.24 ± 0.13^{a}	3.92 ± 0.09^{a}	3.35 ± 0.16	1.31 ± 0.13
WF		6.66 ± 0.12^{b}	3.57 ± 0.09^{b}	3.09 ± 0.16	1.36 ± 0.13
ND		6.96 ± 0.16^{ab}	3.96 ± 0.11^{a}	3.00 ± 0.20	1.48 ± 0.16
p-value		0.006	0.007	0.336	0.710
Among aparasitaemic genotypes and parasitaemic WF					
Crossbreds	Aparasitaemic	7.24 ± 0.12	3.92 ± 0.08^{a}	3.35 ± 0.16	1.31 ± 0.10
WF	Parasitaemic	6.87 ± 0.41	3.15 ± 0.28^{b}	3.72 ± 0.52	0.86 ± 0.33
ND	Aparasitaemic	6.96 ± 0.15	3.96 ± 0.11^{a}	3.00 ± 0.20	1.48 ± 0.13
p-value		0.315	0.029	0.254	0.201

[*]comparison by independent samples t-test; [#]parasitaemic: infected by *T. brucei*; a, b: column means with different superscripts are significantly different ($p < 0.05$); ND: N'Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

None of the parameters differed significantly between anaemic parasitaemic and aparasitaemic cattle. Serum total protein was significantly ($p<0.022$) higher in NDxWF compared to

ND(BOxWF) and aparasitaemic WF cattle but similar to values for other genotypes. Serum albumin was significantly ($p<0.051$) higher in ND(WFxMU) and ND(GUxWFxMU) compared to parasitaemic WF but these did not differ significantly from other genotypes. Serum total protein and albumin were higher in crossbred cattle compared to WF but similar to those of ND.

Aparasitaemic crossbred cattle and ND had significantly ($p<0.029$) higher serum albumin level compared to parasitaemic WF while total protein, globulin and A:G did not differ significantly between the groups.

Aparasitaemic cattle had significantly higher serum ALP but lower creatinine level compared to parasitaemic counterparts (Table 6).

Table 6: Liver enzyme profile of parasitaemic and aparasitaemic cattle genotypes in a trypanosome endemic zone

Genotype	#Parasitaemia	ALT (u/L)	AST (u/L)	ALP (u/L)	Creatinine (mg/dL)
*Across genotypes					
	Parasitaemic	6.51 ± 1.14	24.63 ± 4.99	10.28 ± 3.03^{b}	1.04 ± 0.08^{a}
	Aparasitaemic	7.52 ± 0.31	28.24 ± 0.72	26.48 ± 1.84^{a}	0.47 ± 0.05^{b}
p-value		0.544	0.369	0.012	0.005
*Across anaemic genotypes					
	Parasitaemic	6.51 ± 1.14	24.63 ± 4.99	10.28 ± 3.03^{b}	1.04 ± 0.08^{a}
	Aparasitaemic	7.17 ± 0.78	30.25 ± 2.95	25.03 ± 4.09^{a}	0.42 ± 0.13^{b}
p-value		0.689	0.386	0.017	0.036
Among genotypes					
ND(BOxWF)	Aparasitaemic	5.26 ± 1.63	32.57 ± 3.57^{ab}	31.18 ± 9.68	0.45 ± 0.26^{bc}
ND(GUxWFxMU)	Aparasitaemic	8.67 ± 0.81	33.18 ± 1.78^{a}	26.11 ± 4.84	0.38 ± 0.13^{bc}
ND(WFxMU)	Aparasitaemic	8.02 ± 1.15	27.03 ± 2.52^{ab}	27.32 ± 6.84	0.49 ± 0.19^{bc}
GUxND	Aparasitaemic	8.11 ± 1.63	28.80 ± 3.57^{ab}	39.76 ± 9.68	0.41 ± 0.26^{bc}
ND(GUxWF)	Aparasitaemic	7.15 ± 1.63	25.00 ± 3.57^{b}	34.16 ± 9.68	0.39 ± 0.26^{bc}
NDxWF	Aparasitaemic	7.95 ± 1.63	32.00 ± 3.57^{ab}	20.21 ± 9.68	0.38 ± 0.26^{bc}
3/4ND1/4WF	Aparasitaemic	10.60 ± 1.63	25.20 ± 3.57^{b}	5.25 ± 9.68	1.23 ± 0.26^{a}
WF	Aparasitaemic	6.88 ± 0.48	24.79 ± 1.06^{b}	29.94 ± 2.87	0.57 ± 0.08^{bc}
WF	Parasitaemic	6.51 ± 1.63	24.63 ± 3.57^{b}	10.28 ± 9.68	1.04 ± 0.26^{ab}
ND	Aparasitaemic	7.57 ± 0.62	31.02 ± 1.35^{ab}	21.10 ± 3.66	0.28 ± 0.10^{c}
p-value		0.357	0.003	0.132	0.035
Among aparasitaemic crossbreds and purebred cattle					
Crossbreds		8.16 ± 0.50	30.03 ± 1.08^{a}	26.33 ± 2.98	0.49 ± 0.08
WF		6.88 ± 0.48	24.79 ± 1.06^{b}	29.94 ± 2.87	0.57 ± 0.08
ND		7.57 ± 0.62	31.02± 1.36^{a}	21.10 ± 3.74	0.28 ± 0.10
p-value		0.186	0.000	0.183	0.084
Among aparasitaemic cattle and parasitaemic WF					
Crossbreds	Aparasitaemic	8.16 ± 0.52	30.03 ± 1.25	26.33 ± 2.50	0.49 ± 0.08^{b}
WF	Parasitaemic	6.51 ± 1.70	24.63 ± 4.15	10.28 ± 8.30	1.04 ± 0.25^{a}
ND	Aparasitaemic	7.57 ± 0.64	31.02± 1.57	21.10 ± 3.14	0.28 ± 0.10^{b}
p-value		0.554	0.359	0.121	0.016

*comparison by independent samples t-test; #parasitaemic: infected by *T. brucei*; a,b,c: column means with different superscripts are significantly different ($p < 0.05$); ND: N'Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

A similar result was observed between anaemic parasitaemic and aparasitaemic groups. Serum ALT and ALP levels did not differ significantly among genotypes but serum AST was higher ($p<0.003$) in ND(GUxWFxMU) compared to ND(GUxWF), 3/4ND1/4WF, and WF. Highest ($p<0.035$) serum creatinine value was observed in 3/4NDx1/4WF animals while least value was observed in ND. Aparasitaemic crossbred and ND cattle had higher ($p<0.000$) AST values compared to WF counterparts while parasitaemic WF cattle had higher ($p<0.016$) creatinine level than aparasitaemic crossbred and ND cattle.

Serum total, indirect, and direct bilirubin and urea did not differ significantly between parasitaemic and aparasitaemic cattle across genotypes (Table 7).

Table 7: Bilirubin and urea profile of parasitaemic and aparasitaemic cattle genotypes in a trypanosome endemic zone

		Serum bilirubin			
Genotype	[#]Parasitaemia	Total (mg/dL)	Indirect (mg/dL)	Direct (mg/dL)	Urea (mg/dL)
[*]Across genotypes					
	Parasitaemic	0.22 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	25.67 ± 2.87
	Aparasitaemic	0.24 ± 0.01	0.16 ± 0.01	0.08 ± 0.01	28.40 ± 0.63
p-value		0.553	0.084	0.269	0.429
[*]Across anaemic genotypes					
	Parasitaemic	0.22 ± 0.01	0.11 ± 0.02^{b}	0.11 ± 0.01	25.67 ± 2.87
	Aparasitaemic	0.22 ± 0.02	0.16 ± 0.01^{a}	0.07 ± 0.02	32.04 ± 2.14
p-value		0.992	0.037	0.213	0.175
Among genotype					
ND(BOxWF)	Aparasitaemic	0.21 ± 0.03^{bc}	0.17 ± 0.03^{abc}	0.04 ± 0.03	22.84 ± 3.05^{c}
ND(GUxWFxMU)	Aparasitaemic	0.26 ± 0.02^{abc}	0.15 ± 0.02^{abc}	0.10 ± 0.02	30.49 ± 1.52^{bc}
ND(WFxMU)	Aparasitaemic	0.25 ± 0.02^{abc}	0.14 ± 0.02^{bc}	0.11 ± 0.02	24.71 ± 2.16^{bc}
GUxND	Aparasitaemic	0.27 ± 0.03^{ab}	0.17 ± 0.03^{abc}	0.10 ± 0.03	28.52 ± 3.05^{bc}
ND(GUxWF)	Aparasitaemic	0.30 ± 0.03^{a}	0.21 ± 0.03^{ab}	0.09 ± 0.03	32.50 ± 3.05^{b}
NDxWF	Aparasitaemic	0.19 ± 0.03^{c}	0.16 ± 0.03^{abc}	0.02 ± 0.03	27.20 ± 3.05^{bc}
3/4ND1/4WF	Aparasitaemic	0.26 ± 0.03^{abc}	0.23 ± 0.03^{a}	0.05 ± 0.03	39.68 ± 3.05^{a}
WF	Aparasitaemic	0.22 ± 0.01^{abc}	0.15 ± 0.01^{abc}	0.08 ± 0.01	28.98 ± 0.91^{bc}
WF	Parasitaemic	0.22 ± 0.03^{abc}	0.11 ± 0.03^{c}	0.11 ± 0.03	25.67 ± 3.05^{bc}
ND	Aparasitaemic	0.26 ± 0.01^{abc}	0.19 ± 0.01^{ab}	0.06 ± 0.01	26.06 ± 1.15^{bc}
p-value		0.047	0.016	0.143	0.002
Among aparasitaemic crossbreds and purebred cattle					
Crossbreds		0.25 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.08 ± 0.01	29.29 ± 1.01
WF		0.22 ± 0.01^{b}	0.15 ± 0.01^{b}	0.08 ± 0.01	28.98± 0.99
ND		0.26 ± 0.01^{a}	0.19 ± 0.01^{a}	0.06 ± 0.01	26.06 ± 1.26
p-value		0.038	0.010	0.420	0.109
Among aparasitaemic cattle and parasitaemic WF					
Crossbreds	Aparasitaemic	0.25 ± 0.01	0.17 ± 0.01^{a}	0.08 ± 0.01	29.29 ± 1.01
WF	Parasitaemic	0.22 ± 0.03	0.11 ± 0.03^{b}	0.11 ± 0.03	25.67 ± 3.05
ND	Aparasitaemic	0.26 ± 0.01	0.19 ± 0.01^{a}	0.06 ± 0.01	26.06 ± 1.26
p-value		0.556	0.032	0.210	0.140

[*]comparison by independent samples t-test; [#]parasitaemic: infected by *T. brucei*; a,b,c: column means with different superscripts are significantly different ($p < 0.05$); ND: N'Dama, WF: White Fulani, GU: Sokoto Gudali, BO: Red Bororo.

Serum indirect bilirubin level was significantly ($p<0.037$) higher in anaemic aparasitaemic cattle compared to anaemic parasitaemic counterparts. Among cattle genotypes, total bilirubin was higher ($p<0.047$) in ND(GUxWF) compared to ND(BOxWF) and NDxWF but similar to values for other genotypes. Indirect bilirubin was higher ($p<0.016$) in 3/4NDx1/4WF compared to ND(WFxMU) and parasitaemic WF but similar to values observed in other genotypes. Direct (conjugated) bilirubin did not differ significantly among genotypes. Serum urea was highest ($p<0.002$) in 3/4NDx1/4WF cattle followed by ND(GUxWF) which was higher than the value for ND(BOxWF) but similar to values for other genotypes. Total bilirubin was higher ($p<0.038$) in aparasitaemic ND compared to WF counterparts but similar between these and crossbred counterparts while indirect bilirubin was higher ($p<0.010$) in aparasitaemic ND and crossbred cattle compared to WF. Aparasitaemic crossbred cattle and ND also had higher ($p<0.032$) serum indirect bilirubin level compared to parasitaemic WF cattle.

Discussion

The observed lower serum total protein and albumin in anaemic animals agreed with previous studies on effects of anaemia on serum protein profile of ruminants and other animals (Velayudhan *et al.,* 2015). Decreased serum total protein and albumin levels are had been reported in anaemia resulting from parasitic infections and nutritional deficiencies in animals (Katsogiannou *et al.,* 2018). The studied herd was on high plain of nutrition, as indicated by the serum protein values that were within the reference range for cattle (Nagy *et al.,* 2015; Rocha *et al.,* 2019), and was treated against ectoparasites and gastrointestinal parasites prior to and during the study thus, eliminating nutritional deficiency, ectoparasitism and helminthiasis as possible causes of the anaemia. The finding of trypanosomes in the herd by blood microscopy (even though only in anaemic WF cattle) suggests that the anaemic animals were most likely infected by trypanosomes. The observed higher serum albumin level in anaemic NDxWF, ND(GUxWFxMU) and ND cattle compared to WF counterparts indicate greater capacity to retain body reserves under disease challenge which is an important attribute of trypanotolerance (Igbokwe, 2018). Comparison between crossbred cattle, ND and WF as well as non-anaemic animals belonging to these genotypes also revealed higher total serum protein and albumin in the crossbred cattle and ND compared to WF and this indicates breed differences in agreement with previous studies (Nosike *et al.,*2020; Shafiq *et al.,* 2021). Abwage (2022) reported higher serum proteins in trypanotolerant N'Dama and Muturu compared to trypanosusceptible White Fulani and White Fulani cross. The significantly lower total serum protein observed in anaemic ND compared to anaemic crossbred cattle and WF could result from the lower level of serum globulin observed in this breed and this indicates lower antigenic stimulation by hemoparasite infection probably due to higher innate resistance to trypanosomiasis. The significantly lower serum albumin in anaemic WF compared to crossbred cattle indicate down regulation of albumin synthesis in favour of globulin synthesis in this breed probably in response to inflammation due to hemoparasite infection (Tothova *et al.,* 2016) and this accounts for the significantly higher serum globulin observed in this breed. Albumin is a transport and homeostatic serum protein, a free-radical scavenger (Hankins, 2006; Tothova *et al.,* 2018) and a major source of amino acids for synthesis of various body protein fractions as need arises (Mackiewicz, 1997; Tothova *et al.,* 2018). Synthesis of albumin is down regulated and amino acids channeled to synthesis of immunoglobulins and positive acute-phase proteins such as haptoglobins during inflammatory processes (Tothova *et al.,* 2018).

Globulins (α-, β-, and γ-globulins) are positive acute-phase proteins which protect cells against pathological damage, remove invading parasites, restore homeostasis, and regulate inflammation (Petersen *et al.,* 2004). Synthesis of globulins are up regulated during inflammatory responses (Petersen *et al.,*2004; Tothova *et al.,* 2018) and greater production of acute-phase proteins is normally associated with a decrease in production of negative acute-phase proteins such as albumin (Choi *et al.,* 2021).

The observed serum ALT, AST, ALP, and creatinine values for anaemic and non-anaemic animals in the present study are within the range reported for apparently healthy cattle (Alalade *et al.,* 2021) indicating absence of severe organ pathologies in the studied animals. This could also explain the non-significantly different values for ALT, ALP and creatinine across and among genotypes and the predominantly similar values for serum level of AST among genotypes. The observed significantly higher serum AST level in anaemic NDxWF compared to anaemic WF and non-anaemic ND(WFxMU), ND(GUxWU), 3/4ND1/4WF, and WF could arise from mild hepatocellular inflammation, RBC hemolysis and/or lysis of trypanosome parasites in this genotype as part of cellular defense against hemoparasitism. Our previous report showed that anaemic NDxWF cattle had very low PCV (22.00 ± 2.69 %) and RBC count (4.09 ± 0.70 x10^6/ul) (Ogbu *et al.,* 2023).

Values reported for the various bilirubin species, and urea in the present study were in agreement with reference values for cattle (Debbarma *et al.,* 2020; Shittu *et al.,* 2020) indicating absence of major disturbances in bilirubin and urea metabolism. The higher indirect bilirubin in non-anaemic 3/4ND1/4WF cattle compared to ND(WFxMU) and WF counterparts as well as the higher serum total and indirect bilirubin in ND compared to WF breed was probably due to genetic differences in concord with Zaitsev *et al.* (2020). Elevated serum bilirubin beyond upper reference limit can occur due to over production as in pathologic proteolysis (such as haemolysis), impaired liver uptake, impaired conjugation, and bile clearance defects as in defects of clearance proteins and biliary stasis (Ruiz *et al.,* 2021). On the hand, significantly altered blood urea level indicates renal dysfunction or problems of urea production in hepatocytes. The lower serum values of indirect bilirubin in anaemic ND(GUxWFxMU), WF, and ND could be attributed to the antioxidant activity of serum bilirubin (Guan *et al.,* 2023) in these animals. Serum bilirubin is an important endogenous antioxidant. It is reported that serum bilirubin exerts about one-quarter of the total integral radical scavenging activity in the body (Gopinathan *et al.,* 1994; Kunutsor *et al.,* 2017). The higher level of serum urea in some non-anaemic genotypes could result from higher feed (and therefore protein) intake leading to higher urea production (Higgins, 2016).

Trypanosome parasitaemic cattle had significantly lower serum albumin and non-statistically higher serum globulin similar to findings of Ismail and Eman (2014) in camel. Akinseye *et al.* (2020) indicated that decreased total serum protein and albumin, and increased serum globulin levels were consistent findings. The decrease in albumin could be due to acute phase response in which amino acids are channeled away from albumin synthesis to synthesis of acute phase proteins and immunoglobulins in response to antigenic stimulation and inflammation (Ozdek *et al.,* 2020). The results are however, contrary to Aliyu *et al.* (2022) who reported decreased total protein, albumin, and globulin in naturally infected compared to uninfected cattle. The researchers attributed the hypoproteinaemia, hypoalbuminaemia, and hypoglobulinaemia to trypanosome uptake of albumin-bound fatty acids, and lipoproteins, increase in catabolism of body proteins, or immunologic response due to trypanosome infection. On the other hand, Takeet and Fagbemi (2009) observed no change in albumin level but increased total protein in cattle post infection and this was ascribed to increase in globulin synthesis needed in immune response. The significantly higher total protein in aparasitaemic NDxWF compared to ND(BOxWF) and WF counterparts as well as the higher total protein and albumin in crossbred cattle and ND compared to WF indicate genetic differences probably related to feed intake, protein metabolism, and nutrient retention under natural trypanosome challenge. These findings point to higher trypanotolerance capacity in the crossbred genotypes and ND cattle compared to the WF as alluded to by Abwage (2022). The lower serum albumin in parasitaemic WF compared to aparasitaemic ND(GUxWFxMU) and ND(WFxMU) could be attributed to the utilization of host nutrients by trypanosomes (Akinseye *et al.,* 2020), the channeling of protein resources to synthesis of immunoglobulins (Tothova *et al.,* 2016) in response to trypanosome antigens, loss of appetite and protein deficit or impaired protein synthesis due to hepatic inflammation (Awekew *et al.,* 2017) sequel to trypanosome infection.

The reported values of ALT, AST, ALP, and creatinine for parasitaemic and aparasitaemic cattle across genotypes and anaemic genotypes, as well as the values for the different genotypes were generally close to the lower limit of the reference range for Bovidae: ALT: 3.0 – 54 u/L, and AST: 17 – 144 u/L (Saeed *et al.,* 2022), ALP: 18 – 153 u/L (Mili *et al.,* 2020), creatinine: 0.95 – 1.71 mg/dL (Abd Ellah *et al.,* 2014), and 0.7–1.5 mg/dL (Kuha *et al.,* 2009) indicating suboptimal organ functions but absence of severe organ damage in the studied animals. The lower ALP in anaemic parasitaemic cattle could result from nutritional deficiency arising from anorexia due to trypanosome parasitaemia or suboptimal synthesis in hepatocytes due to cellular inflammation while the higher serum creatinine in this group could result from higher body protein catabolism to make up for the nutritional (energy and protein) deficiency. The higher serum AST in aparasitaemic ND(GUxWFxMU) compared to ND(GUxWF), 3/4ND1/4WF, and WF counterparts as well as the higher levels in crossbred cattle and ND compared to WF could arise from genetic differences (Kuha *et al.,* 2009). The higher serum creatinine observed in 3/4ND1/4WF compared to other aparasitaemic genotypes could be attributed to genetic differences while the higher level in parasitaemic WF compared to aparasitaemic ND could arise from trypanosome induced nutrient deficiency and body protein catabolism in the infected WF breed. Similarly, the higher serum creatinine in parasitaemic WF compared to aparasitaemic crossbred cattle and ND suggest higher endogenous protein catabolism in the WF probably associated with anorexia and nutritional deficiencies as a result of disease severity. We observed more pronounced clinical signs of trypanosomiasis (poor body condition, weight loss, bellowing, salivation, and muscle twitching) in the WF breed compared to the ND and the crossbred genotypes. The observed normal range values for serum levels of liver enzymes and creatinine was in agreement with Fidelis-Junior *et al.* (2016) who reported AST, ALP and creatinine levels that were within range in cattle experimentally infected with *T. brucei*. Bakari *et al.* (2017) however, reported statistically similar ALP but elevated creatinine and lower ALT values in naturally infected cattle compared to uninfected group. In another herd, the authors observed non-significantly different ALT but elevated ALP and creatinine in infected animals. The similar biochemical parameters were attributed to chronicity of trypanosome infection. Our findings also partly agree with Aliyu *et al.* (2022) with regard to AST activity. Anyogu *et al.* (2020) observed similar values of ALP activity but lower AST and ALT activity in *T. congolense* infected rams. Aliyu *et al.* (2022) reported significantly higher ALP and ALT in infected cattle contrary to our findings. Takeet and Fagbemi (2009) had observed elevated ALT and AST post infection in experimentally infected cattle. Elevated aminotransferases in infected compared to uninfected subjects were also reported in naturally infected camel (Ismail and Eman, 2014), and experimentally infected goats (Karaye *et al.,* 2017). The normal range reported for biochemical indices in the present study could be attributed to chronic infection following persistent challenge and development of innate resistance especially in the crossbred and ND cattle. Bakari *et al.* (2017) stated that trypanosome infection in animals often progresses to chronic states with serum biochemical parameters returning to normal ranges.

The observed higher indirect bilirubin in anaemic aparasitaemic cattle compared to parasitaemic counterparts was probably due to the greater binding sites for serum bilirubin provided by the higher serum albumin in the aparasitaemic animals. Bilirubin from reticuloendothelial cells is transported to the liver bound to serum albumin (Westwood, 1991). The higher indirect bilirubin also suggests higher level of haeme protein (e.g., haemoglobin, myoglobin) catabolism in anaemic aparasitaemic cattle probably due to chronic trypanosomiasis. The observed significantly higher total bilirubin in ND(GUxWF) and GUxND compared to NDxWF as well as the higher blood urea in 3/4ND1/4WF and ND(GUxWF) compared to ND(BOxWF) could result from genetic differences between cattle genotypes as suggested by Zaitsev *et al.* (2020) while the significantly lower serum level of indirect bilirubin in parasitaemic WF compared to 3/4NDx1/4WF and ND as well as the lower level in parasitaemic WF compared to aparasitaemic crossbred cattle and ND could be due to consumption of serum bilirubin in free radical scavenging in the parasitaemic animals (Guan *et al.,* 2023) as a result of oxidative stress resulting from trypanosomiasis.

Conclusion

Anaemic and non-anaemic crossbred cattle and ND had higher serum total protein and albumin than WF counterparts while serum globulin was higher in WF indicating higher trypanosome antigenic stimulation. Crossbred cattle and ND also had lower serum creatinine associated with higher body nutrient profile and/or preserved body nutrient reserves, and higher (but within reference range) serum total and indirect bilirubin which suggests lower oxidative stress challenge. Taken together, these findings suggest stronger trypanotolerance capacity in the crossbred genotypes and ND compared to the WF. The crossbred genotypes should be stabilized as breeds expressing trypanotolerance. Molecular studies to further evaluate trypanotolerance in the crossbred genotypes under experimental infection are warranted.

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Ethical Approval

The research protocols and methodology for this study was approved by the Ethics Committee on Welfare and Use of Animals in Research, Michael Okpara University of Agriculture Umudike dated September 3, 2021.

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