



Effects of co-administration of vitamin B₁₂ with diminazene aceturate on packed cell volume and weight gain in cattle experimentally infected with *Trypanosoma congolense*

S. FON-TEBUG^{1,2*}, G.D. MAGANGA¹, O.B. GBATI¹, L.J. PANGUI¹,
J. AWAH-NDUKUM² and T.C. KEAMBOU²

¹ *Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), P.O. Box 5077 Dakar, Sénégal.*
² *University of Dschang, Faculty of Agronomy and Agricultural Science (FASA), P.O.Box 222 Dschang, Cameroon.*

* Corresponding author, E-mail: fon2tebug@yahoo.com, st_fon@hotmail.com

ABSTRACT

The effects of co-administration of cyanocobalamin and/or hydroxocobalamin with diminazene aceturate (DA) on, pack cell volume and weight gain in cattle experimentally infected with *Trypanosoma congolense* was studied. Twenty eight young zebu bulls aged 10-16 months with an average weight of 92.02 ± 14.74 kg were randomly distributed into 4 groups. These bulls were infected with *Trypanosoma congolense* intravenously at a dose rate of 1×10^5 suspended in 4 ml of phosphate buffered saline per animal. Each group was treated with a commercial medication containing DA, cyanocobalamin and/or hydroxocobalamin 10 days post-infection. Haematological examination showed no trypanosomes irrespective of the regimen administered 48 hours post-treatment in all infected cattle. Packed Cell Volume (PCV) and weight gain was highest with the regimen containing DA, cyanocobalamin and hydroxocobalamin. The trypanocidal regimens containing DA co-administered with cyanocobalamin and/or hydroxocobalamin enabled a rapid reconstitution of red blood cells and led to improvement in the weight gain of the trypanosome-infected cattle.
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Keywords: Cattle, diminazene aceturate, packed cell volume, trypanosomiasis, vitamin B₁₂, weight gain.

INTRODUCTION

Trypanosomiasis remains a constraint to ruminant livestock development in large part of Sub-Saharan Africa (Swallow, 2000; Desquesnes and Dia, 2004; Mattioli et al., 2004). Estimates show that 25 to 50 million doses of trypanocides are administered each year to the approximately 45 million cattle at risk of trypanosomiasis (Kristjanson et al., 1999; Gilbert et al., 2001). In infected areas, the herd size as well as meat and milk production is reduced by more than half, causing annual losses of cattle production of more than US\$1 billion (Mortelmans, 1986; Dehaan and Bekure, 1991; Pangui, 2001).

The syndromes associated with the infection vary from chronic to acute and fatal, with clinical signs as progressive weakness, emaciation, fever, anemia, and death (Camargo et al., 2004). Anaemia stands to be the most significant pathological feature bovine trypanosomiasis and is known to be of multifactorial origin ranging from haemodilution, erythrophagocytosis, haemolytic factor, rumen stasis and bone marrow dyserythropoiesis (Murray and Dexter, 1988).

Absolute control of animal trypanosomiasis cannot be achieved with the available current methods, which are

inadequate to prevent the enormous socio-economic losses caused by this disease. Different control strategies including vector control, use of trypanotolerant breeds and chemotherapy have been put in place to fight the disease. (Taylor, 1998 ; Chaka and Abebe, 2003; Camargo et al, 2004). DA alongside Isomethamidium chloride and Homidium bromide have been reported to be the most widely used drugs against trypanosomiasis in cattle and other species (Kinabo, 1993; Perengrine et al. 1993; Chartier et al., 2000; Van den Bossche et al., 2000; Olila et al., 2002; Chaka and Abebe, 2003)

Vitamin B₁₂ (cyanocobalamin and hydroxocobalamin) is a water soluble vitamin and plays an important role in erythrocyte synthesis as well as metabolism of fatty acids and folic acid (Reginald et al., 2000; Moulin and Coquerel, 2002). Cognisant of the significant anaemic effect the disease, pharmaceutical companies turn to associate vitamins especially vitamin B₁₂ to DA. However, information on the use of these trypanocidal regimens combining DA and vitamine B₁₂ is still lacking. The aim of this study was, therefore, to investigate the effects of treatment regimens containing DA and vitamin B₁₂ on the packed cell volume and weight gain of cattle experimentally infected with *T. congolense*.

MATERIALS AND METHODS

Study site

The present study was conducted at the teaching and research farm of Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), Dakar, Senegal. Located at 14°-15 North, 17°-18° West, Dakar has a tropical climate, with two distinct seasons : a hot and humid season (June to October), with rainfall peaking in August (179 mm) and temperatures averaging 27 °C (80 °F), and a cooler season (November to May) with almost no rain (around 1 mm/month).

Animals

Twenty eight zebu bulls (mainly gobra , gouzerat and maure) aged 10-16

months with an average weight of 92.02 ± 14.74 kg were used. These animals were bought from the local markets in Senegal and came from transhumant herds in the sylvo-pastoral area of Senegal and also from neighbouring Mauritania and Mali. These bulls were fed *ad libitum* with rice straw and supplemented with groundnut meal, cotton seed cake, molasses and mineral licks.

Trial management

Upon arrival at the teaching and research farm, bulls were ear tagged, weighed and randomly distributed into 4 groups of 7 animals each. They were given broad spectrum treatment against gastrointestinal and external parasites using Benzal® (*Albendazol*, an imidazol derivative, Laprovet, France). The bulls were clinically observed and fed in their different groups during a period of 21 days prior to infection.

An inoculum of *Trypanosoma congolense* (subtype sat.87/CRTA/237.2) obtained from the CIRDES (*Centre International de Recherche-Développement sur l'Élevage en zone Subhumide*) Bobo Dioulasso, Burkina Faso was used for the study. The inoculum was multiplied by successive passage in 12 mice by intraperitoneal inoculation. Mice with sufficiently high parasitaemia were sacrificed and blood was collected and diluted to obtain a dose of 1×10⁵ per 4 ml in PBS (phosphate buffered saline).

A day prior to inoculation, blood smears were examined microscopically after Giemsa staining for *Theileria*, *Babesia* and *Anaplasma* species with negative results. On the day of infection, each bull was inoculated intravenously with 4ml of the inoculum by intravenous injection into the jugular vein (Murilla et al., 2002). PCV readings, examination for the presence of trypanosomes as well as a general clinical examination was carried daily for the first month, then three times a week till the end of the study. The weight of the bulls was also taken three times a week throughout the study period.

Blood was collected from the ear veins, one week before (week 1) and nine weeks post-infection (weeks 2 to 10) into heparinised capillary tubes, the lower end of tube was sealed with creastaseal (Hawaksly, England) and the tubes were centrifuged in a micro-haematocrit centrifuge at $12\ 000 \times g$ for 10 minutes. PCV determined using a haematocrit reader and values are expressed in percentage (Anosa, 1988). This tube was then cut 1mm below the buffy-coat and the buffy-coat zone was expressed onto a slide covered with a coverslip and examined by dark-ground phase microscopy using the Murray Method (Murray *et al.*, 1977; OIE, 2008).

On the 10th day post-infection when the mean PCV level in all the groups had dropped by 8-10%, animals in groups 2, 3 and 4 were treated with the differernt trypanocidal regimens: group 2 = treated with regimen containing DA without vitamin (negative control) ; group 3 = treated with a regimen containing DA and cyanocobalamin; and group 4 = treated with regimen containing DA, cyanocobalamin and hydroxocobalamin. Animals in group 1 (positive control) were treated and excluded from the study when the mean PCV level dropped below 19.20%. This critical level was reported to present a high mortality risk due to trypanosomiasis (Bengaly *et al.*, 2002; Maganga, 2005) and for animal welfare purposes. All trypanocidal regiments were administered at a dose of 3.5 mg of diminazene per kg body weight following manufacturers indications.

Data analysis

The results were submitted to a one factor Analysis of Variance through general linear model according to the following equation:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where

Y_{ij} = observed performance of an animal

μ = mean performance

T_i = fixed effect of I treatment (i =1, 2, 3)

e_{ij} = effect of residual factors

The mean weekly packed cell volume (PCV), average weight and average weight gains were compared with the test of Duncan.

In all cases, the SPSS statistical package version 12.0 was used. Statements of significance were based on $p < 0.05$.

RESULTS

Haematological examination a week prior to infection showed no trypanosomes in blood samples collected from the bulls. Following syringe challenge, cattle started showing parasitaemia on the 4th day. On the 6th day, all the bulls became parasitaemic accompanied by clinical signs such as ocular discharges, stary haircoat, pale mucosa and anorexia on the 7th day. These clinical signs gradually intensified till 9th day post-infection with pale mucous membranes, conjunctivitis, rough coat and high temperatures (41-42 °C).

Following treatment, trypanosomes were cleared from the blood within 48 hours regardless of the regimen used till the end of the study. A drop in mean PCV values two weeks (weeks 2 and 3) post-infection was observed in all the groups. Mean PCV values increased from week 4 till the end of the study and differed ($p < 0.05$) according to the treatments (Table 1).

The average weight and weight gain is shown in Table 2. The highest average weight gain ($p < 0.05$) at the end of the study was recorded in group 4. A drop in weight gain was recorded in 1st, 2nd and 5th week post-infection in all the groups. Except for group 1, all the groups recorded an increase in weight gain in the 3rd and 4th weeks (Figure 1).

DISCUSSION

Parasitaemia obtained 4 to 6 days post-infection was an indication of successful infection of animals and agreed with earlier studies on cattle (Olubayo *et al.*, 1990; Chaka and Abebe, 2003; Murilla *et al.*, 2002). However, Bengaly *et al.* (2002) reported parasitaemia in cattle between 7 and 11 days post-infection using subcutaneous route. Da Silva *et al* (2009) and Toma *et al.* (2008) also reported parasitaemia in the 2nd and 8th day post-infection in cats and rabbits respectively. In this study, the absence of trypanosomes in blood 48 hours post-treatment regardless of

Table 1: Mean weekly Pack Cell Volumes (PCV) of bulls infected with *T. congolense* and treated with varying regimen containing diminazene aceturate, cyanocobalamin and/or hydroxocobalamin.

Weeks	Group 1	Group 2	Group 3	Group 4
1.	35.1 ± 4.90	35.7 ± 5.06	34.6 ± 3.41	35.4 ± 3.67
2.	32.0 ± 2.80	30.7 ± 5.06	29.6 ± 3.41	33.4 ± 3.67
3.	26.6 ± 2.54	27.4 ± 1.67	27.8 ± 1.1	28.6 ± 2.49
4.	21.3 ± 3.30 ^a	30.4 ± 0.22 ^b	29.3 ± 0.53 ^b	34.7 ± 0.45 ^c
5.	20.7 ± 1.70 ^a	26.3 ± 5.49 ^b	29.1 ± 0.50 ^{bc}	33.5 ± 0.33 ^c
6.	19.7 ± 2.11 ^a	30.4 ± 0.59 ^b	31.1 ± 0.57 ^b	35.0 ± 0.62 ^c
7.	18.64 ± 3.21 ^a	32.3 ± 0.59 ^b	34.9 ± 0.57 ^c	36.7 ± 0.62 ^d
8.	-	32.8 ± 0.60 ^a	35.1 ± 0.30 ^b	37.2 ± 0.30 ^c
9.	-	33.1 ± 0.09 ^a	35.2 ± 0.40 ^b	37.3 ± 0.21 ^c
10.	-	33.7 ± 0.57 ^a	36.0 ± 0.73 ^b	38.1 ± 0.25 ^c

^{a,b,c} Means in the same row for each parameter with different superscripts are significantly different (p < 0.05).

Table 2: Average weight and weight gain of bulls infected with *T. congolense* and treated with varying regimen containing diminazene aceturate and cyanocobalamin and/or hydroxocobalamin.

Group	Number of animals	Average weight		Average weight gain*
		Before	After*	
1	7	91.64 ± 5.34	-	-
2	7	82.6 ± 8.45 ^a	90.8 ± 8.92 ^a	0.570 ± 2.12 ^a
3	7	96.4 ± 17.2 ^a	106.1 ± 17.8 ^b	0.80 ± 3.61 ^b
4	7	93.5 ± 18.6 ^a	105.7 ± 21.2 ^b	1.45 ± 2.81 ^c

^{a,b,c} Figures with same superscript in the same column do not differ significantly (p < 0.05).

* Animals of groups 1 were treated on day 45 and removed from the study.

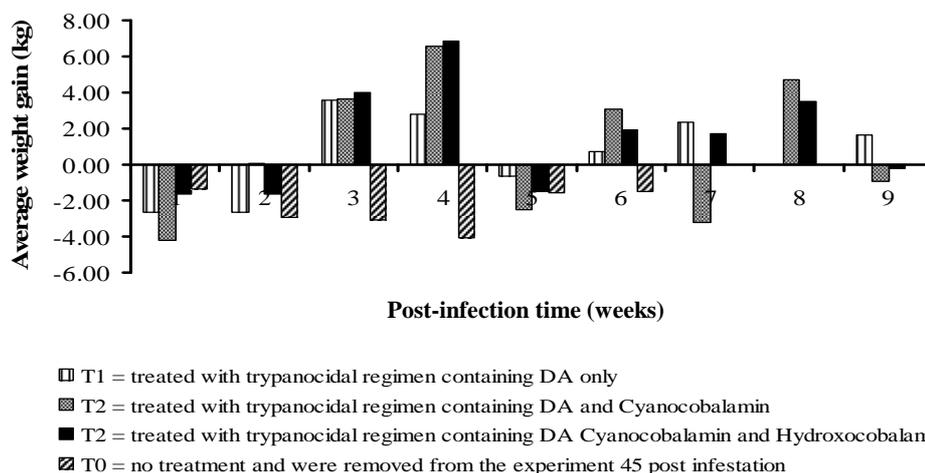


Figure 1: Profile of weight gain in *T. congolense*-infected bulls treated with three diminazene aceturate based regimens.

the treatment regime used is consistent with previous results on the curative properties of diminazene aceturate in the treatment of trypanosomiasis in cattle and other species (Münstermann et al., 1992; Sirivan et al., 1994; Olila et al., 2002; Da Silva et al., 2009).

Packed cell volume (as a measure of anaemia) has been used to determine severity of trypanosomiasis (Katunguka-Rwakishaya, 1999). The drop in PCV values following infestation observed could be associated to intravascular haemolysis caused by the trypanosome, in agreement with previous studies (Dargie, 1979; Olila et al., 2002; Maganga, 2005). The significant increase in PCV values observed in weeks 4, 5 and 6 in groups 2, 3 and 4 as opposed to group 1 could be accounted for by the destruction of trypanosomes, which provokes a regenerative production of erythrocytes or an increase in terminal differentiation of the residual erythroid progenitors (Sauveroche and Wagner, 1993; Seifert, 1996). This could be compared to the progressive increase in PCV in Boran yearlings observed by Andrianarivo et al. (1996). It has been established that Vitamin B₁₂ is essential for normal growth and animals fed with a Vitamin B₁₂ supplement showed a steady weight gain (Thomaskutty and Lee, 1985). In the present study, the presence of cyanocobalamin in the treatment regimen of group 3 and 4 would certainly have boosted erythropoiesis in these animals. These animals attained the initial PCV levels 4 to 6 weeks post-treatment as opposed to reports from a similar study combining DA, vitamin B₆ and B₁₂ (Maganga 2005) where in PCV levels in all treatments remained below the initial level. Moreover, the long acting erythropoietic effect of hydroxocobalamin (Milhaud, 1961; Sureau, 1962) present in the treatment regimen of animals in group 4 would have led to its highest ($p < 0.05$) PCV and weight gain obtained throughout the study. The weight loss registered in the first two weeks post-infection corresponds to the onset clinical signs of the disease. This period also corresponds to the first wave of parasitaemia

in *T. congolense* infection of ruminants in previous studies (Valli et al., 1978; Bengaly et al., 2002) and was marked by anorexia.

Trypanocidal regimens as used in this study had similar effect on the *T. congolense*. Regimens associating cyanocobalamin and/or hydroxocobalamin enabled a rapid improvement in the PCV and weight gain of cattle infected with *T. congolense*; its use should be encouraged.

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