

Influence of Combined Dietary Maize Bran and Wheat Bran Supplementation on the Efficacy of Diminazine Diacetate (Berenil®) in *Trypanosoma congolense* Infection in Rats

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Received: October 18, 2019; **Published:** November 28, 2019

Abstract

The effect of maize bran and wheat bran supplementation on the efficacy of Diminazine diacetate (Berenil®) in experimental *Trypanosoma congolense* infected rats was investigated in 30 days study. Thirty two (32) healthy wistar albino rats of both sexes were randomly divided into eight groups (A-H) of four rats each. Infection caused early onset and progressive parasitaemia accompanied by significant ($P < 0.05$) decrease in Packed Cell Volume (PCV) in the infected not supplemented animals. These effects were improved when subtherapeutic dose of Berenil® (1.75 mgkg⁻¹) was supplemented with Maize bran and or Wheat bran. Thus, Maize bran and Wheat bran supplementation of Berenil® in rats may reduce the level of parasitaemia and its associated anemia.

Keywords: Bran Supplementation; Diminazine Diacetate; *Trypanosoma*; Rats

Introduction

African trypanosomosis in human, livestock and other domestic animals have been a major factor that has devastated the livestock industry in about 10 million square kilometers of tropical Africa [1,2]. It is estimated that 60 million people and over 3 million herds of cattle are at risk [3]. The disease in animals is caused by *Trypanosoma vivax*, *T. congolense*, *T. simiae*, *T. b. brucei*, *T. evansi* and *T. equiperdum* [4]. Nutrition is known to modulate the severity of trypanosomal infection in animals [5-7]. Anosa [8] reported a decreased serum magnesium level in *T. congolense* infected cattle while a higher serum magnesium level was reported in trypanotolerant Keteku than trypanosusceptible White Fulani breed of cattle. Sources of magnesium are rice, wheat bran, cotton seed cake and a host of others.

In another development, serum zinc level correlated positively with resistance of cattle to trypanosomiasis [9]. In light of the foregoing, it is envisaged that combined dietary Maize bran and Wheat bran may improve the efficacy of Berenil® in *T. congolense* infection in rats.

Materials and Methods

Experimental animals

Thirty two (32) healthy Wistar albino rats of both sexes and weighting between 180 - 200g were purchased from the Zoology Department of Faculty of Sciences, Usmanu Danfodiyo University Sokoto. The rats were screened for presence of haemoparasites using standard methods as adopted by Ajagbonna, *et al* [10]. They were fed with commercially formulated ration (Grower mash from Vital Feeds Ltd.) and water was given *ad libitum*. The rats were allowed to acclimatize for a period of 10 days before intervention. After acclimatization, the Packed Cell Volume (PCV) of each rat was determined using microhaematocrit method [11].

Experimental groups

Eight groups of four (4) rats each were formed and were fed *ad libitum*. Groups A, F, G and H were fed with conventional grower mash (not supplemented) while groups B, C, D and E were fed with supplemented feed for 10 days prior to infection and then throughout the experiment.

- **Group A:** Infected with *T. congolense* but not treated (positive control).
- **Group B:** Infected with *T. congolense* and supplemented with Maize bran.
- **Group C:** Infected with *T. congolense* and supplemented with Wheat bran.
- **Group D:** Infected with *T. congolense* and supplemented with Maize bran and Wheat bran combination.
- **Group E:** Infected with *T. congolense*, supplemented with Maize bran and Wheat bran combination and treated once on the 14th day post infection with 1.75 mgkg⁻¹ Diminazine diaceturate (Berenil®).
- **Group F:** Infected with *T. congolense* and treated with 1.75 mgkg⁻¹ Diminazine diaceturate (Berenil®).
- **Group G:** Not infected and not treated (Negative control).
- **Group H:** Infected and treated with 3.5 mgkg⁻¹ Diminazine diaceturate (Berenil®).

Feed supplementation

Maize bran and wheat bran were obtained from Sokoto Kara Market and was identified to be fresh and unadulterated. The supplemented feeds were in three (3) categories:

1. **1st:** Grower mash supplemented with 30% Maize bran; this was done by weighing 700g of grower mash and mixing it with 300g of Maize bran. The combination is thoroughly mixed to give 1 kg of supplemented feed.
2. **2nd:** Grower mash supplemented with 30% Wheat bran; this was done by weighing 700g of grower mash and mixing it with 300g of Wheat bran. The combination is thoroughly mixed to give 1kg of supplemented feed.
3. **3rd:** Grower mash supplemented with 30% Maize bran and 30% Wheat bran; this was done by weighing 400g of grower mash, 300g of Maize bran and 300g of Wheat bran. The combination is thoroughly mixed to give 1kg of supplemented feed.

Experimental infection

The rats were infected with *T. congolense* obtained from the Protozoology unit of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State. Tail blood from moderately infected rats was diluted with normal saline to obtain 10⁶ trypanosomes per millilitre. Each rat was then intraperitoneally inoculated with 0.2 ml of the diluted blood. The parasitaemia levels were then monitored daily by taking blood samples from the tail blood and viewing under the microscope at X40 magnification objective.

The parasitaemia (number of rats positive for trypanosomosis divided by total number of rats inoculated in the group), PCV and mortality were recorded daily during the experiment.

Data obtained were presented as means ± standard deviations and differences between means were compared by analysis of variance (ANOVA).

Results

Only the group G (negative control) showed no sign of parasitaemia throughout the period of the study. Effect of Maize bran, Wheat bran, Berenil® and its combination on *T. congolense* infected rats are shown in table 1.

| Days Groups | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A: Infected not treated | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 1/4 | 2/4 | 4/4 | 4/4 | 3/3 | 2/2 | 1/1 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| B: Infected supplemented maize bran | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 1/4 | 2/4 | 4/4 | 4/4 | 4/4 | 3/3 | 3/3 | 2/2 | 2/2 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| C: Infected supplemented wheat bran | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 2/4 | 2/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 3/3 | 3/3 | 3/3 | 1/1 | 1/1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| D: Infected supplemented maize bran + wheat bran | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 2/4 | 3/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 3/3 | 3/3 | 3/3 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 |
| E: Infected supplemented maize bran, wheat bran +treated with 1.75 mgkg ⁻¹ Berenil® | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | ¼ | 3/4 | 3/4 | 4/4 | 4/4 | 2/4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F: Infected but treated with 1.75mgkg ⁻¹ Berenil® | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 2/4 | 2/4 | 3/4 | ¾ | 3/3 | 3/3 | 3/3 | 3/3 | 2/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| G: Not infected not treated | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |
| H: Infected but treated with 3.5mgkg ⁻¹ Berenil® | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 2/4 | 2/4 | 4/4 | 4/4 | 4/4 | 1/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |

Table 1: Effect of maize bran, wheat bran, Berenil® and combination on the parasitaemia of rats of rats infected with *Trypanosoma congolense*.

$$\text{Key: Parasitaemia} = \frac{\text{number of rats positive}}{\text{total number of rats infected}}$$

Onset of parasitaemia was by the 8th day in the infected not supplemented group (Grp. A), this was however delayed in the supplemented groups.

The group of rats that were infected and treated with 3.5 mgkg⁻¹ Berenil® group (Grp. H) all the rats were completely cured until the end of the end of the experiment. However, in the group treated with half the therapeutic dose (1.75 mgkg⁻¹) of Berenil® alone, only two rats were cured completely. While in the group (Grp. E) that received the combination of half therapeutic dose (1.75 mgkg⁻¹) of Berenil® and supplemented with Maize bran and Wheat bran, all the rats were also completely cured like the group with full therapeutic dose of Berenil®. In the infected and not treated group (Grp. A), the rats exhibited progressive parasitaemia that resulted in early death of all the rats by the 17th day of the experiment.

There was a significant decrease ($P < 0.05$) in PCV (26 ± 4.32) of unsupplemented untreated parasitaemic rats when compared with the values obtained in the uninfected control with a PCV of 46 ± 2.94 . Supplementation with maize bran, wheat bran and treatment with Berenil® however improved the PCV towards the normal control value (Table 2).

| Haemato-logical parameter | Control (Not infected not treated) | Infected not treated | Infected but treated with 1.75 mgkg ⁻¹ Berenil® | Infected but supplemented with maize bran | Infected but supplemented with wheat bran | Infected but supplemented with maize bran and wheat bran | Infected but supplemented with maize bran, wheat bran and treated with 1.75 mgkg ⁻¹ Berenil® | Infected but treated with 3.5 mgkg ⁻¹ Berenil® |
|---------------------------|------------------------------------|----------------------|--|---|---|--|---|---|
| PCV before infection | 46 ± 2.16* | 44 ± 1.63* | 42 ± 2.94* | 43 ± 1.63* | 45 ± 1.41* | 44 ± 1.83* | 47 ± 1.83* | 46 ± 2.16* |
| PCV at peak parasitaemia | 46 ± 2.94* | 26 ± 4.32* | 25 ± 3.61* | 35 ± 1.73* | 36 ± 2.94* | 32 ± 1.41* | 34 ± 1.83* | 26 ± 4.32* |
| PCV after treatment | 48 ± 0.82* | - | 39 ± 1.41* | - | - | 32 ± 1.41* | 40 ± 1.83* | 48 ± 0.82* |

Table 2: Effect of treatment of Diminazine acetate (Berenil®) alone, supplementation of maize bran alone, wheat bran alone and its combination on PCV of rats infected with *Trypanosoma congolense*.

* = $P < 0.05$.

Discussion and Conclusion

Studies indicate that when *T. congolense* is inoculated into susceptible hosts like rats, parasitaemia results and if not treated could result in the death of the host as noted in this study.

The supplemented groups in this study had delayed onset of parasitaemia than the unsupplemented groups. This was an indication that Maize bran and Wheat bran supplementation probably had some effects on the dynamics of parasitaemia in the infected animals. Hecker, *et al.* [12] observed a delayed onset of parasitaemia when maize bran was fed to sheep under Tsetse challenge which he attributed to high Zinc content of the bran as found by Mac Donald, *et al* [13]. Zinc is a trace element which is important in the enzyme, hormonal and immune systems of animals [13].

The parasitological results also indicate that when the infection was treated with 3.5 mgkg⁻¹ Berenil®, 100% the infected rats were completely cured suggesting a strong trypanocidal efficacy of this drug. Treatment with half the therapeutic dose (1.75 mgkg⁻¹) of Berenil®

improved haematological recovery from 25 ± 3.61 before treatment to 39 ± 1.41 after treatment; it was able to completely cure 50% of the infected rats.

Supplementation of this dose of Berenil® (1.75 mgkg^{-1}) with maize bran and or wheat bran not only improved haematological parameter measured in this study but completely cured all the infected rats.

These observations might be attributed to the high magnesium content of wheat bran and also high zinc content of maize bran which serve to improve the efficiency of Berenil® in the treatment of *T. congolense*. Similar findings were reported by Egbenwiyi., *et al.* [5] who reported that high supplementation with magnesium chloride ameliorated the severity of tissue invasive *T. brucei* and non tissue *T. congolense*.

Anosa [8] reported a decreased serum Magnesium level in *T. congolense* infected cattle while Egbe-nwiyi., *et al.* [5] observed that high supplementation with Magnesium Chloride ameliorate the severity of tissue invasive *T. brucei* and nontissue invasive *T. congolense*. Sources of Magnesium are Rice, Wheat bran, Cotton Seed Cake and a host of others [5].

The Magnesium content in the Wheat bran and high Zinc content of the Maize bran might have improved the efficacy of Berenil® in the treatment of *Trypanosoma congolense* in this study.

Recommendation

It is recommended that farmers, especially those within Trypanosomiasis endemic areas, should engage more in supplementing the feed of their animals with either wheat bran, maize bran or both, as this supplementation will help the animals in combating the infection and in quick clearance of parasitaemia during treatment with Berenil®.

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Volume 4 Issue 10 December 2019

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