Nig. J. Anim. Prod. 2022, 49(4): 176-193. https://doi.org/10.51791/njap.v49i4.3714 *Nigerian Journal of Animal Production*

Physiological and Coprological Changes Associated with Single and conjunct experimental infections of trypanosoma brucei and haemonchus contortus in west African dwarf goats

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Abstract

An experimental study on the interaction between Trypanosoma brucei and Haemonchus contortus infections was conducted on 36 male West African Dwarf Goats (WADG) of 8–9 months old. Coprological and physiological observations were made on these animals in different groups of 6 groups of 6 animals each. In this study, experimental single infections of either T. brucei or H. contortus and conjunct infection of both parasites produced an acute infection in WADG with initial parasitaemia occurring 7- 14 days post-infection and initial detection of egg in faeces occurring 14 days post-infection. The pre-patent period of infection of T. brucei and H. contortus were influenced by conjunct infections resulting in the early detection of nematode egg in the faeces and trypanosome in the blood of the infected conjunct groups. A high level of parasitaemia was observed more in group 3 compared to other groups. The faecal egg count and worm burden were more in groups 3, 4, and 5 than group 2. Judging from the degree of clinical manifestation, animals in the conjunct group appeared to be more severely affected. Haematological parameter changes were particularly more marked in conjunct infected groups (3 and 5). These observations may not be unconnected with the stress of the conjunct infections and immunosuppressive effects of trypanosomosis. This was very evident in the significant sustained decrease in the levels of packed cell volume, mean haemoglobin concentration and total red blood cell counts following the onset of parasitaemia and patency in the infected goats. The above observation confirmed the long-held view that both parasites precipitate anaemia and immunosuppression in infected animals.

Keywords: Coprology, *Haemonchus contortus,* Parasitemia, *Trypanosoma brucei*

Changements Physiologiques Et Coprologiques Associes Aux Infections Experimentales Uniques Et Conjointes De Trypanosoma Brucei Et Haemonchus Contortus Dans West African Dwarf Goats

Résumé

Une étude expérimentale sur l'interaction entre les infections à Trypanosoma brucei et à Haemonchus contortus a été menée sur 36 West African Dwarf Goat (WADG) mâles âgées de 8 à 9 mois. Des observations coprologiques et physiologiques ont été faites sur ces animaux dans différents groupes de 6 groupes de 6 animaux chacun. Dans cette étude, des infections expérimentales uniques de T. brucei ou de H. contortus et une infection conjointe des deux parasites ont produit une infection aiguë dans le WADG avec une parasitémie initiale survenant 7 à 14 jours après l'infection et une détection initiale d'œufs dans les fèces survenant 14 jours après. -infection. La période d'infection pré-patente de T. brucei et H. contortus était influencée par des infections conjointes résultant en la détection précoce *d'œufs de nématodes dans les fèces et de trypanosomes dans le sang des groupes conjoints infectés. Un niveau élevé de parasitémie a été observé davantage dans le groupe 3 par rapport aux autres groupes. Le nombre d'œufs fécaux et la charge de vers étaient plus élevés dans les groupes 3, 4 et 5 que dans le groupe 2. À en juger par le degré de manifestation clinique, les animaux du groupe conjoint semblaient être plus gravement touchés. Les modifications des paramètres hématologiques étaient particulièrement plus marquées dans les groupes infectés conjointement (3 et 5). Ces observations ne sont peut-être pas sans lien avec le stress des infections conjointes et les effets immunosuppresseurs de la trypanosomose. Cela était très évident dans la diminution significative et soutenue des niveaux d'hématocrite, de la concentration moyenne d'hémoglobine et du nombre total de globules rouges après l'apparition de la parasitémie et de la perméabilité chez les chèvres infectées. L'observation ci-dessus a confirmé l'opinion de longue date selon laquelle les deux parasites précipitent l'anémie et l'immunosuppression chez les animaux infectés.*

Mots-clés : Coprologie, *Haemonchus contortus*, Parasitémie, *Trypanosoma brucei*

Introduction

Polyparasitism is the most common form of animal parasitosis in tropical areas (Chiejina, 2001). Mixed infections of livestock with various species or the same species of parasites are more common than single parasite infections (Sharma *et al.*, 2000; Okaiyeto *et al.*, 2008; Aga *et al.*, 2014). In mixed infections, the presence of a pathogen may enhance the effect of the other pathogen in the host. This is more likely to occur when the first infection has an immunosuppressive effect on the host thus making the latter more vulnerable to other parasites to which it was resistant (Aga *et al.*, 2014). Conjunct infections involving Haemonchus and trypanosomes are the most common form of polyparasitism (Okaiyeto *et al.*, 2008).

Conjunct interaction between parasites may lead to significant physiological changes in body tissue and fluids which could not be attributed to either of these parasites individually. Under experimental conditions, concurrent infections of T. *congolense* and H. *contortus* resulted in more severe disease in sheep (Aga *et al.*, 2014) and in goats (Sharma et al., 2000) than single infections. Studies on West African Dwarf Goats (WADG) in Gambia and the sub humid derived savanna zone of eastern Nigeria revealed that conjunct infection of WADG with H. *contortus* and T. *brucei* resulted in depressed antibody response to H. contortus infection and strongly increased faecal egg counts (Faye et al., 2002; Chiejina *et al.*, 2005).

The aim of the current study was to investigate the physiological changes associated with single and conjunct infections of WADG with T. *brucei* and H. *contortus*.

Materials and Methods

A total of 36 male WADG aged 8 to 9 months old that looked healthy were used for the current study. Male WADG were used to avoid any interference from normal physiological changes resulting from pregnancy and lactation. The goats were purchased from markets around Umuahia, Abia State, Nigeria. They were housed at Veterinary Parasitology animal house at Michael Okpara University of Agriculture, Umudike, after routine anthelmintic treatment. They were also vaccinated against Pestes des petites ruminants (PPR) (NVR1, VOM) before introducing them to the quarantine pens where they were monitored for 30 days before transfer to the experimental pens. They were acclimatized for four weeks prior to the commencement of the experiment and were fed daily with fresh cut and carry grass and a supplement concentrate mixture comprising of palm kernel cake and grower's mash as described

by Fakae *et al*., 1999. They were given water ad libitum; the pens were all screened against flies.
The nematode parasite (H. *contortus)* and the protozoan parasite (T. *brucei*) were gotten and isolated using the method described by Okaiyeto *et al*, 2008. The isolated parasites were stored until use.

Table 1: Groups and level of Infection

Groups		No of animals	Dose level of *H. contortus* (L_3)	Dose level of *T. brucei* Tryps/ml
1	Infected with *H. contortus* only	6	4500	Nil
2	Infected with *T. brucei* only	6	Nil	1×10^6
3	Infected with *H. contortus* and *T. brucei* on the same day	6	4500	1×10^6
4	Infected with *H. contortus* and after 7 days *T. brucei*	6	4500	1×10^6
5	Infected with *T. brucei* and after 7 days infected with *H. contortus*	6	4500	1×10^6
6	Uninfected control	6	Nil	Nil

Parameters assessed include Body weights (BW), Rectal temperature (RT), Body Condition score (BCS), Faecal egg counts (FEC), Parasitaemia, Packed cell volume (PCV), Red blood cell counts (RBC), Total white blood cell counts (WBC), Haemoglobin concentration (Hb), Differential white blood cell counts, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemonglobin (MCH). All these parameters were determined on Day 0 and subsequently every week till the end of the experiment. At the end of the experiment (day 56), the animals were humanely sacrificed, and worm burdens (WB) were counted and recorded across the groups.

Results

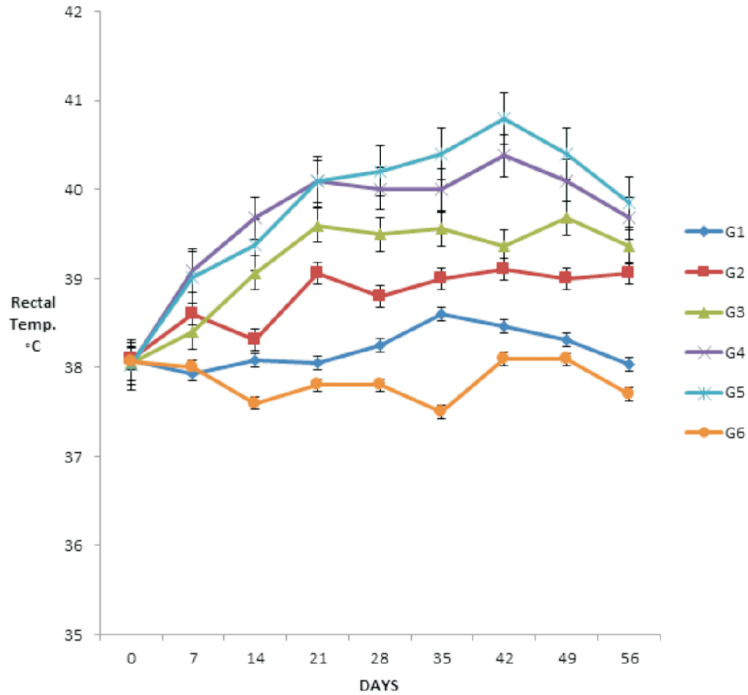
Clinical signs observed in the infected groups from day 14 through the end of the experiment include depression, lethargy, reduced activity, dullness, and loss of body condition. These observable signs occurred more in the conjunct groups 3, 4 and 5 when compared with the single infected groups 1 and 2. In addition, diarrhoeic faeces were observed in some of the goats infected with T. brucei before infecting them with H. *contortus*.

Onset of parasitaemia

Parasites were detectable in the blood of the conjunct infected goats in groups 3, 4, and 5 seven - ten days post infection. Parasitaemia was first noticed on day 7 post infection in the conjunct group 5. By this day 80%, 50%, and 90% of the goats in the conjunct groups of 3, 4 and 5 respectively were parasitaemic. By day 14 post infection, all the goats in the other singly infected group 2 were all parasitaemic.

Patency

Presence of egg was first observed in the faeces of the conjunct infected goats in groups 3, 4, and 5 fourteen – eighteen days post infection. Patency was first noticed on day 14 post infection in the conjunct group 5. By this day 70%, 60% and 80% of the goats in the conjunct groups of 3, 4 and 5 respectively were patent. By day 21 post infection, all the goats in the other singly infected group 1 were all patent.



Body weight

Figure 1 Rectal body temperature of different infection groups from day 0 to day 56

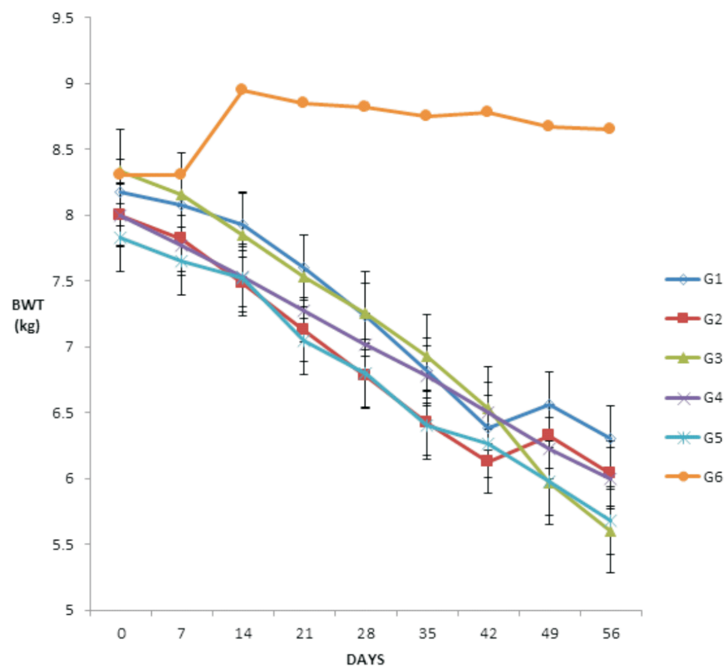


Figure 2: Body weight of different infection groups from DAY 0 to DAY 56

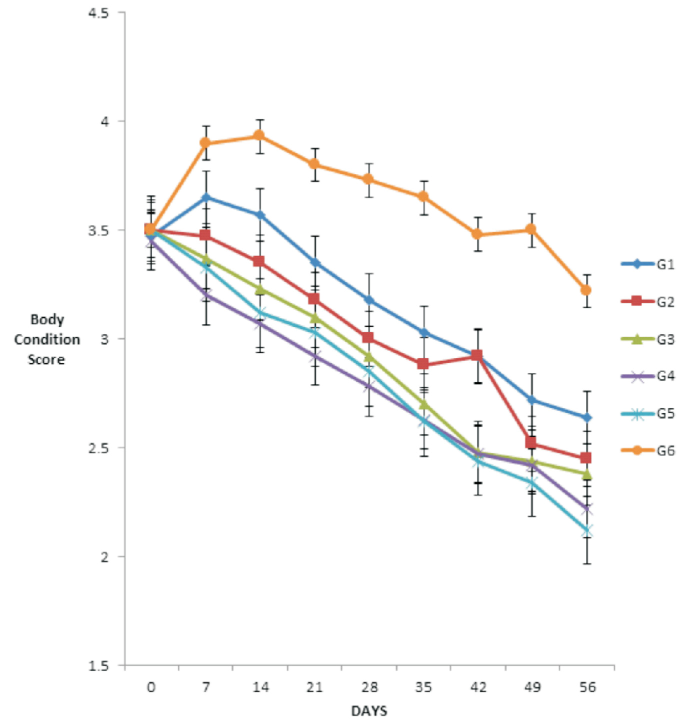


Figure 3: Body condition score levels of different infection groups from DAY 0 to DAY 56

Coprology

Faecal egg count

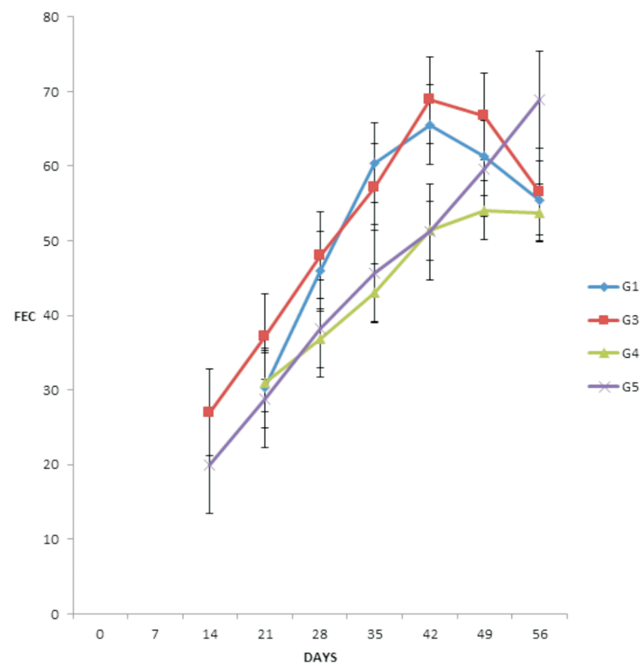


Figure 4: FEC levels of different infection groups from DAY 0 to DAY 56

Worm burden

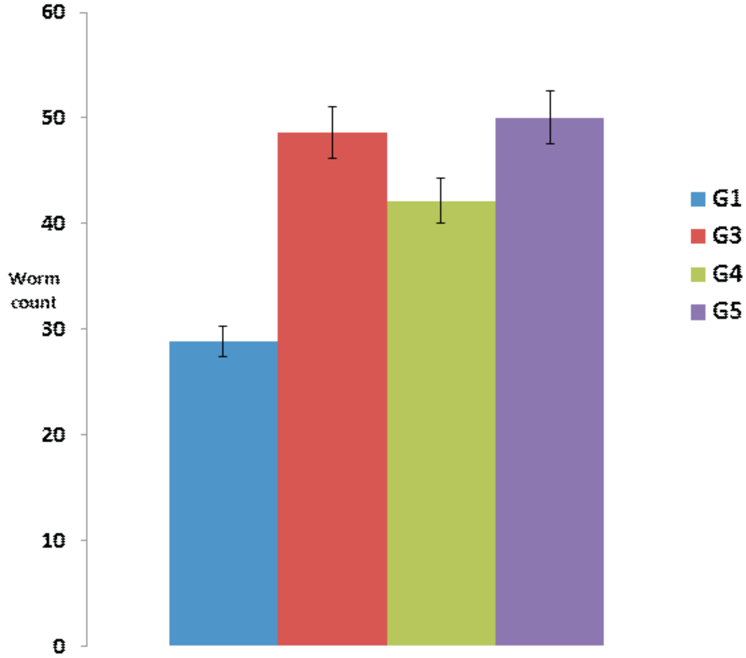


Figure. 5: The postmortem worm count determination for the groups infected with *H. contortus*.

Haematology

Parasitaemia

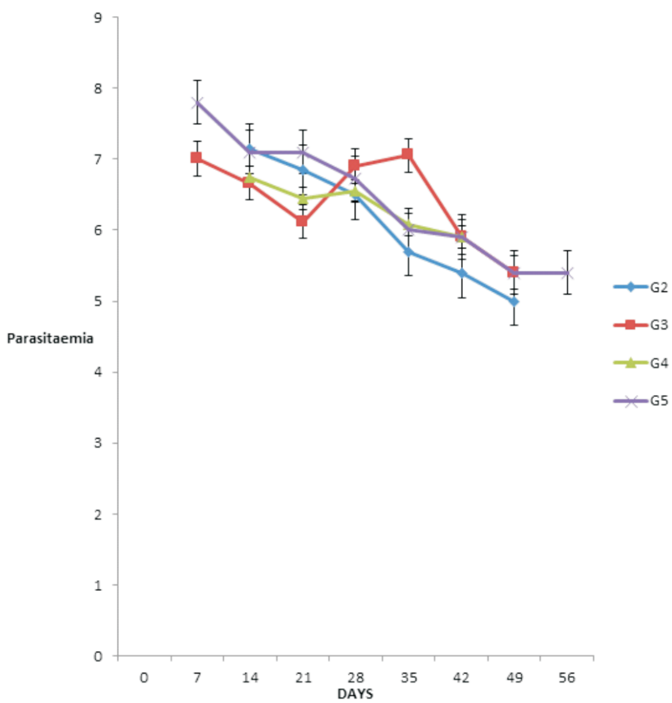


Figure 6: Parasitaemia levels of different infection groups from DAY 0 to DAY 56

Packed cell volume (PCV)

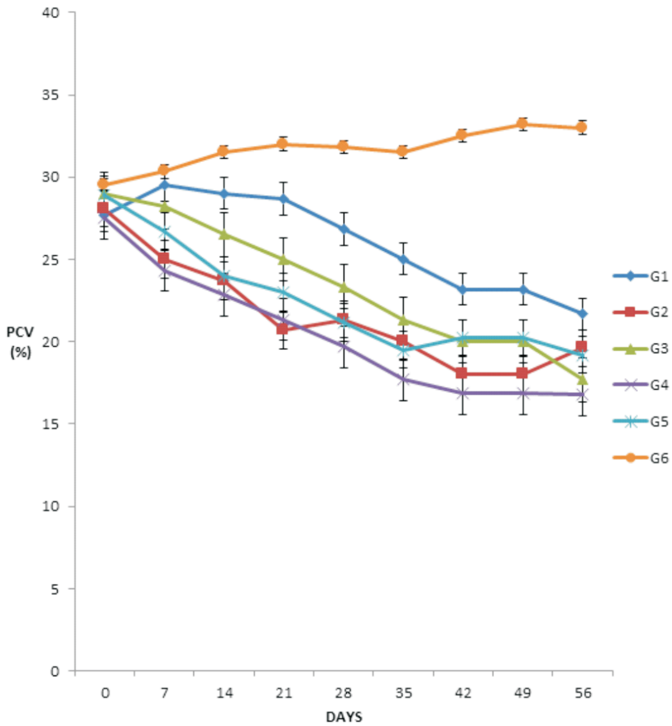


Figure7: PCV levels of different infection groups from DAY 0 to DAY 56

Mean total red blood cell count (RBC)

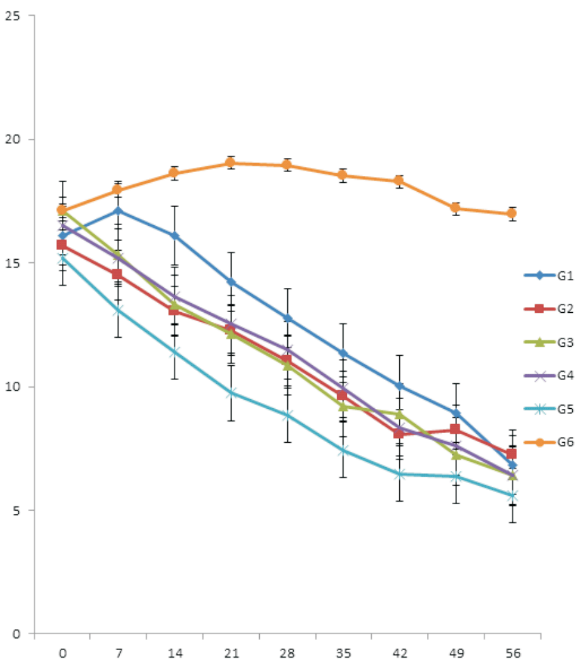


Figure 8: RBC levels of different infection groups from DAY 0 to DAY 56

Mean total white blood cell count (WBC)

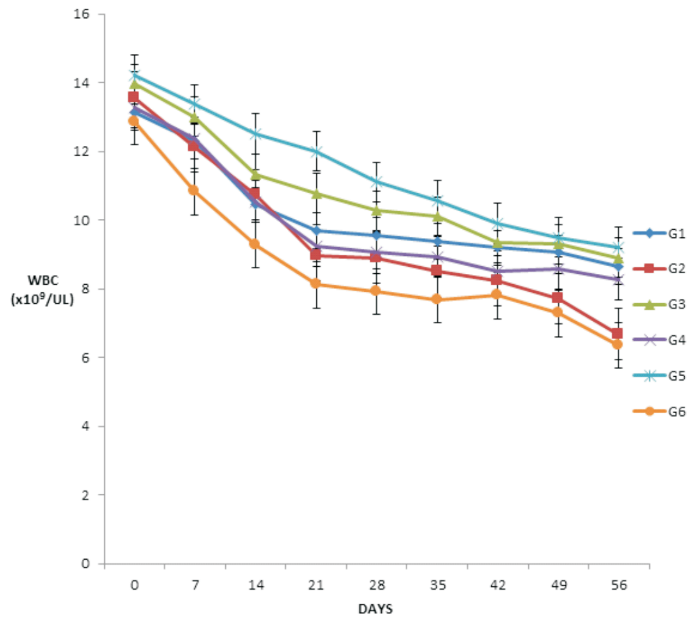


Figure 9: WBC levels of different infection groups from DAY 0 to DAY 56

Mean haemoglobin concentration (MCH)

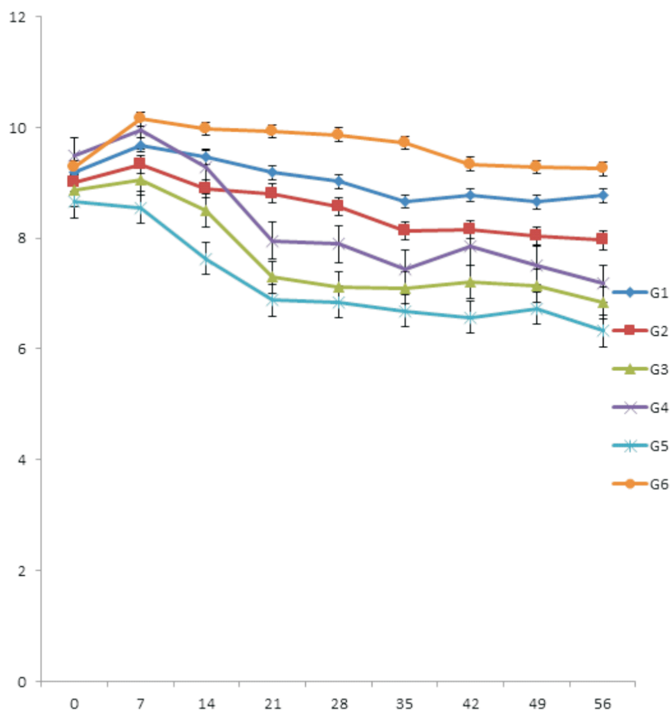


Figure 10: Hb concentration of different infection groups from DAY 0 to DAY 56

Mean corpuscular volume (MCV)

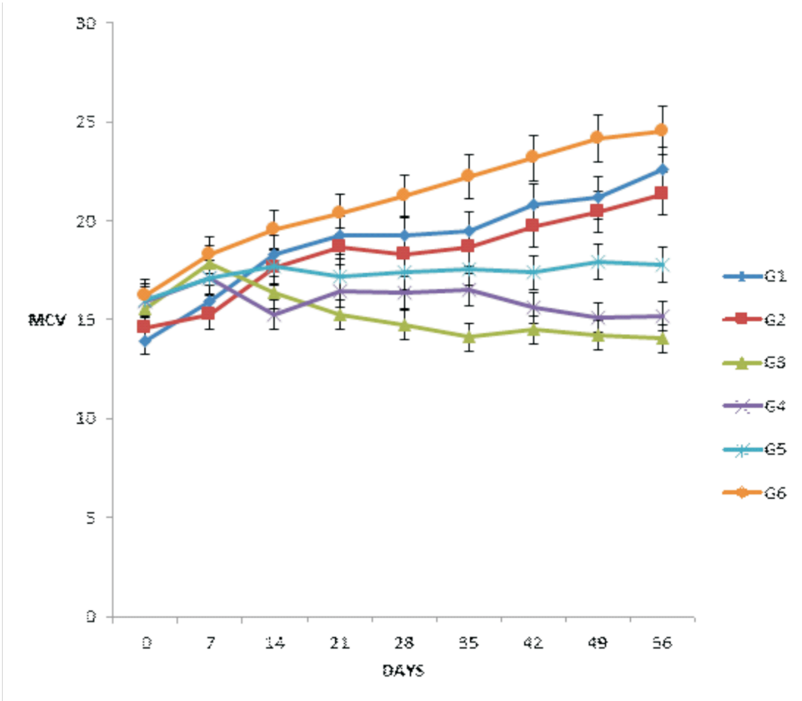


Figure 11: MCV of different infection groups from DAY 0 to DAY 56

Mean corpuscular haemoglobin (MCH)

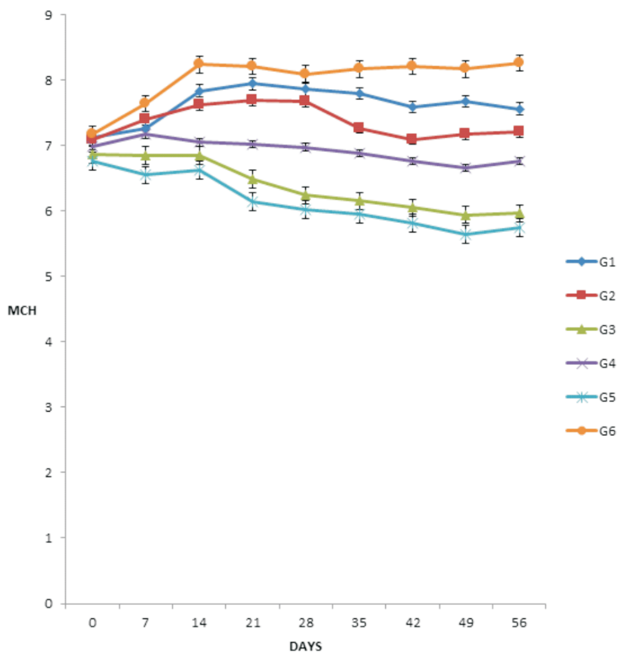


Figure 12: MCH of different infection groups from DAY 0 to DAY 56

Mean corpuscular haemoglobin concentration (MCHC)

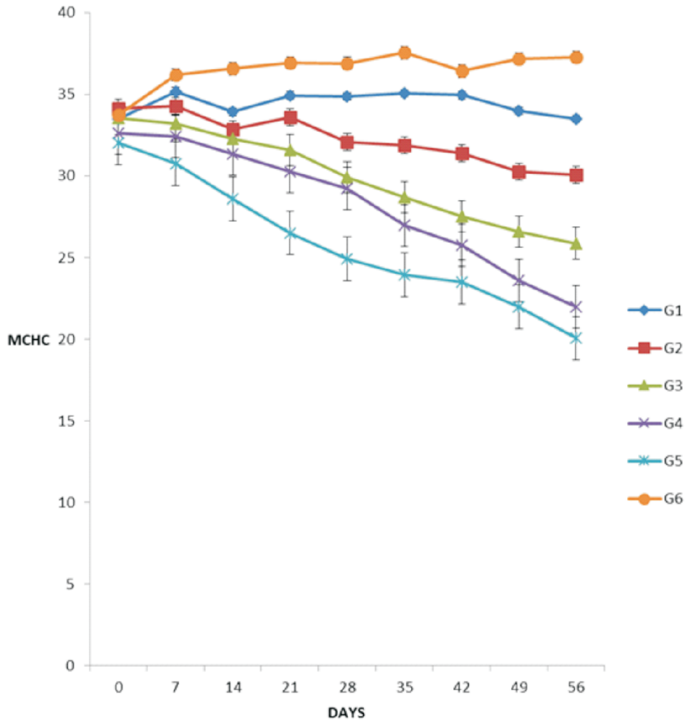


Figure 13 MCHC levels of different infection groups from DAY 0 to DAY 56

Differential leucocyte counts

Eosinophil

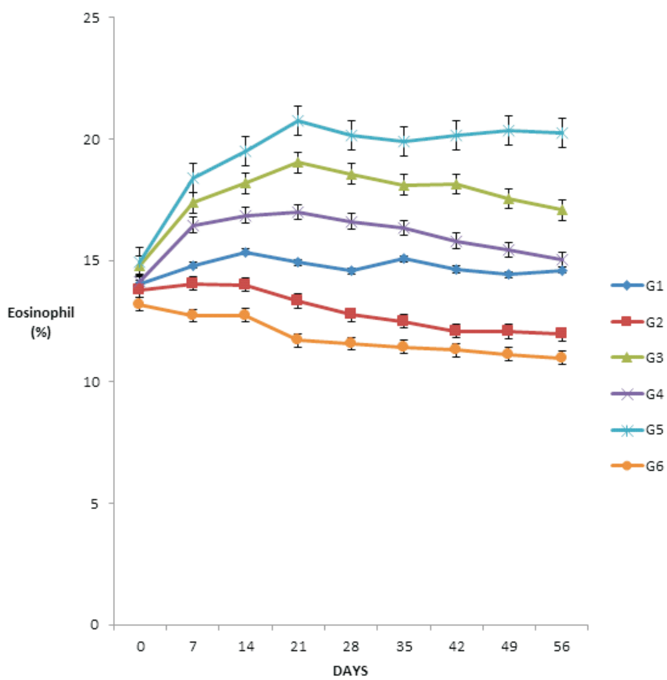


Figure 14: Eosinophil levels of different infection groups from DAY 0 to DAY 56

Basophil

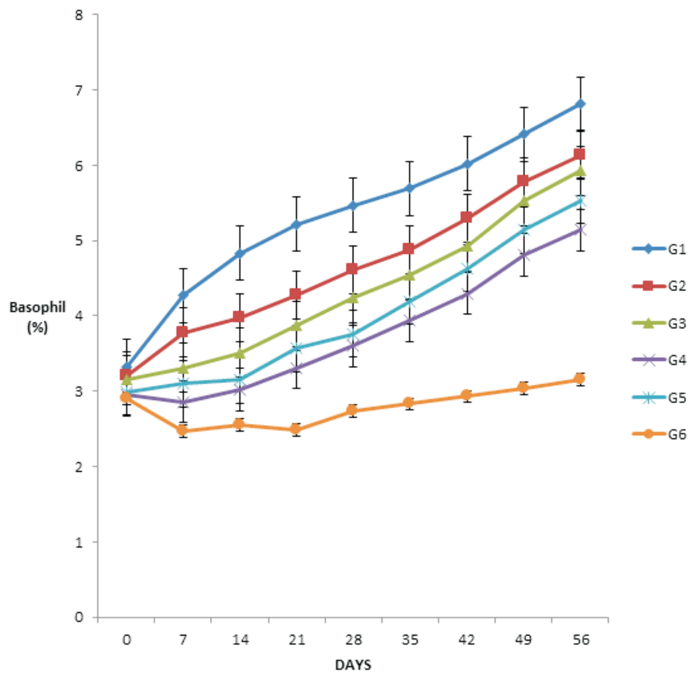


Figure 15: Basophil levels of different infection groups from DAY 0 to DAY 56

Neutrophil

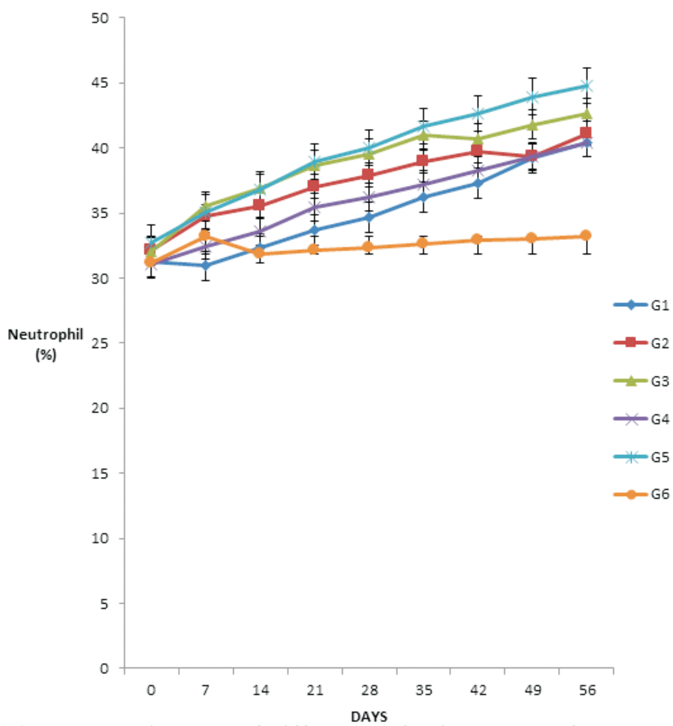


Figure 16: Neutrophil levels of different Infection groups from DAY 0 to DAY 56

Monocyte

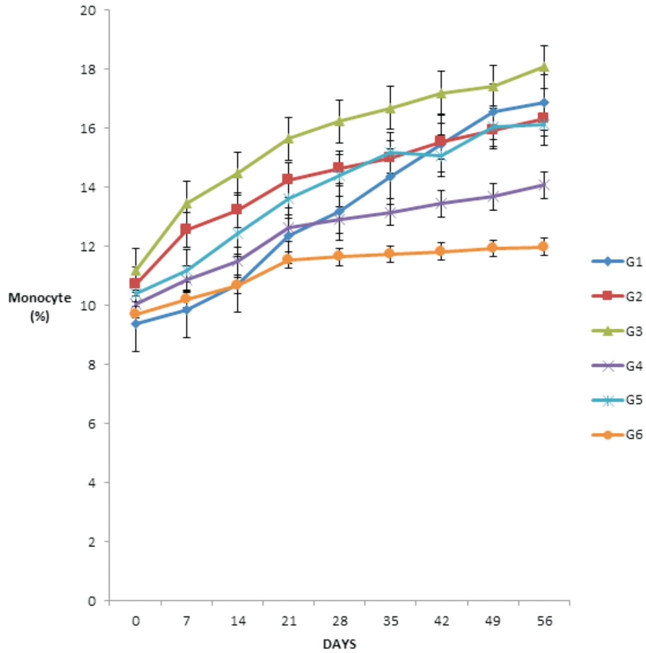


Figure 17: Monocyte levels of different infection groups from DAY 0 to DAY 56

Lymphocyte

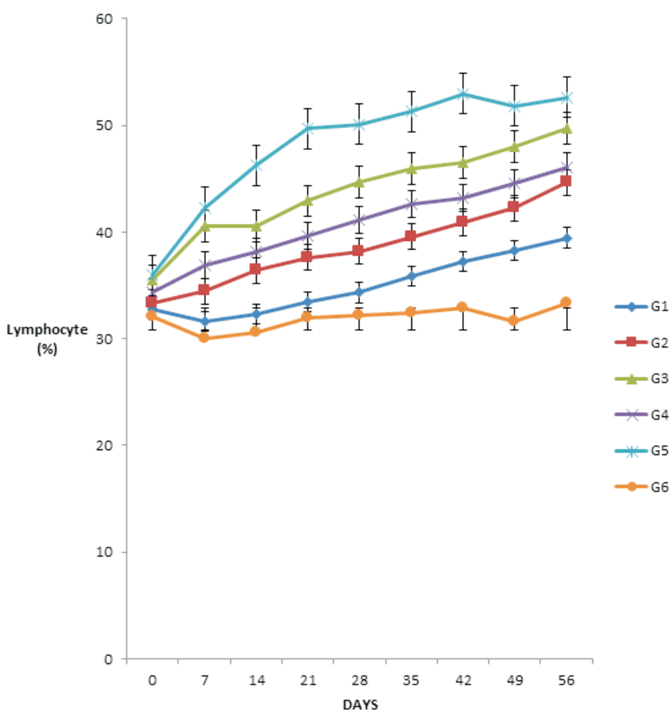


Figure 18: Lymphocyte levels of different Infection groups from DAY 0 to DAY 56

Discussion

The clinical signs of inappetence, anorexia, roughness of the hair coat, diarrhea, paleness of the mucous membrane of the eyes and pyrexia observed in this study were characteristic of haemonchosis (Fakae *et al*; 1999; Urquhart *et al*, 2002) and trypanosomosis (Ezeokonkwo, 2009). These observed clinical signs were however not pathognomonic for haemonchosis and trypanosomosis in goats as they can occur in other diseases of goats. However, these clinical signs were less severe in single infection groups as the goats in those groups were able to adjust and tolerate these infections. This agrees with the findings of Chiejina and Behnke (2011) who reported that WADG breeds were both trypanotolerant and haemonchotolerant. In contrast, conjunct infection with both parasites produced more severe clinical signs. Similar findings were made by Sharma *et al.,* 2000 in goats with conjunct infection of *T. evansi* and *H. contortus*. The immune suppressive effects of trypanosomes may be responsible for this enhanced susceptibility.

Pyrexia observed in this study is also a recognized clinical symptom of trypanosomosis in infected animals (Dinarello; 2009). The result showed that conjunct infection groups (3 and 5) increased the mean rectal temperature significantly ($P < 0.05$) by the 14^{th} day of infection when all the infected goats have become parasitaemic compared to the single infection group. This increase may be due to the stimulation of the thermo-regulatory center of the hypothalamus by pyrogens released during infection.

The result of the study also revealed significant poor body condition score in all the infected groups when compared with the uninfected control. Goats which were singly infected (groups 1, 2) by either parasite had significantly ($P < 0.05$) higher body condition scores than the conjunct infected groups (3, 4, and 5). It has been reported that both *T. brucei* infection and *H. contortus* infection independently have a negative effect on the body condition score of infected goats. This may have been induced by dullness or anorexia observed in the infected animals (Batista *et al*, 2009). The significant ($P < 0.05$) decreases in the body weight of the infected goats observed in this study especially in the conjunct infected groups is in accordance with other reports (Holmes *et al*., 2000; Ezeh *et al*., 2009). This is thought to be associated with anorexia and dullness caused by the infection with the parasites.

The results of this study showed that *H. contortus* infection first occurred in the faeces of the infected WAD goats by day 14 PI and by day 21 PI, patency had occurred in all the infected goats. This relatively short pre-patent period recorded in this study may partly be due to the young age or immune competence of the studied animals. Similar observations have also been made by other workers (Sharma *et al*; 2000) who also used young animals in their study. The stress of the conjunct infections may also have been responsible for the shortened pre-patent period observed in the conjunct infected groups. This disagrees with the findings of Idika *et al.,* (2012) who recorded a slightly longer pre-patent period in WAD sheep. It must be noted that the sheep used by Idika *et al.,* (2012) for the experiment were older.

Parasitaemia due to *T. brucei* infection was first detectable in the blood of the infected WADG seven days post infection and by the 14^{th} day post infection parasitaemia had occurred in all the goats in all the infected groups. This result is consistent with the findings of other workers (Boly, 1993; Sharma *et al*., 2000). Conjunct infection groups (3 and 5) appeared to have a shortened pre-patent period than the single infection groups as parasitemia and patency occurred earlier.

A remarkable observation from this study is that the FEC was influenced by the conjunct infection of hence the significant increase ($P < 0.05$) in the FEC of goats in group 5. This agrees with previous reports which held that concurrent infection led to a marked increase in FEC in concurrently infected animals (Sharma *et al*., 2000; Okaiyeto *et al,* 2008). The immune suppressive effects of *T. brucei* may be responsible for the significant increase in the concurrent infections where the experimental goats were infected first with *T. brucei* and after seven days with *H. contortus*. In contrast to the above findings, Onah *et al* (2004) showed a significant decrease ($P > 0.05$) in FEC of rats concurrently infected with *T. brucei* and *Strongyloides ratti*. The authors opined that the *T. brucei* and *S. ratti* may have interacted in a manner that ameliorated their pathogenic effects resulting in a decrease in the level of FEC. Again, it has been reported that the faecal egg output is inversely proportional to the number of worms that established in the infected animals (Sharma *et al*., 2000). It is thus conceivable in this study, that the poor ability of the worms to establish and mature in the intestine coupled with the immune suppressive effects of trypanosomes and haemoncho-tolerant nature of the WADG may have led to the moderation of the egg output and the worm burden recorded in the infected goats.

Infection with both parasites either singly or concurrently affected the outcomes of the haematological parameters of the infected WADG. There was a significant ($P < 0.05$) reduction in PCV, Hb and RBC values in all the infected groups throughout the study. These results agree with the findings on the impact of each parasite infection on haematological parameters (Sharma et al., 2000; Batista *et al*., 2009). Decrease in values of all the parameters observed in the study is indicative of anaemia which is a major clinical sign of both parasite infections (Fakae *et al*., 1999: Urquhart *et al,* 2002). The anaemia was manifested in the living goats by palour of the mucous membranes of the eye. The reduced values in infected animals may be attributed to the bleeding in the abomasum due to the injuries caused by the *H. contortus* like that described by Abdel (1992) and destruction of red blood cells by trypanosomes in the blood stream of affected animals (Anosa, 1988). The mechanisms that initiate, modulate, and sustain anaemia in trypanosomosis and haemonchosis is widely believed to include erythrocyte destruction by phagocytosis; dyserythropoeisis, disseminated intravascular coagulation and increased plasma volume and haemo-dilution. The significant ($P < 0.05$) increase in the values of PCV, Hb, and RBC beyond the pre-infection values in the uninfected control may be due to the length of time the experiment lasted leading to the increase in the age of the experimental goats by about three months. Also, the improved feeding of the experimental goats during the study may have contributed to the increased levels of the PCV and Hb observed in the study. It must be noted that the conjunct infection of *T. brucei* and *H. contortus* (groups 3 and 5) produced a more severe anaemia than the single infection of either *T. brucei* or *H. contortus*. This may be attributed to the combined effect of the parasites on the vascular system of the goats since *H. contortus* is an avid blood sucker in the intestine and a cause of chronic external blood loss in infected goats (Urquhart *et al*, 2002) while *T. brucei* infection leads to accelerated destruction of red blood cells in infected animals (Anosa and Kaneko, 1989).

An analysis of red blood cell indices from the study indicated that single infection with *H. contortus* produced a macrocytic hyperchromic anaemia. This may have been because of poor dietary uptake of vitamim B_{12} or folic acid consequent upon infection with the parasite. Similar findings have been reported by other workers (Ayub *et al*., 2011). On the other hand, single infection with *T. brucei* produced a microcytic hypochromic anaemia as observed from the results of the study. This may have occurred because of impaired nutrition leading to poor dietary intake of iron. This agrees with the findings of other workers on the pathology of trypanosome infections (Ekanem *et al*., 2008). Concurrent infection with both parasites on the same day led to a more severe microcytic hypochromic anaemia, the presence of both parasites heightening the pathogenic effects of *T. brucei* infection in the affected goats. This suggests an impaired ability of the RBCs to take up as much haemoglobin as is required.

In this study, a low level of parasitaemia was observed in the infected WAD goats throughout the experiment. This agrees with the findings of Talabi *et al.,* (2012) who observed lower levels of parasitaemia in Nigerian Zebu cattle infected with *T. congolense* when compared with the other exotic breeds. This could be because of the reported adaptability or trypano-tolerance of the Nigerian WADG breed to trypanosome infections (Chiejina and Behnke, 2011). Trypanosomes have been reported to display multiple genes that code for different surface coat glycoproteins which enable them to evade the immune responses in their hosts (Aga *et al*., 2014)

Infection of animals produced significantly ($P < 0.05$) elevated leucocyte counts especially in the conjunct infected groups (3 and 5) where in most cases, leucocyte counts were seen to be higher than or comparable to singly infected groups compared to the uninfected control group. Elevated leucocytic parameters could be because of inflammatory responses of the host following introduction of parasite antigens (Tabel *et al*., 2008). Parasitic infections, allergic reactions, and physical stress due to infection have previously been reported to produce elevated leucocyte counts. This agrees with the findings of several workers (Sam-wobo *et al.,* 2010; Bhat *et al*., 2011).

Eosinophils are an important element in the response against parasite infections. The significantly ($P < 0.05$) increased number of circulating blood eosinophils in the present study agrees with the findings of Terefe *et al.,* (2005). The eosinophils mobilized against specific parasites have been frequently found to cause immobility and death of larvae of homologous or heterologous parasites often in association with antibodies and or other factors (Klion and Nutman, 2004). The development of systemic and local tissue eosinophilia is characteristic of the host immune response towards helminth infections. There is also some evidence suggesting that eosinophils may contribute to pathogenesis during parasitic infection.

Increased lymphocyte count observed in the present study agrees with the findings of Bhat *et al*., 2011 and Anthony *et al*., 2008. The increase in lymphocyte count in the conjunct groups seen in this study may be attributable to the combined effects of the proliferation of lymphocytes due to the secretory products of *H. contortus* and the possible antigenic variation due to the *T. brucei*.

Conclusion

Trypanosomosis and haemonchosis have been notably identified as a major constraint on the sustained productivity of small ruminants, especially under intensive and semi-intensive systems of husbandry. Judging from the degree of clinical manifestation, animals in the conjunct group (3 and 5) appeared to be more severely affected.

It was clear from the study that significant alterations in haematological parameters occurred in WADG infected singly or conjunctly with *T. brucei* and *H. contortus*. These changes were particularly more marked in conjunct infected groups (3 and 5). These observations may not be unconnected with the stress of the conjunct infections and immunosuppressive effects of trypanosomiasis. This was very evident in the significant sustained decrease in the levels of packed cell volume, mean haemoglobin concentration and total red blood cell counts following onset of parasitemia and patency in the infected goats. The above observation is in conformity with the long-held view that both parasites precipitate anaemia and immunosuppression in infected animals.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosures

The authors of this manuscript would like to declare that there is no conflict of interest in this work.

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Date received: 18th February, 2022.
Date accepted: 9th March 2022.