

EFFECTS OF DIET FORTIFICATION WITH *MORINGA OLEIFERA* AND *AZADIRACHTA INDICA* LEAF MEALS ON SERUM TOTAL PROTEIN OF RABBITS INOCULATED WITH *TRYPANOSOMA BRUCEI BRUCEI*.

Salifu, A. O^{*}, Carew, S. N.[°] & Adamu, M.^v

^{*}Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Department of Animal African Trypanosomiasis (AAT) Kaduna.

[°]University of Agriculture Makurdi, Benue State, Department of Animal Production.

^vUniversity of Agriculture Makurdi, Benue State, Department of Veterinary parasitology.

ABSTRACT

The anti-trypanosomal effects of dry leaf meals of *Moringa oleifera* and *Azadirachta indica* for serum total protein were investigated using grower rabbits. Forty rabbits were randomized into eight groups (1, 2, 3, 4, 5, 6, 7 and 8) of five animals each with mean live weights of 1.2 Kg. Groups 5, 6, 7 and 8 were inoculated subcutaneously with normal saline diluted blood containing $3.98 \times 10^6 \text{ ml}^{-1}$ of *Trypanosoma brucei brucei*, while groups 1, 2, 3 and 4 were not inoculated. The rabbits were fed on four diets. Diet (D) 1 had no leaf meal (basal) and was fed to groups 1 and 5 (controls). D 2 was fortified with 1% Moringa Leaf Meal (MLM) and was fed to groups 2 and 6. D 3 had 1% Neem Leaf Meal (NLM) and was fed to groups 3 and 7. D 4 had both MLM and NLM at 1% each and was fed to groups 4 and 8. Data were subjected to statistical analysis using SPSS ANOVA and Duncans new multiple range test at 95% confidence limit. The results of total blood proteins gl^{-1} was significantly ($P < 0.05$) higher for rabbits inoculated with *T. brucei brucei* than for non inoculated rabbits except group 6 ($P > 0.05$). The findings in this study reveals that the serum total protein of rabbits in group 6 inoculated with *T. brucei brucei* which was not significant ($P > 0.05$) from group 2 (non inoculated rabbits) supports the hepato protective capability of MLM over NLM.

Keywords: Anti-trypanosomal, serum total protein

INTRODUCTION

The disease Animal African Trypanosomosis (AAT) affects most domestic animals. It is caused by extracellular haemoprotozoan parasites belonging to the genus *Trypanosoma* (Zawua *et al.*, 2015). The most important species of trypanosomes affecting livestock in Nigeria are *T. vivax*, *T. brucei brucei*, *T. congolense*, *T. evansi* and *T. simiae*. Trypanosomes are mainly transmitted cyclically by tsetse fly, a vector belonging to the genus *Glossina*. Trypanosomosis is considered to be among the most important constraint to livestock productivity in sub Saharan Africa (Wellburn *et al.*, 2001). Losses include a decrease in productive potential such as diseased growth rate, poor milk yield, weight loss in young growing animals and death (Kaewthamasorn and Wongsamee, 2006). Several ethno medicinal plants have been researched on to find alternative treatments for trypanosomosis as against the three major salts of Diminazene, Homidium and Isomethamidium chloride produced in 1950s and still used as trypanocides to treat AAT (Nok *et al.*, 1993; Geerts *et al.*, 2001 and Atawodi, 2005).

The shrub *Moringa oleifera* is a pan-tropical species that is reputed to possessing nutritional and medicinal properties (Gafar and Itodo, 2011). It belongs to the family moringaceae. It is widely cultivated in Asia, Africa and other tropical parts of the world (Makker and Becker, 1997). Anti-trypanosomal trial of moringa leaves prolonged the lifespan of rats and was reported to be non toxic (Olugbemi *et al.*, 2010).

The neem tree scientifically called *Azadirachta indica* has been highly valued in India for over 4,000 years and is referred to as miraculous healing plant (Gunguli, 2002). It belongs to the mahogany family meliceae and can reach a height of 15-20 meters. Reports had been made on the trypanocidal potentials of *Azadirachta indica* leaves extract (Nok *et al.*, 1993). Domestic rabbits (*Oryctolagus cuniculus*) were used as model laboratory animals to represent animals naturally infected by trypanosomosis. The aim of this study was to investigate the antitrypanosomal effect of serum total protein in prophylactic treatment of moringa and neem leaf meals against *T. brucei brucei* experimentally infected rabbits.

MATERIALS AND METHODS

Experimental Site

This study was conducted at the Nigerian Institute for Trypanosomiasis Research (NITR) -Vom in Plateau State, Nigeria.

Preparation of Moringa and Neem Leaf Meals

Fresh moringa and neem leaves were harvested from residential houses in hudco quarters north bank, makurdi, Benue state. The leaves were air dried on clean surfaces in the laboratory indoors until crisp. The dried leaves were then pulverized and stored in sealed polythene bags until they were used in feed preparation.

Experimental Animals and their Management

Forty weaner rabbits were purchased from Dagwom farm in Vom. They were vaccinated against Coccidiosis using Coccinor, treated with long acting Oxytetracycline and Ivermectin following manufacturer's directives against mange and gastro-intestinal parasites. The animal house, hutches and all handling equipments were washed with detergent and disinfected with 3.5% Sodium Hypochlorite before arrival of the rabbits. Each rabbit were housed individually in wire mesh cages. An acclimatization period of three weeks was observed. The animals were maintained according to the CIOMS guidelines (1985).

Proximate Composition and Energy Content of Leaf Meals

The proximate composition and energy value of *Moringa oleifera* Leaf Meal (MLM) and Neem Leaf Meal were determined according to AOAC (2002) at the Biochemistry Department of the National Veterinary Research Institute (NVRI) Vom.

Experimental Diets, Animal Groups and Inoculation with Trypanosomes

Four types of diet and eight groups of rabbits fed with diet 1 which contained no leaf meal (basal diet) served as control. The second diet (D 2) contained 1% Moringa Leaf Meal (MLM). The third diet (D 3) had 1% Neem Leaf Meal (NLM). The fourth diet (Diet 4) had approximately 1% each of MLM and NLM. Diet 1 was fed to groups 1 and 5, diet 2 was fed to groups 2 and 6, diet 3 was fed to groups 3 and 7 and diet 4 was fed to groups 4 and 8 respectively.

The *T. brucei brucei* (Federe Strain) used was obtained from the parasitology division of Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The parasites were maintained by serial passage in albino rats bred in NITR-Vom. Blood was obtained from the serial passage rats by tail bleeding into normal saline and the parasitaemia adjusted to 3.98×10^6 trypanosomes per milliliter (ml) by the method of Herbert and Lumsden (1974). Each infected rabbit received 0.5ml of saline containing *T. brucei brucei*. The infection was by intraperitoneal injection. The duration of the experiment was 12 weeks (3 weeks pre-infection and 9 weeks post-infection periods).

Collection of blood samples

Trypanosomes were monitored daily and detected using a rapid approximation method (Herbert and Lumsden, 1974). At the end of the experiment, 5ml of blood was collected from the heart of three rabbits each per group and euthanized, for serum total protein test into blood sample tubes.

Biochemical technique

The total plasma protein was estimated using the Golberg refractometer method as described by Kohn and Allen (1995).

Statistical analysis

The data collected were subjected to statistical analyses by Analysis Of Variance (ANOVA) and Duncans New Multiple Range Test (DMRT) at 95 % confidence limit using SPSS 24 (2016). Data are presented as Mean $\pm$ standard error ba

RESULTS AND DISCUSSION

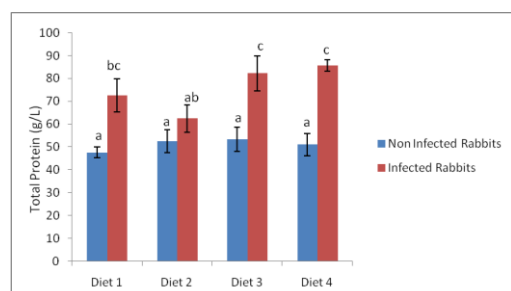
The results of proximate composition of neem and moringa leaf meals are presented in Table 1. The moringa leaf meal had higher moisture, crude protein, ether extract, ash, Nitrogen Free Extract (NFE) and metabolizable energy than the neem leaf meal.

Table 1: Proximate composition of Neem and Moringa leaf meals.
Weight per 100grams of sample.

Neem Leaves	Moringa Leaves	
Proximate analysis	Result (g/100g)	
Moisture	10.4	15.0
Crude Protein	16.2	25.4
Ether Extract	4.1	6.3
Crude Fibre	14.5	8.6
Ash	7.4	8.8
NFE	47.3	35.9
Metabolizable Energy Kcal/Kg	2596.1	2694.5

M.E was calculated using the method of Ponzenga, 1985. M.E (Kcal/Kg) = $37 \times \% \text{CP} + 81.8 \times \% \text{EE} + 35 \times \% \text{NFE}$.

The results on serum total protein effects of moringa leaf and neem leaf meals are presented in figure 1. It revealed significantly ($p < 0.05$) higher total protein for the rabbits inoculated with *Trypanosoma brucei* than the non inoculated rabbits for groups fed on diets 1, 3 and 4 except for diet 2 which had no significant difference ($p > 0.05$) between the non inoculated and the *T. brucei* inoculated rabbits that fed on the diet containing the 1% moringa leaf meal. The significantly higher ($P < 0.05$) serum total protein of the infected rabbits in this study agrees with the report of Takeet and Fagbemi (2009) who also had increase in serum total protein of rabbits infected with trypanosomes. This strongly suggests an increase in some forms of the serum protein reacting to the presence and activities of the trypanosomes in the body system of animals.



Different a, b and c means are considered significant at $p < 0.05$, I error bar.

Figure 1. Effects of diets 1(basal), 2 (basal and 1% Moringa Leaf Meal MLM), 3 (basal and 1% Neem Leaf Meal NLM) and 4 (basal, 1% MLM and 1% NLM) on serum total protein of rabbits after 9 weeks post inoculation (pi) with *Trypanosoma brucei brucei*.

CONCLUSION AND RECOMMENDATION

The findings in this study reveal the superior hepato protective capabilities of MLM over NLM in *T. brucei brucei* infection constraint. In conclusion, the serum total protein revealed greater potentials of moringa over neem in trypanosomosis infection of rabbits. It is recommended that MLM should be selected to ameliorate trypanosomosis disease over NLM.

REFERENCES

- AOAC. (2002). Association of Official Analytical Chemist. Official method of Analysis. 16 edition, William Tryd. Press Washington D.C U.S.A. Chapter 4 PP. 17-34.
- Atawodi, S. E. (2005). Comparative In vitro trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian Savannah plants. *African Journal of Biotechnology*. 4(2): 177-182.
- CIOMS (1985). Council for International Organizations of Medical Sciences International guiding principles for biomedical research involving animals.
- Gafar, M. K. and Itodo, A. U. (2011). Proximate and Mineral Composition of Hairy Indigo Leaves. In *Electronic journal of Environmental, Agricultural and Food Chemistry (EJEAChe)*. 10(3): 2007-2018.
- Geerts, S., Holmes, P.H., Eisler, M. C. and Diall, O.(2001). African bovine trypanosomiasis: The problem of drug resistance. *Parasitology. Today.*, 17(1): 25-28.
- Gunguli, S. (2002). Neem. A therapeutic for all seasons. *Journal of current science*. 82 (11): 1304-1306.
- Herbert, N.J. and Lumsden, W.H. (1976). *Trypanosoma brucei*: A rapid "Matching" Method for Estimating the Host's parasitemia. *Experimental Parasitology* 40: 429-431.
- Kaewthamasorn, M. and Wongsamee, S. (2006). A preliminary survey of gastrointestinal and haemoparasites of beef cattle in the tropical livestock farming system in Nan province, Northern Thailand. *A journal of parasitological Research*.
- Kohn, R. A. and Allen, M. S. (1995). Enrichment of proteolytic activity relative to nitrogen in Preparations from the rumen for in vitro studies. *Animal feed science technology*, 52(1/2): 1-14.
- Makkar, H. P. and Becker, K. (1996, 1997). Nutritional value and anti-nutritional components of whole and ethanol extraction of *M. oleifera* leaves. *Journal of Animal Feed Science and Technology* 63: 311-322.
- Nok , A.J., Esievo, K.A., Hondjet, I., Arowosafe, S., Onyenekwe, P. C., Gimba C. E., and Kagbu, J.A. (1993). Trypanocidal potential of *Azadirachta indica*: Invivo activity of leaf extract against *T. brucei brucei*. *Journal of Clinical Biochemistry and Nutrition.*, 15: 113-118.
- Ogbuewu, I. P., Okoli, I. C. and Iloeje, M. U. (2008). Serum Biochemical Evaluation and organ weight characteristics of Buck Rabbits fed Graded levels of Neem (*Azadirachta indica*) leaf meals Diets. www.priory.com/neem_rabbits.
- Olugbemi, T.S., George, P. and Dauda, O.M. (2010). Anti-trypanocidal potentials of aquaeous and ethanolic leaf extracts of *Moringaoleifera* in wistar stock albino rats. www.ajol.info/journalhome 8: (2).
- Pauzenga, U. (1985). Feeding parent-stock. Zootenicia International, December 1985. Pp. 22-24.
- Takeet, M. I. and Fagbemi, B. O. (2009). Haematological, Pathological and Plasma Biochemical changes in Rabbits experimentally infected with *Trypanosoma congolense*. *Science World Journal*, 4(2):1597-6343.
- Wellburn, S. C., Coleman, P. G., Fevre, E. and Maudlin I. (2001). Sleeping Sickness- a tale of two diseases. *Trends Parasitology*. 17:19-24.
- Zawua, T. P., Amali, O., Amuta, E. U. and Sar, T. T. (2015). Haemoparasites of cattle Slaughtered for sale within Gboko Metropolis of Benue State, Nigeria. *Nigerian journal of parasitology*. 36 (1): 72-76.