DIRECT AND RESIDUAL EFFECT OF *AZANZA GARCKEANA* (GORON TULA) FRUIT MEAL ON LIPID PROFILES OF NEW ZEALAND WHITE RABBITS IN THE HUMID TROPICS

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ABSTRACT
This study was conducted to examine the direct and residual effects of Azanza garckeana fruit on the serum lipid profile of New Zealand white rabbit bucks. Sixty (60) New Zealand white rabbit bucks, aged 2-3 months were randomly divided into 4 treatment groups (T_1, T_2, T_3 and T_4) of 15 bucks, with 3 replicates of 5 rabbits per replicate in a Completely Randomized Design (CRD) Experiment. The experimental diets were formulated to meet the nutrient requirement of rabbit bucks and supplemented with Azanza garckeana fruit meal at 0, 100, 200 and 300g/kg respectively, representing T_1, T_2, T_3 and T_4. The study was conducted in two experimental phases of 8 weeks each, which were- the direct and the residual phases. At the end of each experimental phase, blood samples were collected, the sera extracted and analyzed for the various lipid profile parameters. The data collected were subjected to Analysis of Variance (SPSS 23). Results showed that the different supplementations of Azanza garckeana fruit meal up to 300g/kg in the diets significantly reduced the serum total cholesterol, triglycerol, high density lipoprotein cholesterol and very low density lipoprotein cholesterol at the direct and residual phase. It was concluded that Azanza garckeana fruit meal at 100g/kg, 200g/kg and 300g/kg significantly reduced the serum total cholesterol, triglycerol, high density lipoprotein cholesterol and very low density lipoprotein cholesterol at the direct and residual phase without detrimentally affecting the physiology of the New Zealand White rabbit bucks in the humid tropics.
Keywords: Serum, Lipid profile, *Azanza garckeana,* Rabbit Bucks, Goron Tula

INTRODUCTION

Serum lipid profile is the amount of fats in the blood. It indicates the levels of total cholesterol, triglycreride, High Density Lipoprotein Cholesterol (HDL-C): Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) in the blood. The serum lipid profile helps to evaluate the cardiovascular health, risk prediction and indicators, by analyzing the cholesterol in the blood. Serum lipid profile indicates certain genetic, cardiovascular disease, certain forms of pancreatitis and other diseases (Nigam, 2011).

Azanza garckeana (Goron Tula) is an indigenous fruit shrub with rich sources of proteins, carbohydrates, fat, vitamins, minerals and other trace elements (Michael *et al.,* 2015; Alfred, 2017). *Azanza garckeana* has been reported to possess a wide range of pharmacological activities justifying some of its ethno-medicinal uses (Mutindi, 2014; Ochokwu, *et al.,* 2014). The fruits of *Azanza garckeana* have been documented to possess significant amounts of alkaloids, phytosteroids, tannins, Phenols, Saponins and Cyanogenic glucosides (Nkafamiya *et al*., 2015; Ahmed and Hamid, 2020). Some other researchers have also reported the presence of Terpenoids, triterpenes, Cumarins, Cardiac glycosides, Carotenoids and Flavonoids in *Azanza garckeana* fruit extracts (Idris *et al*., 2015; Dikko *et al*., 2016). These phytosteroids, pharmacological chemical compounds and antinutrients may affect the blood profiles of rabbit bucks when used in rabbit nutrition. Hence, this study aimed to examine the physiological growth potentials and nutritional impact of *Azanza garckeana* fruit meal on the serum lipid profile of New Zealand white rabbit bucks in the humid tropics.

MATERIALS AND METHODS

Experimental site

This research was conducted at the Rabbit Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria. The area is located in the South-Easthern part of Nigeria on latitude $5^0 27^1$ north, longitude 7^0 32' East, an altitude of 123m above sea level with an annual rainfall of 2177mm, temperature of 22^0C – 36^0C and relative humidity of 50 – 90%. Climatic data were collected from the Meteorological Center of National Root Crop Research Institute, Umudike, Abia State (NRCRI, 2018).

Experimental Animals and Management

A total number of 60 growing New Zealand White rabbit bucks aged 2-3 months were used for this study. Two weeks pre-experimental period were used to acclimatize the experimental animal, treatment against ecto and endo-parasite using Ivomectin and Levamisole (0.1mL/kg body weight), respectively was done. The experimental rabbits were housed singly in pens of colony hutches for ease of identification. The condition of housing and management were similar for all the experimental rabbits. Experimental diets and clean water were offered *ad-libitum.* All routine management practices were strictly adhered throughout the experimental period. The field work lasted for 16 weeks.

Experimental design

The design for the study was a Completely Randomized Design (CRD) experiment with 4 treatments consisting of T_1, T_2, T_3 and T_4 respectively. T_1 served as the control. Fifteen (15) growing rabbits bucks were randomly assign to each treatment, balanced in weights and replicated 3 times, with 5 rabbits per replicate. The ages of the rabbits were between 2-3 months. The rabbits in T_1 (Control) were given no *Azanza garckeana* fruit meal. Rabbits in T_2, T_3 and T_4 were given 100g/kg, 200g/kg, and 300g/kg *Azanza garckeana* fruit meal per kilogram of feed, respectively.

The experimental model were as follows:

$\mathbf{Y_{ij} = \mu + Ti + e_{ij}}$

Where:

$\mathbf{Y_{ij}}$ = individual rabbit observation

$\boldsymbol{\mu}$ = overall mean

$\mathbf{T_i}$ = treatment effect

$\mathbf{e_{ij}}$ = Experimental error.

The research was carried out in two (2) phases:

Phase 1: *Azanza garckeana* fruit meal administration phase

This experimental phase lasted for 8 weeks. In this phase, the ground *Azanza garckeana* fruit were mixed with the diets and offered daily to the rabbits for 8 weeks. At the end of this phase, blood samples were collected and analyzed for lipid profile parameters. The compositions of the experimental diets for the direct phase are presented in Table 1.

Table 1: Gross Composition and Calculated Nutrients of Experimental Diets for Rabbit Bucks in Phase 1

Ingredients	T_1 (0.0g/kg)	T_2 (100g/kg)	T_3 (200g/kg)	T_4 (300g/kg)
Maize	44.94	44.94	44.94	44.94
Soya bean meal	17.31	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00	32.00
Fishmeal	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00
Vit/min Premix*	0.25	0.25	0.25	0.25
Common salt	0.50	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**	**100.00**
Ground *Azanza garckeana* (g/kg feed)	0.00	100.00	200.00	300.00
Calculated nutrients:				
Crude Protein (%)	17.00	17.00	17.00	17.00
Metabolizable Energy (ME) (Kcal/kg diet)	2505.42	2505.42	2505.42	2505.42
Crude fiber (%)	11.36	11.36	11.36	11.36
Lysine (%)	0.514	0.514	0.514	0.514
Methionine (%)	0.199	0.199	0.199	0.199

Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

Phase 2: Residual phase

This experimental phase lasted for another 8 weeks, which continued immediately after the phase 1. In this phase, no *Azanza garckeana* fruit meal were supplemented on the diets. The rabbits in each treatment were fed the control diet. This was done to examine for possible side effects or residual effects of *Azanza garckeana* fruit after the first 8 weeks administration. At the end of this phase, blood samples were also collected and analyzed.

Sourcing and Preparation of *Azanza garckeana* Fruit Meal

The *Azanza garckeana* fruit were sourced from the rural and urban markets in Abia State. The *Azanza garckeana* fruits were dried at room temperature to a constant weight. *A. garckeana* fruits were ground to a fine powder using a mechanical grinding machine to produce *Azanza garckeana* fruit meal. The ground *Azanza garckeana* fruit meal were incorporated into the experimental diets and used as the test ingredients for the experiment.

Data Collection and Analysis

Three (3) blood samples were collected from each replicates. The different lipid profile parameters were analyzed from the sera using the standard laboratory procedures, and used to determine the Total Cholesterol, Triglycerol, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol.

Data Analysis

Data collected on the various serum lipid profile parameters were subjected to Analysis of Variance (ANOVA) using IBM SPSS statistics (version 23). Significant means were separated using Duncan's Multiple Range Test at 5% level of significance (Duncan, 1955). All statistical analysis were in accordance with the methods of Steel and Torie, (1980).

RESULTS AND DISCUSSION

The direct effects of *Azanza garckeana* fruit meal on the Serum Lipid profile of New Zealand white rabbit bucks are shown in Table 2.

Table 2: Serum Lipid profile of New Zealand White Bucks Fed Diets Supplemented with *Azanza garckeana* Fruit Meal

Parameters	T_1 (0.0g/kg)	T_2 (100g/kg)	T_3 (200g/kg)	T_4 (300g/kg)	SEM
Total Cholesterol (mg/dL)	76.03^{a}	74.73^{b}	74.54^{b}	72.04^{c}	0.46
Triglycerol (mg/dL)	74.90^{a}	53.65^{b}	53.10^{b}	52.29^{b}	3.75
HDLC (mg/dL)	38.43^{a}	30.76^{b}	31.75^{b}	31.67^{b}	1.38
LDLC (mg/dL)	30.32	33.07	32.05	29.94	0.66
VLDLC (mg/dL)	12.19^{a}	10.77^{ab}	10.63^{b}	10.40^{b}	0.28

^{abc}: Means with different superscripts along rows are significantly different ($p<0.05$). SEM: Standard error of treatment means. HDLC: High Density Lipoprotein Cholesterol; LDLC: Low Density Lipoprotein Cholesterol; VLDLC: Very Low Density Lipoprotein Cholesterol

The results showed that no significant differences ($p>0.05$) were observed on the low density lipoprotein cholesterol following the supplementations of *Azanza garckeana* fruit meal on the diets of rabbit bucks compared with the control. The serum total cholesterol reduced significantly ($p<0.05$) in T2, T3 and T4 compared with the bucks in T1; while the serum triglycerol were observed to reduce significantly ($p<0.05$) from T1 (74.90 mg/dL), T2 (53.65 mg/dL), T3 (53.10 mg/dL) and T4 (52.29 mg/dL), with T4 having the least value. The High Density Lipoprotein Cholesterol were also observed to have reduced significantly in T2 (30.76mg/dl), T3 (31.75mg/dL) and T4 (31.67mg/dL) compared to the High Density Lipoprotein Cholesterol of the rabbit bucks in T1 (38.43mg/dL). The similar trend of significant reductions ($p<0.05$) across the treatment groups following the different supplementations of *A. garckeana* fruit meal were also observed on the serum Very Low Density Lipoprotein Cholesterol. The significant reductions followed a definite pattern. A closer observation

on the results indicated that the serum total cholesterol, triglycerol, HDLC and VLDLC significantly reduced ($p<0.05$) as the levels of supplementation of the *Azanza garckeana* fruit meal in the diets of rabbit bucks increased. The significant reductions observed on the lipid profile of T2, T3 and T4 might have suggested that the rabbits in T2, T3 and T4 may have utilized and converted the total cholesterol, triglycerol and the differential lipoproteins to metabolizable energy, heat energy, work energy and for different muscular activities. Furthermore, the significant reduction on the lipid profile following the supplementations may be attributed to the presence of appreciable amounts of tannins, alkaloids, phenols and flavonoids in *Azanza garckeana* fruit as documented by Dikko *et al.* (2016) and Iyojo, *et al*. (2022). *Azanza garckeana* could decrease cholesterol by diminishing cholesterol synthesis and inhibition of hydroxy-methyl-glutaryl-coenzyme –A- reductase (HMGCoA), an enzyme controlling the rate of cholesterol formation (Tuduri *et al.,* 2018). Reports have also shown that stigmasterol enhances Glucose transporter Type 4 (GLUT 4) translocation, an insulin regulated glucose transporter found in adipose tissues. Thiamine normalizes cholesterol and triacylglycerol levels, while nonyl flavanones and other flavonoids generally regulate glucose metabolism and improve the hepatic enzyme activity in diabetes complications (Yusuf *et al*., 2023). The mechanisms of action and the pharmacological properties of tannins, alkaloids, phenols and flavonoids might have been responsible for the significant reductions observed on the serum lipid profiles of the rabbits. The reports documented by Jiang and Youling (2016) confirmed the present results as they suggested that *A. garckeana* supplementation decreased total cholesterol, triglycerol, HDLC and VLDLC. Based on this, the results herein suggests that *A. garckeana* fruits can exhibited a remarkable protection against dyslipidemia, suggesting a possible innovative remedy for arteriolosclerosis.

The residual effects of *Azanza garckeana* fruit meal on the Serum Lipid profile of New Zealand White bucks are shown in Table 3.

Table 3: Residual Effects of *Azanza garckeana* Fruit Meal on Serum Lipid Profile of New Zealand White Bucks

Parameters	T_1 (0.0g/kg)	T_2 (100g/kg)	T_3 (200g/kg)	T_4 (300g/kg)	SEM
Total Cholesterol (mg/dL)	90.09^{a}	73.15^{b}	71.42^{b}	70.02^{b}	2.62
Triglycerol (mg/dL)	59.69^{a}	49.30^{b}	48.91^{b}	47.20^{b}	1.55
HDLC (mg/dL)	40.58^{a}	39.48^{ab}	37.67^{b}	34.60^{c}	0.77
LDLC (mg/dL)	24.39	25.77	22.25	19.81	1.46
VLDLC (mg/dL)	11.86^{a}	9.85^{b}	9.85^{b}	9.64^{b}	0.29

^{abc}: Means with different superscripts along rows are significantly different ($p<0.05$). SEM: Standard error of treatment means. HDLC: High Density Lipoprotein Cholesterol; LDLC: Low Density Lipoprotein Cholesterol; VLDLC: Very Low Density Lipoprotein Cholesterol.

The result showed that the total cholesterol, triglycerol, high density lipoprotein cholesterol and very low density lipoprotein cholesterol showed a significant different ($p<0.05$) compared with those of the control group. The total cholesterol significantly reduced ($p<0.05$) in T2 (73.15 mg/dL), T3 (71.42 mg/dL) and T4 (70.02 mg/dL) compared to those of the rabbit bucks in T1 (90.09 mg/dL). The triglycerol significantly reduced ($p<0.05$) in T2 (49.30 mg/dL), T3 (48.91 mg/dL) and T4 (47.20 mg/dL) compared to those of the rabbit bucks in T1 (59.69 mg/dL), with the highest reductions observed in T4. The High Density Lipoprotein Cholesterol were also observed to have reduced significantly in T2 (39.48mg/dl), T3 (37.67mg/dL) and T4 (34.60mg/dL) when compared to the High Density Lipoprotein Cholesterol of the rabbit bucks in T1 (40.58mg/dL). Similar significant reductions ($p<0.05$) were also observed on the VLDLC; while the LDLC were not residually affected. The reasons for this significant reductions may be similar and aligned with those of the direct phase (Table 2). This observations inferred that *Azanza garckeana* fruit meal had residual effects on the serum lipid profile of the New Zealand White rabbit bucks in the humid tropics.

CONCLUSION

Based on the results and observations from this study, it is concluded that *Azanza garckeana* fruit meal at 100g/kg, 200g/kg and 300g/kg significantly reduced the serum total cholesterol, triglycerol, high density lipoprotein cholesterol and very low density lipoprotein cholesterol at the direct and

residual phase without detrimentally affecting the physiology of the New Zealand White rabbit bucks in the humid tropics.

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