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Original Article

Proximate analysis of wheat supplemented diet and its anti-trypanosomal effect on infected rat

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ABSTRACT

Anti-trypanosomal properties of wheat supplemented diet and its proximate composition were investigated. The percentage proximate composition of wheat seed were estimated as 72.503 ± 0.005% carbohydrate, 11.690 ± 0.017% lipid, 7.756 ± 0.005% Moisture content and 5.876 ± 0.005% Protein. Whereas the percentage composition of the wheat supplemented diet were 53.443± 0.005 carbohydrate, 13.656 ± 0.011% lipid, 8.460 ± 0.010% Moisture content and 18.316 ± 0.005% Protein. Prophylactic treatment with wheat supplemented diet extended the lifespan of infected rats by 4 extra days from the Control (infected untreated). While early stage treatments also extended the lifespan by 4 days. This study has demonstrated that wheat as part of regular diet may be useful in the management of African Trypanosomiasis.

Key words: Anti-trypanosomal, Wheat-supplemented diet, *T. brucei*, Parasitemia

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INTRODUCTION

African Trypanosomiasis, also known as sleeping sickness, is a severe disease that is fatal if left untreated (Pentreath and Kennedy, 2004). The mechanisms of pathogenicity and adverse reactions resulting from some drug treatments are only partially understood and remain a considerable challenge to sustainable control of the disease (Pentreath and Kennedy, 2004). There are indications that organs are invaded by trypanosomes with the central nervous system involvement leading to coma and death (Sternberg, 2004).

Wheat and its products are part of the regular western diet. Fermented wheat germ extract called avemer has been reported to control cell growth and proliferation mainly by inhibiting ribonucleotide reductase, needed to make new DNA to support replication (Sukkar and Edoardo, 2004). Scholten *et al.* (1999; 2002), also, reported that fermented liquid diets have positive influence on gastrointestinal health, by lowering gastric pH, increasing contents of short chain fatty acids, reducing microbial activity and improving mucosal architecture. We have earlier reported that the administration of ethylacetate extract of fermented wheat seed could be a potential candidate in the management of African sleeping sickness (Yusuf and Ekanem, 2010). In this report, we assessed the effect of wheat-supplemented diet on parasite replication in *T. brucei* infected rats.

MATERIALS AND METHODS

Feed Composition

The feed was formulated with the different classes of food: corn starch (maize and wheat) was used as source of carbohydrate, dried milled soya beans was used as protein, pure soybean oil was used as lipid and dried rice husk was used as fibre. Other

ingredients of the feed included DL-methione and vitamin/mineral mix .

Table 1: Feed Composition (g/kg)

Feed component	Weight(g/kg)
Corn/wheat starch	516
Soya bean	250
Oil	40
Cellulose (Rice husk)	40
Vitamin/mineral mix	50
DL – Methionine	4
Sucrose	100
Total	**1000**

Source: Ekanem *et al*. (2006)

Inoculation of Rat

Parasite-infected blood was obtained from the tail of infected rats at high parasitaemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritonneal cavity of uninfected rat weighing approximately 180 to 200g. The suspension contained 3 to 4 trypanosomes per view at ×40 magnification (approximately 10^6 cells per ml) .

Parasite Count

Parasitaemia was determined by counting the number of trypanosomes per view under light microscope at ×40 magnification from thin blood smear obtained from the tip of the tail of infected rats.

Proximate Analysis

Proximate analysis was carried out as described in AOAC (1999) to estimate moisture, crude fibre, fat and ash content. The carbohydrate content was estimated using anthrone method.

Experimental Design

In the experiment, there were three groups of rats and each comprised of four rats. Group 1 comprises of infected rats fed with Control diet (control), group 2 were infected early treated (treatment commenced the first day of sighting parasite in the blood) and group 3 were infected prophylactic treated (treatment commenced 72hr before infection).

Statistical Analysis

The group means $\pm$ standard error (SEM) was calculated for each analysis and significant difference between means was evaluated by analysis of variance (ANOVA). Post test analysis was done using the Duncan multiple comparison tests. Values of ($p \leq 0.05$) were considered as statistically significantly (Adamu and Johnson, 1997).

RESULTS

Proximate composition of wheat

The proximate composition of wheat used for this work (Table 2) shows that carbohydrate forms the highest 72.503 ± 0.005%, lipid (11.690 ± 0.017%), Moisture content (7.756 ± 0.005%), and Protein (5.876 ± 0.005%), composition.

Table 2: Proximate composition of wheat used in this study

FOOD CLASS	PERCENTAGE (%) ± S.D
Carbohydrate	72.503 ± 0.005
Protein	5.876 ± 0.005
Lipid	11.690 ± 0.017
Crude fibre	1.340 ± 0.017
Ash	0.833 ± 0.005
Moisture content	7.756 ± 0.005

Each value is a mean of 3 determination ± S.D

Proximate composition of wheat-supplemented diet and control diet

The result of proximate composition of wheat supplemented diet and control diet shows that wheat supplemented had high percentage of carbohydrate (53.443 ± 0.005%), lipid (13.656 ± 0.011%),Moisture content (8.460 ± 0.010%), and Protein (18.316 ± 0.005%) compared to the Control diet that had carbohydrate (57.990 ± 0.020%), lipid (12.336 ± 0.005%),Moisture content (5.686± 0.005%), and Protein (16.800 ± 0.010%).

Table 3: Proximate composition of wheat supplemented diet and control diet.

FOOD CLASS	WHEAT DIET Percentage (%) ± S.D	CONTROL DIET Percentage (%) ± S.D
Carbohydrate	53.443 ± 0.005	57.990 ± 0.020
Protein	18.316 ± 0.005	16.800 ± 0.010
Lipid	13.656 ± 0.011	12.336 ± 0.005
Crude fibre	2.653 ± 0.050	3.350 ± 0.010
Ash	3.470 ± 0.010	3.836 ± 0.005
Moisture content	8.460 ± 0.010	5.686 ± 0.005

Each value is a mean of 3 determination ± S.D

Parasitaemia

Figure 1 showed the results of parasite count in the infected rats fed with wheat-supplemented diet compared with infected untreated (Control diet) rats. Wheat supplemented diet was fed to infected rats to assess its efficacy against *T.brucei* infection. The prophylactic (72hr before infection) and early (first day parasite sighted in blood) treatment shows low proliferation or replication of parasite with extension of surviving days from 6 days of the control (infected untreated) to 10 days for wheat supplemented diet rats (Fig 1).

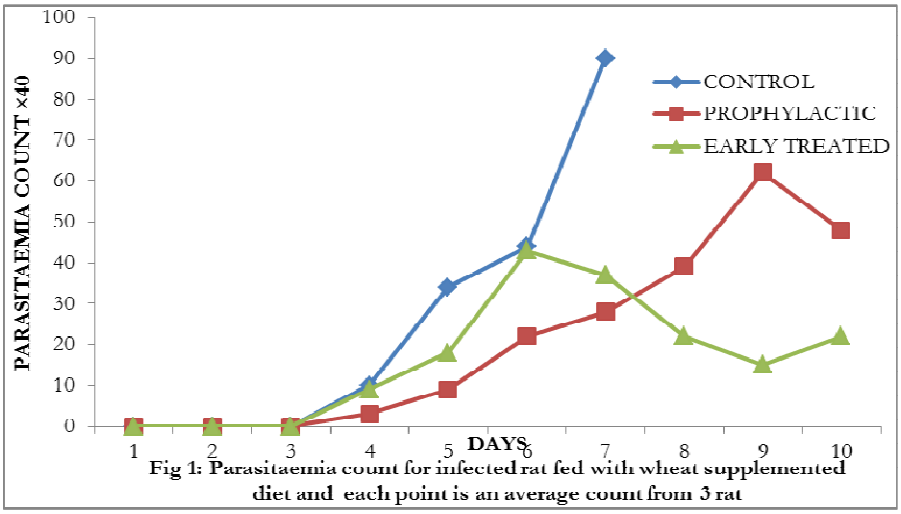

Fig 1: Parasitaemia count for infected rat fed with wheat supplemented diet and each point is an average count from 3 rat

DISCUSSION

Plant species have been reported to posses' trypanocidal activity (Freigburghaus *et al.,* 1996; Hoet *et al.,* 2004). Wheat is part of the regular western diet and has been reported to have therapeutic properties but no research has been done to ascertain the effect of wheat-supplemented diet on *T. brucei* infection. In the literature, mainly the effect of wheat feed intake has been studied (Scholten *et al.,* 2002), whereas in the present study the nutrient level of wheat seed and supplemented diet were investigated. The composition of wheat supplemented diet changed compared to the seed used in the feed formulation in such a way that the carbohydrate level decreased from 72.503±0.005 for seed to 53.443± 0.005 for supplemented diet. Whereas, the protein and ash contents increased significantly in formulated diet than the seed. (Table 1 and 2).

In the present study, the proximate composition of wheat supplemented diet shows high percentage of protein and moisture content compared with corn supplemented (Control) diet. The level of carbohydrate of the Control diet (corn supplemented) increased significantly 57.990±0.020 compared to 53.443±0.005 of wheat-supplemented diet (Table 2).

The effect of feeding of wheat supplemented diet on *T. brucei*-infected rats shows anti-trypanosomal properties by reducing parasite replication and extending the life span of the infected treated host. The present study demonstrated that the addition of wheat to diet *for T. brucei* infected rats changed the nutrient

composition of the diet and this had a positive effect on the parasitaemia level. These findings seem to agree with the previous studies that the administration of ethylacetate extract of wheat seed reduced parasitaemia level and extends the life span of *T. brucei*. (Yusuf and Ekanem, 2010).

DEDICATION

This paper is dedicated to the cherished memory of late venerable teacher Dr J.T. Ekanem. He was my B.Sc teacher, M.Sc teacher and supervisor and Ph.D guide and mentor.

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