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Hematological and clinical studies in West African Dwarf sheep experimentally infected with *Trypanosoma brucei* and treated with diminazene aceturate, levamisole, and vitamin A

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Abstract: The effects of diminazene aceturate (DA), levamisole, and/or vitamin A on hematological and clinical parameters of West African dwarf sheep experimentally infected with *Trypanosoma brucei* were studied. Twenty-four adult sheep were randomly assigned to 6 groups of 4 animals. They were infected with 1×10^6 trypanosomes intravenously (groups 2–6) or were uninfected (group 1). Treatment was administered 2 weeks post infection (PI) in all treated groups, except group 5, which was treated 3 weeks PI. Group 2 (positive control) received 7 mg/kg DA. Group 3 received 7 mg DA and 5 mg levamisole per kilogram of body weight. Group 4 received 7 mg/kg DA and 50,000 IU of vitamin A. Group 5 received 7 mg/kg DA and 50,000 IU of vitamin A. Group 6 received 7 mg/kg DA, 5 mg/kg levamisole, and 50,000 IU of vitamin A. Parameters monitored were parasitemia, respiratory and pulse rates, rectal temperature, body weight changes, packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell counts, and clinical signs. Treatments were successful in all groups with no relapse recorded. Sheep in groups 6, 3, and 4 had significantly ($P < 0.05$) higher PCV and Hb than those treated with DA only or with DA and vitamin A at 3 weeks PI.

Key words: *Trypanosoma brucei*, West African dwarf sheep, clinical studies, diminazene aceturate, levamisole, vitamin A

1. Introduction

African animal trypanosomosis is one of the most important arthropod-borne diseases of cattle and other domestic animals in sub-Saharan Africa (1) and contributes greatly to its underdevelopment (2). It causes reduction in milk production, weight gain, and reproduction and eventually death of the affected animals. Up to 30 million sheep and 40 million goats are at risk of *Trypanosoma vivax*, *Trypanosoma congolense*, and *Trypanosoma brucei* infections across the tsetse belt (3).

In the absence of effective vaccination, control of trypanosomosis is principally by means of either chemoprophylactic or chemotherapeutic agents (4). However, drug resistance, which leads to relapse of infection, is a major challenge often encountered in the treatment of trypanosomosis (5). The situation throughout Nigeria, for instance, has been particularly troubling because resistant strains of the major species of trypanosomes of ruminants (*T. congolense*, *T. vivax*, and *T. brucei*) have been isolated (6). These strains do not respond to curative doses of the three main drugs used for animal trypanosomosis: homidium chloride (Novidium), homidium bromide (Ethidium), and diminazene aceturate (Berenil). Therefore, use of available trypanocides requires careful management in order to minimize the incidence of resistance (7).

The problems of drug resistance have stimulated much research into alternative methods of disease control such as avoidance of subtherapeutic doses, use of sanative pairs, reduction of treatment frequency, use of trypanotolerant animals, tsetse control, and stimulation of host immunity through good nutrition (8). Substances possessing immunopotentiating or immune-enhancing properties such as vitamin A (9) and levamisole (10) may be beneficial when combined with known trypanocides in the treatment of trypanosomosis.

This study was carried out to investigate the efficacy of diminazene aceturate (DA) in combination with levamisole and/or vitamin A in the treatment of West African dwarf (WAD) sheep infected with *Trypanosoma brucei*.

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2. Materials and methods

2.1. Experimental animals

Twenty-four adult WAD sheep of both sexes were used. They were purchased from a local market (Nkwo Ibagwa) in the Nsukka Local Government Area of Enugu State. They were housed in the experimental animal unit of the department. Acclimatization was for some weeks, during which time they were dewormed with albendazole at 15 mg/kg body weight per os (Tuyil Pharm Ind. Ltd., Nigeria) and ivermectin at 1 mL/50 kg body weight subcutaneously. Deticking was done by use of a tick and flea powder (Propets Product & Serv., Nigeria). Long-acting oxytetracycline at 1 mL/10 kg body weight intramuscularly (TETROXY LA, Bimeda, the Netherlands) was also administered to treat bacterial infections. Blood samples collected from the animals were negative for trypanosomes. Feed and water were provided ad libitum. They were humanely cared for in accordance with the principles of laboratory animal care (11). All procedures performed were in accordance with the ethical standards of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

2.2. Trypanosomes

The *T. brucei* used in this study was a local isolate obtained from a clinically infected pig presented at the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The parasites were identified in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The parasites were maintained in albino rats, from which the WAD sheep were first infected.

2.3. Infection of experimental animals

Infected blood from donor rats was collected from the retrobulbar plexus via the median canthus of the eye into vacutainer tubes containing ethylene diamine tetra acetate (EDTA). Infected blood was then diluted in phosphate-buffered saline (PBS). Each sheep was infected with 1×10^6 trypanosomes suspended in 2 mL of PBS intravenously via the jugular vein. Estimation of parasitemia was done by the rapid matching technique (12).

2.4. Experimental drugs

Three drugs used in this experiment were diminazene aceturate (Trypazen, Pantex, the Netherlands), levamisole hydrochloride (Levaject 100, Farvet, the Netherlands), and vitamin A (Aquasol-A, US Vitamins Ltd., India).

2.5. Experimental design

The 24 WAD sheep were randomly assigned into six groups (groups 1–6) of four animals each. Sheep in groups 2–6 were infected with 1×10^6 trypanosomes intravenously while group 1 was left as the uninfected control. Treatment was administered at 2 weeks post infection (PI) in all treated groups except group 5, which was treated at 3 weeks PI. Infected sheep in groups 2–6 were treated with 7 mg/kg diminazene aceturate intramuscularly. Sheep in groups 3, 4, and 6 received additional treatments with levamisole at 5 mg/kg intramuscularly, vitamin A at 50,000 IU/animal intramuscularly, or a combination of levamisole and vitamin A at the same doses, respectively. Sheep in group 5 received similar treatment as those in group 4 at 3 weeks PI.

2.6. Parameters monitored

The efficacy of treatment was assessed weekly by monitoring parasitemia, packed cell volume (PCV) (13), hemoglobin (Hb) concentration (14), body weight, and total red blood cell (RBC) count (15).

2.7 Clinical signs

Rectal temperature and respiratory and pulse rates were monitored daily using a clinical thermometer and stethoscope. Physical examinations were carried out to note the color of mucous membranes, size and consistency of superficial lymph nodes through palpation, and general observations of animals for other clinical signs.

2.8. Statistical analysis

The results of this study were analyzed statistically with SPSS using one-way analysis of variance (ANOVA). Variant means were separated using Duncan's multiple range tests. The level of significance was considered to be $P < 0.05$.

3. Results

3.1. Clinical signs

Intermittent pyrexia was observed in all infected animals from 1 week PI. Other clinical signs observed from the second week of infection included pale mucous membranes, increased respiratory and pulse rates, depression, dullness, edema of the face, enlargement of the prefemoral and prescapular lymph nodes, and nervous signs like muscle spasms, circling, torticollis, and prostration. A mortality rate of 25% was recorded in groups 2 and 3 while in group 5 it was 50%. In group 5 animals, which were treated at 3 weeks PI, complete anorexia, drowsiness, emaciation, and lethargy were also observed. These clinical signs disappeared following treatment.

3.2. Parasitemia

An average prepatent period of 5 days was recorded in all infected sheep. Parasitemia progressed steadily until 2 weeks PI when all the infected sheep were treated, except those in group 5, which were treated 3 weeks PI (Table 1). Trypanosomes were completely cleared from the blood within 1 week of treatment and treated sheep remained aparasitemic throughout the experiment. There was no relapse of infection in all the treatment groups.

3.3. Rectal temperature

The mean rectal temperature of infected sheep (groups 2–6) was significantly higher ($P < 0.05$) than that of uninfected group 1 from week 1 to week 2 PI before the onset of treatment (Table 2). By week 3, there was no significant

Table 1. Number of *T. brucei*-infected WAD sheep before and after treatment with DA and either levamisole or vitamin A combinations.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0	0/4	0/4	0/4	0/4	0/4	0/4
1	0/4	4/4	4/4	4/4	4/4	4/4
2	0/4	3/3	3/3	4/4	3/3	4/4
3	0/4	0/3	0/3	0/4	2/2	0/4
4	0/4	0/3	0/3	0/4	0/2	0/4
5	0/4	0/3	0/3	0/4	0/2	0/4
6	0/4	0/3	0/3	0/4	0/2	0/4
7	0/4	0/3	0/3	0/4	0/2	0/4
8	0/4	0/3	0/3	0/4	0/2	0/4
9	0/4	0/3	0/3	0/4	0/2	0/4
10	0/4	0/3	0/3	0/4	0/2	0/4
11	0/4	0/3	0/3	0/4	0/2	0/4
12	0/4	0/3	0/3	0/4	0/2	0/4

Numerator: Number of sheep positive; denominator: number infected and surviving.
DA: Diminazene aceturate, 7 mg/kg, i.m.
Levamisole: 5 mg/kg, i.m.
Vitamin A: 50,000 IU, i.m.

Table 2. Mean rectal temperature (°C) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	38.68 ± 0.50^{a}	39.05 ± 0.13^{a}	38.80 ± 0.11^{a}	39.00 ± 0.18^{a}	39.05 ± 0.12^{a}	38.88 ± 0.75^{a}
1	39.08 ± 0.15^{a}	39.93 ± 0.37^{b}	40.80 ± 0.09^{c}	40.83 ± 0.85^{c}	40.65 ± 0.65^{c}	40.34 ± 0.65^{c}
2**	39.25 ± 0.12^{a}	40.63 ± 0.88^{c}	40.00 ± 0.26^{b}	39.93 ± 0.48^{b}	40.93 ± 0.67^{c}	39.88 ± 0.21^{b}
3***	28.85 ± 0.65^{a}	39.97 ± 0.33^{b}	39.17 ± 0.32^{a}	39.30 ± 0.20^{a}	39.85 ± 0.15^{b}	39.03 ± 0.06^{a}
4	39.15 ± 0.16^{a}	39.00 ± 0.06^{a}	39.07 ± 0.22^{a}	39.35 ± 0.22^{ab}	39.85 ± 0.05^{b}	39.98 ± 0.18^{a}
5	38.98 ± 0.31^{a}	38.90 ± 0.06^{a}	39.30 ± 0.15^{a}	39.25 ± 0.20^{a}	39.20 ± 0.20^{a}	39.08 ± 0.08^{a}
6	39.03 ± 0.33^{a}	39.27 ± 0.37^{ab}	40.00 ± 0.06^{b}	39.03 ± 0.17^{a}	38.90 ± 0.00^{a}	39.28 ± 0.16^{ab}
7	38.85 ± 0.06^{a}	39.67 ± 0.23^{b}	39.70 ± 0.10^{b}	38.98 ± 0.03^{a}	38.95 ± 0.05^{a}	39.18 ± 0.16^{a}
8	39.55 ± 0.19^{a}	39.47 ± 0.29^{a}	39.83 ± 0.07^{a}	39.35 ± 0.20^{a}	39.90 ± 0.10^{a}	39.43 ± 0.20^{a}
9	39.45 ± 0.26^{a}	39.43 ± 0.30^{a}	39.27 ± 0.22^{a}	39.13 ± 0.19^{a}	39.70 ± 0.20^{a}	39.35 ± 0.18^{a}
10	39.75 ± 0.25^{a}	39.30 ± 0.17^{ab}	38.87 ± 0.32^{b}	39.40 ± 0.15^{ab}	39.55 ± 0.05^{ab}	39.30 ± 0.25^{ab}
11	39.25 ± 0.24^{a}	39.27 ± 0.37^{a}	39.57 ± 0.28^{a}	39.57 ± 0.17^{a}	38.85 ± 0.05^{a}	39.38 ± 0.18^{a}
12	39.60 ± 0.24^{a}	39.50 ± 0.20^{a}	39.67 ± 0.28^{a}	39.28 ± 0.16^{a}	39.60 ± 0.10^{a}	39.05 ± 0.10^{a}

Different superscripts $^{a, b, c}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

difference in rectal temperatures of treated sheep (groups 3, 4, and 6) and that of the uninfected control. Mean rectal temperature of the animals in DA-treated groups was higher than that in the other experimental groups. By weeks 4 and 6, mean rectal temperature was significantly ($P < 0.05$) higher in groups 5 and 3, respectively, when compared with the other experimental groups. From week 7 to the end of the experiment, there was no significant difference in rectal temperatures of any of the experimental groups.

3.4. Body weight

From weeks 7 to 8 mean proportional body weight was significantly ($P < 0.05$) lower in group 4 than in the other experimental groups (Table 3), while from weeks 10 to 12, it was significantly ($P < 0.05$) higher in group 5 than in the other groups.

3.5. Respiratory rate

There was a significant ($P < 0.05$) increase in the mean group respiratory rate of sheep in the infected groups from week 1 to week 2 PI when compared with that of the uninfected control (Table 4). From week 3 to the end of the study, there was no significant difference in respiratory rates of any of the experimental groups.

3.6. Pulse rate

There was a significant increase ($P < 0.05$) in mean pulse rates of sheep in the infected groups when compared with that of the uninfected group from week 1 to week 3 (Table 5). From week 3 to the end of the experiment, there was no significant difference in pulse rates of any of the experimental groups.

3.7. Mean group packed cell volumes

Mean group PCV was significantly ($P < 0.05$) lower in the infected and treated groups from week 1 to the end of the study when compared with the uninfected group (Table 6). By weeks 4, 6, 7, 8, and 9 the PCV of sheep in group 4 was significantly ($P < 0.05$) lower than those of the other treated groups. By week 5, PCVs of groups 4 and 5 were significantly ($P < 0.05$) lower than those of the other treatment groups. By weeks 9, 10, and 11 the PCVs of groups 2 and 4 were significantly ($P < 0.05$) lower than those of the other treatment groups.

3.8. Mean group hemoglobin concentrations

Mean group Hb was significantly ($P < 0.05$) lower in the infected groups when compared with that of the uninfected control by week 1 (Table 7). From week 2 to week 3 PI there was no significant difference in the Hb of the uninfected and treated groups, except groups 4 and 5. By week 4, the Hb concentrations of groups 1, 3, and 6 were significantly ($P < 0.05$) higher than those of groups 2, 4, and 5. By weeks 5, 6, 7, 10, and 11 the Hb concentrations of sheep in groups 2 and 4 were significantly ($P < 0.05$) lower than those of the other experimental groups.

Table 3. Mean proportional body weight gain (kg ± SE) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1	1.28 ± 0.29^{ab}	1.34 ± 0.03^{ab}	1.40 ± 0.06^{ab}	1.21 ± 0.06^{a}	1.42 ± 0.08^{b}	1.35 ± 0.1^{ab}
2**	1.34 ± 0.05^{ab}	1.22 ± 0.08^{ab}	1.34 ± 0.08^{ab}	1.18 ± 0.05^{a}	1.42 ± 0.06^{b}	1.30 ± 0.09^{ab}
3***	1.31 ± 0.05^{a}	1.36 ± 0.07^{a}	1.34 ± 0.09^{a}	1.12 ± 0.07^{a}	1.34 ± 0.14^{a}	1.30 ± 0.09^{a}
4	1.34 ± 0.05^{a}	1.15 ± 0.05^{a}	1.19 ± 0.06^{a}	1.13 ± 0.07^{a}	1.33 ± 0.24^{a}	1.32 ± 0.10^{a}
5	1.33 ± 0.09^{a}	1.33 ± 1.12^{a}	1.27 ± 0.09^{a}	1.12 ± 0.07^{a}	1.22 ± 0.22^{a}	1.32 ± 0.10^{a}
6	1.33 ± 0.09^{ab}	1.31 ± 0.10^{ab}	1.45 ± 0.08^{b}	1.08 ± 0.07^{a}	1.33 ± 0.18^{ab}	1.35 ± 0.10^{ab}
7	1.34 ± 0.05^{a}	1.42 ± 0.14^{a}	1.51 ± 0.05^{a}	1.00 ± 0.07^{b}	1.40 ± 0.25^{a}	1.30 ± 0.09^{ab}
8	1.38 ± 0.08^{a}	1.42 ± 0.14^{a}	1.51 ± 0.05^{a}	1.00 ± 0.07^{b}	1.36 ± 0.21^{a}	1.28 ± 0.11^{ab}
9	1.37 ± 0.09^{ab}	1.44 ± 0.16^{ab}	1.53 ± 0.07^{b}	1.04 ± 0.09^{a}	1.39 ± 0.25^{ab}	1.26 ± 0.12^{ab}
10	1.34 ± 0.05^{ab}	1.42 ± 0.14^{bc}	1.51 ± 0.05^{bc}	1.04 ± 0.09^{a}	1.71 ± 0.00^{c}	1.26 ± 1.24^{ab}
11	1.38 ± 0.08^{a}	1.42 ± 0.14^{a}	1.51 ± 0.07^{a}	1.20 ± 0.06^{a}	1.86 ± 0.00^{b}	1.35 ± 0.10^{a}
12	1.38 ± 0.80^{ab}	1.44 ± 0.16^{ab}	1.53 ± 0.07^{b}	$1,15 \pm 0.11^{a}$	1.86 ± 0.00^{c}	1.30 ± 0.09^{ab}

Different superscripts $^{a, b, c}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

Table 4. Mean respiratory rates (breaths/min) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	28.5 ± 0.65^{a}	27.75 ± 0.48^{a}	27.50 ± 0.65^{a}	27.25 ± 1.55^{a}	27.00 ± 0.71^{a}	29.25 ± 0.48^{a}
1	28.50 ± 0.87^{a}	31.00 ± 0.71^{ab}	32.00 ± 0.91^{b}	31.50 ± 1.55^{ab}	31.50 ± 0.65^{ab}	33.50 ± 0.87^{b}
2**	28.00 ± 1.81^{a}	33.00 ± 0.58^{bc}	32.00 ± 0.58^{bc}	31.00 ± 0.41^{b}	33.33 ± 0.67^{c}	31.00 ± 0.41^{b}
3***	28.00 ± 1.08^{a}	29.67 ± 1.67^{a}	29.00 ± 0.58^{a}	28.75 ± 0.48^{a}	29.50 ± 0.50^{a}	28.25 ± 0.49^{a}
4	27.00 ± 0.58^{a}	28.33 ± 0.33^{a}	28.67 ± 0.89^{a}	28.50 ± 0.96^{a}	29.00 ± 1.00^{a}	29.00 ± 0.41^{a}
5	28.25 ± 0.63^{a}	28.67 ± 0.88^{a}	28.00 ± 1.15^{a}	29.25 ± 0.95^{a}	27.50 ± 0.50^{a}	29.00 ± 0.41^{a}
6	27.50 ± 0.50^{a}	29.00 ± 2.08^{a}	27.67 ± 0.88^{a}	28.25 ± 0.63^{a}	29.00 ± 1.00^{a}	27.75 ± 0.25^{a}
7	28.75 ± 1.03^{a}	29.33 ± 1.33^{a}	28.33 ± 0.33^{a}	29.00 ± 0.96^{a}	28.50 ± 1.00^{a}	28.25 ± 0.63^{a}
8	29.25 ± 1.11^{a}	31.00 ± 1.53^{a}	28.67 ± 0.88^{a}	28.50 ± 0.65^{a}	29.00 ± 0.00^{a}	29.75 ± 0.25^{a}
9	27.50 ± 0.50^{a}	30.67 ± 1.20^{b}	28.00 ± 0.00^{a}	28.75 ± 0.48^{a}	28.00 ± 0.00^{a}	28.25 ± 0.48^{a}
10	27.25 ± 1.03^{a}	29.00 ± 1.00^{a}	28.00 ± 0.58^{a}	28.00 ± 0.82^{a}	28.50 ± 1.50^{a}	28.50 ± 0.65^{a}
11	27.50 ± 0.50^{a}	29.67 ± 1.20^{a}	28.00 ± 1.15^{a}	29.25 ± 0.85^{a}	29.50 ± 1.50^{a}	29.25 ± 0.48^{a}
12	29.25 ± 1.11^{ab}	31.33 ± 1.33^{b}	28.33 ± 0.67^{a}	28.50 ± 0.29^{a}	29.50 ± 0.50^{ab}	29.00 ± 0.41^{ab}

Different superscripts $^{a, b, c}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

Table 5. Mean pulse rates (pulsations/min) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	82.25 ± 3.47^{a}	81.50 ± 1.71^{a}	85.50 ± 2.21^{a}	86.00 ± 2.68^{a}	84.25 ± 3.07^{a}	85.00 ± 2.80^{a}
1	86.50 ± 1.32^{a}	94.25 ± 2.21^{ab}	96.00 ± 1.58^{ab}	93.75 ± 2.50^{b}	96.25 ± 3.07^{b}	98.00 ± 3.39^{b}
2**	85.50 ± 2.73^{a}	93.33 ± 1.76^{ab}	93.33 ± 3.28^{ab}	95.75 ± 3.33^{b}	99.00 ± 0.41^{b}	100.50 ± 3.97^{b}
3***	84.25 ± 1.93^{a}	89.00 ± 1.00^{ab}	89.00 ± 1.00^{ab}	90.00 ± 0.41^{b}	92.50 ± 2.50^{b}	92.50 ± 2.04^{b}
4	86.25 ± 1.43^{a}	89.00 ± 1.15^{a}	87.00 ± 1.00^{a}	84.00 ± 2.36^{a}	87.00 ± 3.00^{a}	88.00 ± 0.49^{a}
5	81.50 ± 2.75^{a}	83.33 ± 2.91^{a}	79.00 ± 7.00^{a}	86.00 ± 2.48^{a}	88.00 ± 2.00^{a}	84.25 ± 2.32^{a}
6	80.50 ± 2.96^{a}	88.00 ± 1.15^{a}	79.50 ± 5.50^{a}	88.25 ± 0.63^{a}	87.00 ± 1.00^{a}	81.25 ± 2.95^{a}
7	83.50 ± 2.06^{a}	85.00 ± 3.61^{a}	84.50 ± 5.50^{a}	85.75 ± 2.39^{a}	89.00 ± 1.00^{a}	84.00 ± 2.92^{a}
8	83.00 ± 3.49^{a}	84.67 ± 3.38^{a}	85.50 ± 2.50^{a}	81.25 ± 2.93^{a}	85.00 ± 5.00^{a}	82.50 ± 1.66^{a}
9	83.75 ± 2.53^{a}	85.67 ± 3.38^{a}	87.50 ± 0.50^{a}	88.50 ± 0.29^{a}	88.50 ± 0.50^{a}	89.00 ± 0.60^{a}
10	88.50 ± 0.65^{a}	83.67 ± 2.85^{ab}	82.00 ± 6.00^{a}	86.75 ± 0.25^{ab}	86.50 ± 0.50^{ab}	87.25 ± 0.48^{ab}
11	86.25 ± 0.85^{a}	89.33 ± 0.67^{a}	87.50 ± 2.50^{a}	88.50 ± 0.96^{a}	89.00 ± 1.00^{a}	88.00 ± 1.22^{a}
12	82.75 ± 3.64^{a}	87.67 ± 0.88^{a}	81.00 ± 2.00^{a}	89.25 ± 0.48^{a}	88.00 ± 2.00^{a}	82.25 ± 3.47^{a}

Different superscripts $^{a, b}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

Table 6. Mean PCV (%) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	35.50 ± 1.26^{a}	31.75 ± 3.07^{a}	33.75 ± 3.33^{a}	33.25 ± 3.77^{a}	34.75 ± 3.90^{a}	31.75 ± 2.95^{a}
1	37.50 ± 0.96^{a}	25.00 ± 4.12^{b}	22.50 ± 3.80^{b}	21.50 ± 3.40^{b}	25.25 ± 1.93^{b}	21.75 ± 3.90^{b}
2**	39.50 ± 0.50^{a}	19.67 ± 4.18^{b}	18.67 ± 0.33^{b}	21.25 ± 3.20^{b}	22.67 ± 1.45^{b}	21.50 ± 3.20^{b}
3***	39.50 ± 0.29^{a}	22.33 ± 2.85^{b}	20.00 ± 2.89^{b}	18.50 ± 2.53^{b}	22.50 ± 0.50^{b}	20.50 ± 2.63^{b}
4	38.75 ± 0.48^{a}	22.00 ± 2.52^{b}	22.67 ± 1.76^{b}	18.25 ± 2.32^{b}	20.50 ± 0.50^{b}	22.75 ± 2.63^{b}
5	38.00 ± 0.48^{a}	25.00 ± 1.53^{b}	27.33 ± 1.45^{b}	18.00 ± 2.27^{c}	19.00 ± 0.00^{c}	23.25 ± 1.60^{bc}
6	38.50 ± 0.29^{a}	24.67 ± 0.33^{cd}	28.00 ± 1.15^{bc}	22.75 ± 1.15^{d}	26.00 ± 1.00^{bcd}	39.00 ± 1.47^{b}
7	37.50 ± 0.29^{a}	26.30 ± 0.33^{d}	30.00 ± 1.15^{bc}	22.00 ± 1.08^{c}	32.00 ± 2.00^{b}	28.25 ± 0.75^{c}
8	38.25 ± 0.29^{a}	28.67 ± 0.33^{bc}	27.33 ± 1.15^{c}	23.75 ± 1.08^{d}	30.50 ± 2.00^{b}	27.50 ± 0.75^{c}
9	38.00 ± 0.48^{a}	26.00 ± 0.58^{c}	30.00 ± 1.15^{b}	24.00 ± 0.48^{c}	29.50 ± 0.50^{b}	30.00 ± 0.71^{b}
10	38.00 ± 0.00^{a}	24.66 ± 0.33^{d}	32.00 ± 1.15^{b}	23.75 ± 0.25^{d}	29.00 ± 0.00^{c}	29.75 ± 0.85^{c}
11	36.50 ± 0.65^{a}	26.67 ± 1.20^{d}	32.00 ± 0.58^{b}	25.25 ± 0.25^{d}	29.50 ± 0.50^{c}	29.25 ± 0.48^{c}
12	37.50 ± 0.29^{a}	26.67 ± 0.88^{dc}	31.33 ± 0.67^{b}	26.00 ± 0.58^{c}	28.50 ± 0.50^{cd}	29.75 ± 0.85^{bc}

Different superscripts $^{a, b, c, d}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

Table 7. Mean group hemoglobin concentration (g/dL) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	9.65 ± 0.09^{a}	9.70 ± 0.14^{a}	9.75 ± 0.10^{a}	8.98 ± 0.41^{a}	9.83 ± 0.66^{a}	9.63 ± 0.90^{a}
1	10.10 ± 0.14^{a}	8.70 ± 0.12^{b}	8.68 ± 0.12^{b}	7.00 ± 0.18^{c}	8.85 ± 0.57^{b}	8.05 ± 0.61^{bc}
2**	10.88 ± 0.43^{a}	11.17 ± 0.44^{a}	11.83 ± 0.95^{a}	8.43 ± 0.19^{b}	8.83 ± 0.17^{b}	11.83 ± 0.31^{a}
3***	11.50 ± 0.65^{a}	11.17 ± 0.44^{a}	11.17 ± 0.74^{a}	8.05 ± 0.50^{b}	7.40 ± 0.40^{b}	11.98 ± 0.18^{a}
4	12.75 ± 0.25^{ab}	10.17 ± 0.44^{c}	11.83 ± 0.52^{b}	10.08 ± 0.15^{c}	10.40 ± 0.50^{c}	13.13 ± 0.16^{a}
5	12.88 ± 0.13^{a}	11.33 ± 0.67^{c}	13.27 ± 0.39^{ab}	10.00 ± 0.08^{d}	14.25 ± 0.25^{b}	13.93 ± 0.15^{b}
6	12.53 ± 0.21^{ab}	10.67 ± 0.33^{d}	11.73 ± 0.37^{b}	9.73 ± 0.15^{c}	14.00 ± 0.20^{a}	12.90 ± 0.31^{a}
7	12.70 ± 0.12^{a}	11.00 ± 0.29^{b}	12.17 ± 0.49^{a}	10.05 ± 0.16^{c}	12.65 ± 0.15^{a}	12.80 ± 0.27^{a}
8	13.30 ± 0.12^{a}	12.03 ± 057^{b}	12.07 ± 0.54^{b}	10.70 ± 0.12^{c}	14.00 ± 0.20^{a}	13.40 ± 0.13^{a}
9	14.00 ± 0.00^{a}	12.00 ± 0.50^{b}	12.27 ± 0.64^{b}	10.70 ± 0.12^{c}	14.00 ± 0.20^{a}	13.90 ± 0.12^{a}
10	14.53 ± 0.21^{a}	11.63 ± 0.37^{c}	13.23 ± 0.43^{b}	10.78 ± 0.10^{a}	13.75 ± 0.25^{b}	13.75 ± 0.13^{b}
11	14.63 ± 0.13^{a}	11.43 ± 0.47^{c}	12.67 ± 0.23^{b}	10.08 ± 0.34^{d}	13.00 ± 0.00^{b}	13.05 ± 0.24^{b}
12	14.95 ± 0.50^{a}	11.77 ± 0.43^{d}	13.23 ± 0.43^{b}	12.18 ± 0.17^{cd}	12.75 ± 0.25^{bc}	13.10 ± 0.27^{b}

Different superscripts $^{a, b, c, d}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

3.9. Mean group RBC counts

Mean group total RBC count was significantly ($P < 0.05$) lower in the infected groups when compared with the uninfected animals by weeks 2, 4, and 5 of the experiment (Table 8). By weeks 3, 6, 7, 8, 9, 10 11, and 12, however, there was no significant difference in mean group RBC counts of any of the experimental groups.

4. Discussion

Experimental infection of WAD sheep with *T. brucei* was achieved perhaps due to the high number of parasites inoculated (1×10^6). Trypanotolerance is known to be limited by high challenge rates (16). In semidomesticated red-fronted gazelles subjected to the stress of captivity and experimental infection with 1.5×10^6 *T. b. brucei*, trypanotolerance was not absolute and severe trypanosomosis similar to that found in domestic animals occurred (17). When subjected to high doses of infective organisms, trypanotolerant animals succumb to infection and show clinical disease (16). Their resistance is particularly effective in the face of mild pathogenic species of trypanosomes or may be developed against particular prevalent strains/species of trypanosomes in a locality, in which case infection with new strains/species will not confer immunity (16).

The prepatent period observed here agrees with the report of Mohammed et al. (18) in *T. congolense*-infected sheep. Differences in prepatent periods may be attributed to the number of parasites inoculated, the pathogenicity of the parasite, and the immune status of the host (19). No differences in time of parasite clearance were observed among the treatment groups.

Pyrexia is a cardinal clinical sign in trypanosomosis (3,20). Pyrexia was intermittent and lasted between weeks 1 and 3 PI. Fever is due to the stimulation of the thermoregulatory center of the hypothalamus by pyrogens released during trypanosome infection. Treatment reversed pyrexia in treated animals and hence the return to normal temperature values occurred from week 5 of the experiment.

There was no significant ($P > 0.05$) change in mean group proportional body weight gains of all the experimental animals from weeks 1 to 10 PI. This result agrees with the findings of Akpa et al. (21), who reported no loss of weight in bovine trypanosomosis, but it differs from those of some researchers who reported cases of weight loss in trypanosomosis (22). Significant ($P < 0.05$) increase in proportional body weight gain was recorded from week 11 to the end of the study in group 5 (DA + vitamin A 3 weeks PI) when compared with all the other experimental groups. Studies in cattle have shown that weight changes in trypanosomosis are markedly influenced by levels of protein and energy intake (23). Low levels of both nutrients can exacerbate the adverse effects

Table 8. Mean RBC counts ($\times 10^6$/μL) of sheep experimentally infected with *T. brucei* and treated with DA, levamisole, and vitamin A.

Weeks	Experimental groups					
	Uninfected, untreated	DA	DA + levamisole	DA + vit A 2 weeks PI	DA + vit A 3 weeks PI	DA + vit A + levamisole
0*	12.26 ± 1.50^{a}	12.91 ± 1.14^{a}	11.79 ± 1.17^{a}	11.74 ± 1.17^{a}	12.44 ± 1.07^{a}	11.32 ± 1.08^{a}
1	11.95 ± 1.26^{a}	11.06 ± 0.76^{ab}	8.72 ± 0.55^{c}	9.08 ± 0.43^{bc}	10.01 ± 0.47^{ab}	8.62 ± 0.23^{c}
2**	13.36 ± 0.95^{a}	8.44 ± 0.26^{b}	8.90 ± 0.52^{b}	9.05 ± 0.13^{b}	8.71 ± 0.11^{b}	9.11 ± 0.07^{b}
3***	12.85 ± 0.67^{a}	10.45 ± 0.73^{bc}	9.51 ± 0.30^{c}	11.37 ± 0.35^{ab}	10.00 ± 0.30^{bc}	10.52 ± 0.28^{bc}
4	13.89 ± 0.39^{a}	9.65 ± 0.41^{b}	10.69 ± 1.28^{b}	11.38 ± 0.24^{b}	10.73 ± 0.15^{b}	11.14 ± 0.78^{b}
5	13.80 ± 0.36^{a}	10.70 ± 0.61^{b}	11.12 ± 1.19^{b}	11.86 ± 0.10^{ab}	11.41 ± 0.36^{ab}	11.51 ± 1.09^{ab}
6	13.30 ± 0.50^{ab}	11.27 ± 0.67^{b}	11.50 ± 0.87^{ab}	13.35 ± 0.18^{ab}	13.62 ± 0.35^{a}	12.06 ± 0.78^{ab}
7	13.33 ± 0.20^{ab}	12.70 ± 0.35^{ab}	12.61 ± 0.38^{a}	13.90 ± 0.10^{ab}	14.00 ± 0.00^{b}	13.15 ± 0.65^{ab}
8	13.37 ± 0.24^{a}	11.93 ± 0.93^{b}	13.46 ± 0.32^{a}	13.17 ± 0.17^{ab}	14.25 ± 0.25^{a}	13.74 ± 0.22^{a}
9	13.94 ± 0.60^{ab}	13.21 ± 0.45^{a}	13.98 ± 0.29^{ab}	13.63 ± 0.88^{ab}	14.45 ± 0.11^{b}	14.24 ± 0.28^{b}
10	13.95 ± 0.18^{a}	14.00 ± 0.00^{a}	13.96 ± 0.04^{a}	13.72 ± 0.15^{a}	14.78 ± 0.22^{b}	14.73 ± 0.23^{b}
11	14.21 ± 0.26^{a}	14.10 ± 0.11^{a}	14.06 ± 0.60^{a}	14.12 ± 0.19^{a}	14.83 ± 0.18^{b}	14.61 ± 0.08^{b}
12	14.23 ± 0.13^{a}	14.34 ± 0.17^{a}	14.52 ± 0.06^{ab}	14.27 ± 0.30^{a}	15.00 ± 0.00^{b}	14.97 ± 0.35^{b}

Different superscripts $^{a, b, c}$ in a row indicate significant differences between the means of the groups at $P < 0.05$.
*Experimental infection, **early treatment, ***late treatment.

of trypanosomosis on body weight (24). Levamisole has been reported to improve weight gain when administered at repeated doses of 2.5 mg/kg (10), but in this work it was only administered once.

Respiratory and pulse rates were also significantly ($P < 0.5$) increased in infected animals when compared with the uninfected from 1 to 3 weeks of the experiment. This is at variance with the work of Chukwudi et al. (25), who reported no significant changes in pulse rates of WAD sheep infected with *T. congolense*, but in agreement with Lazim et al. (26), who recorded similar observations of increased pulse and respiration rates during an experimental infection of goats with *T. vivax*. Hyperpnea and tachycardia are associated with cardiac insufficiency resulting from anemia, which is a cardinal feature of trypanosomosis.

Significant reductions in total RBC counts, PCV, and Hb observed after infection are indicative of anemia and are in agreement with earlier reports that trypanosomosis causes anemia (1,27). Some factors reported to be responsible for these reductions include erythrocyte injury caused by the lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation, and malnutrition (28). The severity of anemia depends on the level and duration of parasitemia in trypanosome-infected animals. Reductions in total RBC counts, PCV, and Hb were, however, reversed by treatment. Sheep treated with DA, levamisole, and vitamin A combinations had higher values than those treated with DA alone. Those treated with DA and levamisole also had higher blood indices than those treated with DA and vitamin A. This finding agrees with the report of Adieme et al. (29) in rats infected with *T. brucei* treated with levamisole and/or vitamin C combination with DA.

Clinical signs of pale mucous membranes, anorexia, facial edema, and enlargement of prefemoral and prescapular lymph nodes observed are characteristic of trypanosomosis in animals (22,25). Muscular spasms, circling, and torticollis observed in some sheep before treatment agrees with the findings of Desquesnes et al. (30), who reported nervous signs in *T. evansi* infections of sheep and goats. Clinical signs were more severe with mortality of up to 50% in the group that was treated 3 weeks PI. Late treatment of trypanosomosis is disadvantageous to livestock because parasites may invade various tissues and organs, including the brain, where they become sequestered and often inaccessible to drugs (21), causing relapse of infection after treatment. All clinical signs observed were reversed following treatment.

Early treatment of trypanosomosis in WAD sheep using combinations of DA, levamisole, and/or vitamin A significantly ($P < 0.05$) increased the PCV and Hb values of treated animals when compared with those of the other experimental groups.

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