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Effect of dietary supplementation of neem oil (*Azadirachtaindica*) on the haematology, serum biochemistry and carcass characteristics of weaned rabbits

Oluwafemi R. A and Oluwayinka E. O
Department of Animal Science, Faculty of Agricultural Sciences, University of Abuja, FCT-Abuja. Nigeria.
Corresponding author: E-mail: olaoluwayinkamail@gmail.com

ABSTRACT: The objective of the present study was to determine the effect of dietary supplementation of neem oil (*Azadirachtaindica) on the haematology, serum biochemistry and carcass characteristics of weaned rabbits.* A total of 50 weaned male cross bred rabbits between 5-6 weeks with an average weight of 565.4g-566.8gwere divided into five dietary groups of ten (10) weaned rabbits each in a completely randomized design. The dietary treatments include a control, T1 (basal) diet with no neem oil (NOL), T2, T3, T4 and T5 were fed basal diet supplemented with NOL at 0.1%, 0.2%, 0.3% and 0.4% respectively. Feed and water were offered *ad libitum* throughout the experiment which lasted for 12 weeks. The data obtained was used to evaluate the haematology: packed cell volume, Haemoglobin, Red Blood Cell, White Blood Cell, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration., serum biochemistry: Total protein, Globulin, Cholesterol, Glucose and Serum electrolytes (Sodium and chloride) and carcass characteristics, final weight, head, dressing percentage, liver, kidney, heart, lungs and spleen were significantly ($P<0.05$) different among the treatments. All the haematological parameters evaluated differs significantly ($P<0.05$) except haemoglobin, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration which were not influenced ($P>0.05$) by the dietary supplementation of neem oil. The serum biochemistry parameters evaluated differs significantly ($P<0.05$) among the treatments, except total protein values which were not significantly affected ($P>0.05$) by neem oil ($P>0.05$). Result on carcass evaluation revealed that T5 had the highest weight gain (755.90 g) followed by T4 (734.0g), T3 (705.90g), T2(705.0g) and T1(621.80g) respectively. Highest mortality was recorded in T1 (2%) followed by T2 (1%), none was recorded in T3, T4 and T5. Neem oil significantly influenced ($P<0.05$) all the parameters measured. It was concluded that neem oil contains some essential nutrients and bioactive chemicals and could be included in the diets of rabbits at 0.4 % without causing any deleterious effect on the performance and health of the animal.

Key words: Rabbits, neem seeds, *Azadirachtaindica*, haematology, serum biochemistry, carcass.

Introduction

With so many research carried out to find endogenous feed alternatives for rabbit production as the high cost of raw materials that are used for commercial feed increase the production cost., there is also an urgent need to embark on scientific findings on livestock's natural growth promoter which will ensure increasing growth rate, enormous availability of meat and other high value bye-products for the entire populace at relatively conservative cost. Producers use growth promoters to increase growth rates and improve overall efficiency and product quality without leaving any toxic residue in the body system of livestock. Their inclusion in feedstuffs should be designed so as to feature a pharmacological characteristic that enhance the immunity of the animal and to help in minimizing the use of the conventional antibiotics in prevention and treatment of diseases of livestock. The conventional artificial growth promoters are known to have deleterious effect on human who are the secondary consumer of residues of artificial growth promoter in the body of livestock (Sinniah, 1981).

Neem belongs to the kingdom: Plantae; Division: Magnoliophyta; Order: Sapindales; Family: Meliaceae; Genus: *Azadirachta*; Species: *indica*., It is a tropical evergreen related to mahogany. Native to east India and Burma, it grows in much of Southeast Asia and West Africa; a few trees have recently been planted in the Caribbean and several Central American countries, including México. The name *Azadirachtaindica*is derived from a Persian term "Axaddarakth" (free tree). In Ayurveda it is known as the 'Arishta', which means "relieving sickness" in Sanskrit. It is a medium sized or large evergreen tree with irregular rounded crown, attaining a height of 14m-20m. It is a hardy tree that grows well in sandy, stony shallow soil, and is tolerant to alkaline, saline and acidic soil and it grows well on black cotton soil (Patnaik, 1993). Neem is ubiquitous in Northern Nigeria. The Neem tree popularly referred to in Hausa language as *Dogonyaro* is a tree in the mahogany family with broad dark brown stem and widely spread branches.According to Subbalakshmi*et al.*, (2012), all parts of neem like seeds, flowers, bark and leaves are beneficial due to their medicinal properties. Research has shown that neem will boost the immune system by stimulating the production of T-cells when challenged with infections (Upadhyay, 1990). The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Demiray*et al.*, 2009). The bark of the neem has been reported to have higher phenolic and antioxidant activity compared to the leaf (Ghimeray*et al.*, 2009; Olabinri*et al.*, 2009). Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation and skin infections (Biswas *et al.*, 2002). The neem tree contains more than 100 bioactive ingredients and the most important bioactive compound is azadirachtin (Nahak and Sahu, 2010). The Neem leaves, neem oil and de-oiled neem seed cake are used as animal feeds (Ogbuewu *et al.*, 2010a). The neem leaves contain appreciable amounts of proteins, minerals, carotene and adequate amount of trace minerals (Ogbuewu *et al.*, 2010). Neem tree as one of the most researched tree in the world has attracted world-wide prominence due to its vast range of medicinal propertieslike antibacterial, antiviral, antifungal, antiprotozoal,hepatoprotective and other various properties without showing any adverse effect (Kale *et al.*, 2003).

The compounds in neem have been divided into two major classes; isoprenoids and others (Singh *et al.*, 1996). The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and Csecomeliacins such as nimbin, salanin and azadirachtin. The none-isoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc). (Zengin*et al.*, 2016a, Zengin *et al.*, 2016b).

MATERIALS AND METHODS

Experimental Site

The study was carried out at University of Abuja Teaching and Research farm, Airport road, Abuja., in Gwagwalada area council of the Federal Capital Territory, Abuja. Gwagwalada is situated in the North central zone of Nigeria., Lying at the latitude N 9.0765 and longitude E 7.3986 at an average elevation of 476m above sea level.

Collection and processing of neem oil (NOL)

Neem seeds were collected from Gwagwalada, Abuja and identified at the department of biological sciences, University of Abuja, Nigeria. The seeds of Neem were separated from the seed coats manually and sundried for 2 weeks. The dried seeds were granulated into coarse particles using a blender (Model Ap-DKL, Samsung). Oil was extracted using the soxhlet extraction method; it was later poured into a well labeled container for further analysis.

Experimental animals and their management

Fifty (50) apparently healthy, cross bred weaned male rabbits with average initial body weight of 565.4g-566.8g were used for the study and were randomly allotted into Five Treatments with ten (10) rabbits per treatment designated as treatment 1, 2, 3, 4 and 5 in a Completely Randomized Design (CRD), animals were kept in an all wired hutch measuring 35 × 35 × 55cm (width × length × height).All treatments have 5 replicates with two (2) rabbits per replicate. After 14 days of acclimatization, all rabbits were fed diets corresponding to their treatments and given prophylactic treatment with broad-spectrum medication (Kepromec®) against endoparasites and helminthes infestation before the commencement of the experiment. Feed and water was given *ad libitum* and all other management practices were strictly adhered to.

Experimental diets

Basal were formulated to meet the nutritional requirement for rabbits according to NRC (1977).

Treatment 1 – Basal diet + 0 % NOL

Treatment 2 – Basal diet + 0.1 % NOL

Treatment 3 – Basal diet + 0.2 % NOL

Treatment 4 – Basal diet + 0.3 % NOL

Treatment 5 – Basal diet + 0.4 % NOL

Haematology and serum biochemistry

Blood samples were collected from the ear vein of each animal with a sterilized disposable syringe and needle. In order to minimize the standard error in values, the animals were fasted for 12 hours prior to blood collection. Five milliliter of blood sample were collected into bottles containing anti–coagulant Ethylene Diamine Tetra Acetate (EDTA) for haematological analysis while the blood samples for serum analysis were collected into sterile bottlesfor analysis at specific days intervals of 0, 35, and 70 days of the study and were accurately labelled. Samples were let to coagulate and centrifuged for 15min and serum was separated and stored immediately at -20^0C till analyzed. The haematological parameters which were measured include packed cell volume,Haemoglobin, Red Blood Cell, White Blood Cell, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration.The serum biochemical constituents observed were Total protein, Globulin, Cholesterol, Glucose and Serum electrolytes (Sodium and chloride). All the parameters were estimated in an automated biochemical analyzer (Accurex — Sphera Automated Clinical Chemistry Analyzer Italy), using commercial kits according to manufacturer instruction.

Carcass characteristics

At the end of the experiment, fifteen rabbits (i.e 3 rabbits per treatment) across the treatments were randomly selected for slaughtering. They were deprived of feed for 12 hours as recommended by Joseph *et al*., (1994). Withholding feed for 12 hours before slaughter reduces the volume of gut contents and hence, bacteria and therefore reduces the risk of contamination of the carcass during dressing without adversely affecting meat yield and quality (FAO, 1991; Joseph *et al*., 1994). The rabbits were weighed and slaughtered humanely (Mann, 1960). After slaughtering, incision was carefully made around the abdomen with a pen knife to create space through which the visceral were removed. The weight of the kidney, heart, liver, lungs and pancreas were taken. The organs were weighed using the electronic sensitive weighing scale and their respective weights were recorded and expressed as a percentage of fasted live weight. The dressed carcass is the portion of the rabbit remaining after the removal of the head, feet, fur, tail and visceral organs. The dressed carcasses were splits into retail cuts such as shoulder/forelegs, thigh/hind leg, rack and loin as described by Blasco*et al.* (1993).

$$\text{Dressed percentage} = \frac{\text{Dressed carcass weight (g)}}{\text{Live weight (g)}} \times 100$$

STATISTICAL ANALYSIS

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (18.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

RESULTS AND DISCUSSION

Proximate composition of experimental diet

Table 1 shows the proximate composition of experimental diet. The proximate components contained crude protein (18.22 %), crude fibre (13.22 %), ether extract (3.20 %), ash (6.15 %) and energy (2566.5 kcal/kg). The crude protein, crude fibre and energy values reported in this experiment is in agreement with the findings of Ahmed et al. (2018); Alagbe and Oluwafemi (2019) but contrary to the reports of Ahmed *et al.* (2019) when thyme oil was fed to growing rabbits. However, all values were within the nutritional requirement of growing rabbits according to NRC (1977). Adequate intake of dietary fibre lowers the serum cholesterol level, risk of coronary heart disease, constipation and colon and breast cancer (Fashola, 2011; Alagbe, 2019; Olanipekun s*et al.*, 2016). Ash content gives an indication of the amount of minerals present in a feed, which are important in many biochemical reactions functioning as co-enzyme and aid physiological functioning of the major metabolic processes in the body (Ojewuyi *et al.*, 2014).

Table1: Chemical composition of experimental diet

Materials	Quantity (Kg)
Maize	30.0
Wheat offal	20.0
Soya meal	16.25
Groundnut cake	10.0
Palm kernel meal	20.0
Bone meal	2.00
Limestone	1.00
Lysine	0.01
Methionine	0.01
*Premix	0.25
Salt	0.25
Total	100.0
Calculated analysis	
Crude protein (%)	17.22
Crude fibre (%)	13.20
Ether extract (%)	3.02
Ash (%)	6.15
Energy (Kcal/kg)	2566.5

*Premix supplied per kg diet: Vit A, 7,000 I.U; Vit E, 5mg; Vit D3, 3000I.U, Vit K, 3mg; Vit B2, 5.5mg; Niacin, 25mg; Vit B12, 16mg; Choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu,
2.6g; Folic acid, 2mg; Fe, 5g; Pantothenic acid, 10mg; Biotin, 30.5g; Antioxidant, 56mg.

Effect of different levels of neem (*Azadirachtaindica*) oil on the haematological parameters of weaned rabbits

Table 2 shows the haematological indices of growing rabbits fed diets containing neem oil. The blood indices show normal physiological ranges as established by Kronfield and Mediway (1975); Mitruka and Rawnsley (1977) and Hewitt *et al.*,

(1989). With respect to PCV, there was no significant difference (P>0.05) when T4 and T5 were compared. Similarly, there was no significant difference (P>0.05) between T2 and T3., PCV values recorded for T2 and T3 were significantly (P<0.05) different when compared to the values obtained for T4 and T5 but PCV values for T1 (diet + 0% neem oil) is significantly (P<0.05) different when compared to T2, T3, T4 and T5. With respect to Hb, a significant difference occurred when T2 and T3 were compared to T4 and T5 but not the same (P>0.05) among T2 and T3 so also, T4 and T5. However, T1 is significantly (P<0.05) different when compared to T2, T3, T4 and T5.

There was no significant difference (P>0.05) among treatments with respect to RBC. The WBC showed that there was a significant difference (P<0.05) comparing T2 to T3 and T4 to T5. But the mean value obtained in T1(0%) was significantly (P<0.05) different compared to other treatment groups. The MCV, MCH and MCHC were not significantly different (P>0.05) among the treatments.Okoli *et al.,* (2002); Omokore and Alagbe (2019) reported that neem leaf was traditionally used as human blood building tonic especially for weak toddlers. The results of this study disagree with the findings of Biu*et al.,* (2009) and Gowda *et al.,* (1998) that neem preparations fed to laying birds significantly reduced the content of haemoglobin, erythrocyte and packed Cell Volume.

Therefore, the results of this haematological parameters support that 0.40% neem oil could be included in rabbit diet to enhance growth performance and haematological values in rabbits without endangering the animal productive potentials.

Table 2: Haematological parameters of rabbits fed diets containing neem oil

Parameters	T1 (0%)	T2 (0.10%)	T3 (0.20%)	T4 (0.30%)	T5 (0.40%)	SEM
PCV (%)	36.80^{c}	37.62^{b}	38.42^{b}	39.03^{a}	39.60^{a}	0.28
Hb(g/dl)	11.00^{c}	13.47^{b}	13.97^{b}	14.18^{a}	14.34^{a}	1.13
RBC (x 10^{6}µL)	4.65	4.73	4.89	5.10	5.92	0.13
WBC (x 10^{9} µL)	7.80^{c}	9.18^{b}	10.12^{a}	9.34^{b}	10.62^{a}	0.23
MCV (fl)	76.46	81.23	80.98	83.94	86.00	2.76
MCH (pg)	27.38	28.14	29.97	29.33	30.04	1.43
MCHC (pg)	35.79	35.79	35.81	36.21	37.10	0.68

^{abc}means with different superscripts on the same row are significantly different (P < 0.05)

Key:

PCV = Packed Cell Volume; Hb = Haemoglobin, RBC = Red Blood Cell,WBC = White Blood Cell, MCV = Mean Corpuscular Volume,MCH = Mean Corpuscular Haemoglobin,MCHC = Mean Corpuscular Haemoglobin Concentration

Serum biochemistry of rabbits fed graded levels of neem oil

Data on the effects of neem oil on serum biochemical constituents of rabbits are presented in Table 3. The total protein was not significantly (P>0.05) different among the treatments. Considering globulin, there was significant (P<0.05) difference when T1 and T2 were compared to T3, T4 and T5. However, there was no significant difference (P>0.05) between T1 and T2, also similarly to T3, T4 and T5.The cholesterol level decreases with increase in neem oil inclusion level across the treatment. There was significant difference (P<0.05) in cholesterol level among T1, T2 and T3. Meanwhile, there was no

significant (P>0.05) difference between T4 and T5. The serum glucose level obtained is inversely proportional to increasing neem oil inclusion across treatments T3, T4 and T5. There was significant (P<0.05) differences when T3, T4 and T5 were compared to T1 and T2. Although, there was no significant (P>0.05) difference between T3, T4 and T5 also similarly to T1 and T2.

The non-significant difference observed in serum protein in this study could be compared to earlier report of protein retained in animals. Awosanya *et al.*, (2002); Oluwafemi et al. (2020) reported the dependence of blood protein on the quality and quantity of dietary proteins. The reduction in serum cholesterol value of the rabbits fed diets containing neem oil is an indication that neem oil could reduce the deposition of cholesterol in the skin and muscles. The reduction in serum cholesterol is a positive development since low cholesterol meat is healthier for consumption.

Table 3: Serum biochemical characteristics of weaned rabbits fed graded levels of neem oil

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dl)	6.10	6.90	6.10	6.20	6.20	0.50
Globulin (g/dl)	2.49^{b}	2.53^{b}	3.00^{a}	3.02^{a}	4.70^{a}	0.06
Cholesterol (mg/dl)	174.60^{a}	115.20^{b}	95.40^{c}	56.50^{d}	54.10^{d}	2.45
Glucose (mg/dl)	80.00^{a}	83.10^{a}	78.30^{b}	76.80^{b}	75.80^{b}	1.10
Chloride (mmol/l)	117.10^{b}	112.00^{b}	119.20^{b}	129.30^{a}	134.50^{a}	3.88
Sodium (mmol/l)	155.50^{c}	198.60^{b}	203.40^{b}	232.20^{a}	269.20^{a}	11.3

^{abcd} means with different superscripts on the same row are significantly different (P < 0.05)

Carcass characteristics of weaned rabbits fed diets graded levels of neem oil

The carcass characteristics of weaned rabbits fed diets different gradedlevels of neem oil inclusion are shown in Table 4. The mean final weight of the experimental animals fed diet containing 0.40% (T5) neem oil had the highest final body weight and the final body weight of rabbits in treatment 4, fed diet containing 0.30% neem oil was also better (1322.1^{a} and 1300.5^{a} respectively) and not significantly (P>0.05) different but significantly (P<0.05) higher than the final body weight of rabbits in the control treatment (T1). Rabbits in treatment 2 (0.10%) and 3 (0.20%) respectively had similar final body weight and not significantly (P>0.05) different. There were significant (P<0.05) differences in the statistical values of the liver, kidney, heart, lungs and spleen of the rabbits fed diets containing different inclusion levels of neem oil.

Dressing percentage of rabbits fed diet containing 0.40% (T5) neem oil inclusion level and those fed diet including 0.30% (T4) neem oil were higher and similar to each other (56.33^{a} and 56.00^{a} respectively)., both values were however not statistically (P>0.05) different. The dressing percentage values obtained for rabbits in T2 (0.10%) and T3 (0.20%) were not significantly (P>0.05) different. There was significant difference (P<0.05) when T4 (0.30%) and T5 (0.40%) were compared to T2 (0.10%) and T3 (0.20%). Rabbits in treatment 3 fed diets containing 0.20% neem oil had dressing percentage better than dressing percentage of rabbits in treatment 1 (0%) and 2 (0.10%) but lower than the dressing percentage of rabbits in treatments 4 (0.30%) and 5 (0.40%).

The result for dressing percentage increases across the treatments as inclusion level of neem oil in diets increases. This could be attributed to the phytosterols in neem oil which might render the nutrients available for the animals' utilization (Alikwe*et al.*, 2014; Musa et al., 2020). Other carcass parameters; head, liver, kidney, heart, lungs and spleen increases with increasing levels of neem oil in diets which may be as a result of the appreciable levels of Azadirachtin in neem oil which positively affects the final product (carcass). This result contradicts the report by Obun *et al.*, (2013); Olatunji et al. (2015) that decrease in the relative organs weights of liver, heart, pancreas and gizzard with increasing neem leaf inclusion in the diets could be an indication of residual bioactive components (Azidirachtins, tannins and limonoids) in the leaf meal which may have depressed these parameters.

Table 4: Carcass characteristics of weaned rabbits fed diets containing graded levels of neem oil

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	SEM
Final wgt (g)	1188.40^{c}	1270.40^{b}	1272.70^{b}	1300.50^{a}	1322.01^{a}	23.45
Head	4.87^{c}	6.61^{b}	6.12^{b}	6.01^{b}	7.98^{a}	1.56
D.P (%)	48.56^{c}	51.34^{b}	53.56^{b}	56.00^{a}	56.33^{a}	9.11
Liver (%)	2.11^{b}	3.67^{a}	3.88^{a}	3.79^{a}	3.66^{a}	0.04
Kidney (%)	0.09^{b}	1.36^{a}	1.34^{a}	1.31^{a}	1.30^{a}	0.10
Heart (%)	0.29^{c}	0.40^{b}	0.42^{b}	0.41^{b}	0.49^{a}	0.03
Lungs (%)	1.01^{b}	1.04^{b}	1.06^{b}	2.09^{a}	2.04^{a}	0.01
Spleen (%)	0.26^{b}	0.31^{a}	0.34^{a}	0.35^{a}	0.34^{a}	0.02

D.P: dressing Percentage

^{a,b,c,} means along the same row with different superscripts are significantly different ($P<0.05$)

CONCLUSION

Bioactive chemicals in neem oil which may have acted singly or in synergy with one another to produce the effects observed. *A. indica*in diets of growing rabbits increases some haematological parameters which were within the normal range. And also, *A. indica*showed positive effects on the carcass with reduction in serum cholesterol.

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