

Effects of selected herbal diets on performance of Trypanosome-challenged West African Dwarf Sheep

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ABSTRACT

Herbal remedies play a major role in animal production with many of these herbs serving as treatment for some ailments and also allowing animals become more resistant to deleterious effects of disease. This study was conducted to evaluate the effect of herbal based diet on physiological parameters (temperature and pulse rate), feed intake, weight gain and feed conversion ratio respectively in West African Dwarf sheep infected with trypanosomes. Fifteen (15) West African Dwarf sheep separated into 5 groups fed various herbal diets in sole or mixed formulations. Temperature and pulse rate, feed intake, weight gain and feed conversion ratio were measured on a daily basis. Animals placed on Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) had a significantly higher average daily weight gain (24.32g, $p < 0.05$) when compared across all treatments while animals placed on Diet D (*Vernonia amygdalina* + *Citrus aurantifolia*) had a significantly higher ($p < 0.05$) average daily feed conversion ratio (35.02). A combination of *Occimum gratissimum* + *Vernonia amygdalina* and *Vernonia amygdalina* + *Citrus aurantifolia* aided in ameliorating the deleterious effects of trypanosomosis on production in sheep.

Keywords: Herbal diets, Sheep, Trypanosomosis, Weight gain, Feed conversion, physiological parameters

INTRODUCTION

Herbal based diets are constituted with certain percentage of one or more plants of medicinal relevance to the animals (Biobaku *et al.*, 2014). A medicinal plant which is a component of the diet is one whose one or more of its organs contains phytochemical substances that could be useful for therapeutic purpose (Ogunkunle and Ladejobi, 2006). The phytochemical principles in plants useful in prevention and treatment of diseases in animals are foreign to the body of the animals. It is a well-known fact that phytochemical substances being xenobiotics is capable of altering physiological parameters of the animals depending on the plant, species, health status of the animal, nutritional status and environmental influences (Biobaku *et al.*, 2008). The alteration of physiological parameters as compensatory mechanisms might alter metabolism and nutritive cellular demand cumulating to an alteration in feeding habit of the animal (Biobaku *et al.*, 2008). Similarly, *Khaya senegalensis*

directly affect the psychology of the animals and is used as psychotherapy in horses to improve their appetite (Muraina & Mamman, 2007). There are also bitter principles meliacines that could affect the secretion of exocrine hormones thereby affecting the feeding habits of the animals (Muraina & Mamman, 2007). This subsequently could affect weight of the animal.

The herbal diet containing various plants could affect haematological parameters in a previous study by Ajagbonna *et al.* (2005) it was observed that one or more of the phytochemical principles could improve the haematological parameters in trypanosoma infected experimental animals. In another study using phenylhydrazine induced anaemia model, it was observed that *Khaya senegalensis* improved the total erythrocytes count (Sanni *et al.*, 2005). Saponins from plant sources can have deleterious effect on the membrane of erythrocytes causing haemolysis (Nwude, 1997; Wanamaker & Massey, 2009). With the aforementioned, it is of utmost

importance to assess feed intake, weight gain and basic haematological indices on the effect of herbal based diets of *Trypanosome* infected animals.

The aim of this experiment is to evaluate the effect of herbal based diet on physiological parameters (temperature, pulse rate, feed intake and weight gain respectively) in West African Dwarf sheep infected with trypanosomes.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The study was conducted at the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin. The city of Ilorin (8.4799 °N, 4.5418 °E) is located in the Guinea Savannah zone of Nigeria characterised by high rainfall and humidity for about 10 months of the year. The duration of the study was three (3) months. The study was carried out humanely in accordance with the guidelines, governing the welfare of research animals by the University of Ilorin, and as approved by the Ethics Research Committee of the University of Ilorin, Ilorin, Nigeria.

EXPERIMENTAL ANIMALS AND MANAGEMENT

Fifteen (15) West African Dwarf sheep used for this study were purchased from local market within Ilorin city and were acclimatized for 4 weeks. All experimental animals were treated against any gastrointestinal parasites and any haemoprotozoan parasites such as *Babesia* and *Trypanosoma*. *Pestes des petit ruminant* (PPR) vaccine purchased from the National Veterinary Research Institute, Vom Plateau State, Nigeria was administered to all sheep. Antibiotics (Long acting oxytetracycline at 1ml/10kg body weight), anthelmintics (Mebendazole at 12.5mg/kg body weight (Jubaili Agrotec, Kano, Nigeria)) and Acaricide dips (Ethion made by Jubaili Agrotec, Kano, Nigeria) were also administered as clinically indicated. The sheep were randomly assigned to five (5) groups with each group having three (3) animals in a completely randomized design.

INOCULATION PROCEDURE

Strains of *Trypanosoma brucei* was obtained from the Nigerian Institute of Trypanosomiasis Research (NITR), Vom, Jos. The parasites were inoculated into two albino rats. The rats were later bled during the first wave of parasitaemia into an EDTA container (Ethylene diamine tetra acetic acid). The phase contrast buffy coat technique (Biobaku *et al.*, 2008) was used to detect and quantify trypanosomes in the blood samples using standard methods as described by Murray *et al.* (1998). WAD sheep previously screened for *Trypanosome* were inoculated intramuscularly with 3mls of phosphate buffered Saline containing 1.5×10^6 *Trypanosomes*. The Specie of *Trypanosome* was confirmed in the infected WAD sheep following laboratory blood test (Murray *et al.*, 1998) on the 6th day post-infection.

PREPARATION OF PLANT SPICES

Some of the plant samples (leaves from *Citrus aurantifolia* with voucher no: UILH/003/983; *Occimum gratissimum* with voucher no: UILH/002/1984; *Vernonia amygdalina* with voucher no: UILH/001/1023) were collected from the herbarium of University of Ilorin, Nigeria. The samples were identified at the Department of Plant Science, University of Ilorin, Nigeria. The collected samples were washed in a running tap to remove soil and dust particles. It was later air dried on the laboratory bench for five days. The dried samples were milled with pestle and mortar into a powdery form. The powdered samples were stored in a dry and clean covered container. These were mixed with prepared diets at 5% inclusion rate as shown in Table I below. The diets were fed to the animals based on the group they belonged to.

EXPERIMENTAL DIETS AND FEEDING

Throughout period of the experiment, the experimental animals were fed experimental diets based on 3% of their body weight; twice per day and water was given to them *ad libitum*. They were on these diets until the experiment was terminated at the end of 3rd week.

DATA COLLECTION

Body temperature was taken daily from the rectum by the use of a clinical thermometer (Hanimax Thermometer Solan Spain) which was inserted into the rectum and allowed to touch rectum mucous membrane and stay in it for 2 minutes. Parasitaemia was determined on a weekly basis according to the method described by Murray *et al.* (1977).

FEED INTAKE

Average daily feed intake was recorded daily as the difference between the initial known weight of feed (3% of Animal Body weight) offered to the animals and the left-over collected.

WEIGHT GAIN

Body weight was recorded on a weekly basis with the use of a weighing scale (Camry emperors, China for 1.00kg and above). Record obtained was used to calculate weight gain.

DATA ANALYSES

Data generated from this study were expressed as means \pm SEM and analysed using one –way analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant. The software Graph Pad Prism (version 5.3) was used for the analysis of the data.

Table 1. Composition of the Experimental diets for West African Dwarf Sheep

	A (Control)	B (CA)	C (OG)	D (VA+CA)	E (OG+VA)
Cassava waste	60.00	55.00	55.00	55.00	55.00
Plant extract	-	5.00 ^b	5.00 ^c	5.00 ^d	5.00 ^e
Soyabean meal	16.00	16.00	16.00	16.00	16.00
Rice husk	22.00	22.00	22.00	22.00	22.00
Vitamin-Mineral Premix	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

B-(*Citrus aurantifolia*), C-(*Occimum gratissimum*), D-(*Occimum gratissimum* + *Vernonia amygdalina*), E- (*Vernonia amygdalina* + *Citrus aurantifolia*).

CA: *Citrus aurantifolia*; OG: *Occimum gratissimum*; VA+CA: *Vernonia amygdalina*+ *Citrus aurantifolia*; OG+VA: *Occimum gratissimum*+ *Vernonia amygdalina*

RESULTS

Table 2 represents the performance indices of *Trypanosoma brucei* infected WAD sheep placed on the various herbal based diets. There was significant difference in body temperature ($p>0.05$) of the WAD sheep infected with *Trypanosoma brucei* across all treatments (highest in animal placed on Diet E and lowest in Control Diet A); all the animals had an average temperature of 39°C. Furthermore, a significant difference ($p>0.05$) was observed in the pulse rates of the sheep across all treatments, however animals placed on Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) had a significant lower pulse rate (69.54, $p<0.05$) while those placed on Diet C (*Occimum gratissimum*) had a significant higher pulse rate (78.22, $p<0.05$). The control Diets A and other diets had respectively pulse rate of 74.06, 76.40 and 39.20 cycle/minute.

Feed intake also followed the same trend as no significant difference ($p>0.05$) was observed across all treatments, however animals on Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) treatment showed numerical higher (856.4, $p>0.05$) feed intake while those on Diet D (*Vernonia amygdalina*+ *Citrus aurantifolia*) treatment had numerical lower feed intake (678.0, $p>0.05$).

Animals placed on Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) had a significantly higher average daily weight gain (24.32g, $p<0.05$) when compared across all treatments; while the animals placed on Diet B (*Citrus aurantifolia*), Diet C (*Occimum gratissimum*) and Diet D (*Vernonia amygdalina*+ *Citrus aurantifolia*) herbal treatments were not significantly different ($p>0.05$) from the control Diet A, however, those placed on diet B (*Citrus aurantifolia*) showed a significant lower ($p<0.05$) average daily weight gain (17.08g/wk).

Animals placed on Diet D (*Vernonia amygdalina*+ *Citrus aurantifolia*) had a significantly higher ($p<0.05$) average daily feed conversion ratio (35.02) closely followed by Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) with the control group Diet A having the lowest feed conversion ratio.

Table II: Performance indices of West African Dwarf Sheep placed on the various Experimental Diets

Diet	Temperature (°C)	Pulse rate (cycle/minute)	Feed intake (g/d)	Weight gain (g/d)	Feed conversion ratio g feed/g BW
Control (A)	39.06 ± 0.21 ^d	74.06 ± 2.08 ^d	830.0 ± 2.55 ^b	19.14 ± 2.03 ^b	43.36 ± 3.1 ^e
CA (B)	39.18 ± 0.19 ^c	76.40 ± 4.11 ^b	734.0 ± 5.79 ^d	17.08 ± 3.98 ^c	42.97 ± 5.45 ^d
OG (C)	39.14 ± 0.31 ^c	78.22 ± 3.98 ^a	750.0 ± 6.11 ^c	20.82 ± 4.22 ^b	36.02 ± 4.77 ^c
VA+CA (D)	39.26 ± 1.76 ^b	75.04 ± 3.76 ^c	687.0 ± 6.32 ^e	19.62 ± 4.22 ^b	35.02 ± 5.55 ^a
OG+VA (E)	39.34 ± 1.88 ^a	69.54 ± 4.33 ^e	856.4 ± 5.88 ^a	24.32 ± 4.02 ^a	35.21 ± 6.23 ^b

Means of 15 determinations with different superscripts along the same column are significant ($P<0.05$)

CA: *Citrus aurantifolia*; OG: *Occimum gratissimum*; VA+CA: *Vernonia amygdalina*+ *Citrus aurantifolia*; OG+VA: *Occimum gratissimum*+ *Vernonia amygdalina*

DISCUSSION

Fairly constant body temperature exhibited by the WAD sheep (38.3-39.9°C) used in this study which is within the normal body temperature for sheep is suggestive of the tendency to suppress parasitaemia as described by Biobaku *et al.*(2009). Pulse rates (70-80cycle/minute) and average daily feed intake, is suggestive of effect of the phytochemical present in the herbs used in the study, most of which promote trypanotolerance mechanism in animals.

Although, the difference observed in the aforementioned indices were significant ($P < 0.05$), herbal mixtures particularly those of E treatment showed better acceptability, feed conversion and overall metabolic response when compared to other herbal diets. The ethanolic leaf extract of *O. gratissimum* was found effective in inhibiting/preventing disease condition after infection through immunostimulatory mechanisms (Oladunmoye, 2006) which in combination with VA reported to increase performance in broiler chickens (Osho et al., 2014). This might be the reason the sheep fed this combination had an excellent feed conversion during the study despite being challenged with *T. brucei*.

Table III: Level of *Trypanosoma brucei* parasitaemia following administration of feed

	A (Control)	B (CA)	C (OG)	D (VA + CA)	E (OG + VA)
Pre-inoculation	Nil	Nil	Nil	Nil	Nil
6 th Day Post-inoculation	+++	+++	+++	+++	+++
3 rd week on diet	+++	+	+	+	Nil

+ (Mild Parasitaemia), ++ (Moderate Parasitaemia). +++ (Severe Parasitaemia)

The body temperature of the herb-treated WAD sheep were fairly stable, as with the control and this could be due to tissue. Concurrently, phytochemicals present in herbs could act as immunostimulants which effectively delayed parasitaemia and yet simultaneously acting as insulin-inhibitors thereby reducing glucose uptake in the blood and enhancing body weight gain (Morrison *et al.*, 1978). This was the trend observed in Diet E (*Occimum gratissimum* + *Vernonia amygdalina*) as weight gain was significantly higher (24.32g, $P < 0.05$), even though feed intake of animal on this diet was comparable with those on other Diets. A number of plant phenolics present in these herbs have been shown to possess certain anti-microbial potency, which when compared with chemotherapeutic drugs commonly used are relatively less toxic to the GIT and possess reduced risk of pathogen resistance (Legros *et al.*, 2002; Kennedy, 2004). It should be noted that just as in the rat experiment, initiation of host cell type II anti-inflammatory response to combat and stabilize the excessive production of pro-inflammatory cytokines which could shut down the body system was

necessary for the WAD sheep (Janeway & Medzhitor, 2002). The usual trend according to reports by Murray *et al.*(1998) for trypanosome infected animals include pathological symptoms as mainly loss of body weight, fever (pyrexia), reduced locomotory activity, hence by evading such symptoms suggest efficacy of the herbs in treating the disease, especially Diet E (*Occimumgratissimum* + *Vernonia amygdalina* treatment (Wright and Philipson, 1990;Hoet *et al.*, 2007). Tijani *et al.*(2009) observed that the stem barks of *F. albida* was effective in the management of *T. brucei* anaemia in rats and in related study of Hoet *et al.* (2007), 100mg/kg dose of herbal extract was trypanostatic because parasitaemia was kept relatively low compared to the untreated animals. Herbal mixtures exhibit trypanostatic effect which is often associated with reduction in anaemia and increased weight gain (Ogbadoyi *et al.*, 1999). Physiologically, this is done by reducing the parasitic load and by neutralizing the toxic metabolites produced by the trypanosomiasis. The development of fever during the course of trypanosome infection is associated with increased heat production and increased metabolisable energy for

maintenance. The consequence of this is that the proportion of feed that would be used for growth is reduced as it is metabolized to provide extra energy for maintenance, hence greater growth retardation (Verstegen *et al.*, 1991). Phytochemical screening of the herbs revealed that it is rich in flavonoids and terpenes and the pharmacological actions inherent

are believed to be due to the presence of these phytochemicals (Ajagbonna *et al.*, 2003; Biobaku *et al.*, 2009, 2010).

CONCLUSION

It was thereby evident that sheep preferred E (OG+VA) mixture for better acceptability and performance during experimental trypanosomosis and its management. These sheep ate more and also a significantly high feed conversion ratio for this diet. Therefore, showing a combination of *Occimum gratissimum*+*Vernonia amygdalina*and *Vernonia amygdalina*+*Citrus aurantifolia* aided in ameliorating the deleterious effects of trypanosomosis on production in sheep.

CONFLICT OF INTEREST

No conflict of interest

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