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Research Article
Effect of Diets with Iron Tree (*Prosopis africana*) Pulp on Performance and Blood Characteristics of Growing Rabbits

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Abstract
Background and Objective: Animal protein tends to rank better than plant protein because of its higher essential amino acids. Some plant parts have been reported to have phytogenic and growth promoting properties. The study aimed to determine the influence of iron tree (*Prosopis africana*) pulp containing diets on growth indices, feed intake and blood characteristics of rabbits. **Materials and Methods:** Twenty four male rabbits were used for this study. They were randomly divided into 4 experimental groups of 6 animals each. Each group was assigned to one of the experimental diets containing *Prosopis africana* Pulp (PAP) at 0% (control), 10, 20 and 30% for 50 days in a Completely Randomized Design (CRD). Blood samples were obtained from each rabbit on the last day of the trial. **Results:** It was observed that PAP is abundant in phytochemicals. Body weight gain and feed conversion ratio were ($p<0.05$) best for rabbits on PAP containing diets. However, feed intake was best ($p>0.05$) for rabbits in the control group. Haemoglobin, red blood cells, mean cell haemoglobin concentration, lymphocytes and neutrophils showed significant ($p<0.05$) difference across the groups. Sodium, potassium and cholesterol showed significant ($p<0.05$) difference and tended to be higher in T_1. **Conclusion:** The results indicated that PAP enhanced body weight gain, feed intake and feed conversion. In addition, all the haematological and serum biochemical parameters examined fell within the normal physiological range indicating that the diets were not deleterious to the animals.

Key words: Phytochemicals, phytogenics, rabbits, body weight, feed intake, blood indices

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Animal protein tends to rank better than plant protein because of its higher essential amino acids needed by man for survival and healthy living. However, poor intake of animal protein has been identified as a major challenge to Nigerians and many other citizens of developing countries. This problem has lingered owing to the fact that rabbit production has been neglected over the year in many developing countries[1]. Today, rabbit production is gaining popularity not only because it is cheap source of animal protein but also because the meat is very healthy due to its lower level of cholesterol and sodium contents[2,3]. Similarly, the ability of the rabbit to utilize fibre better than pigs and poultry due to its enlarged caecum has enhanced its popularity[4].

Phytogenic plant materials have continued to gain great attention today in animal production not only because of their growth promoting abilities but also due to their pharmaceutical and medicinal properties. *Prosopis africana* is one of the phytogenic plants and also a member of family Fabaceae. The genus *Prosopis* has 44 species of which 40 are native to north and south Americas, 3 to Asia and one originates from Africa[5]. In Africa, the only record of the genus is *Prosopis africana*. *P. africana* is a fast growing hardy and drought resistant plant. *P. africana* is leguminous tree of about 4-20 m in height, found mainly in the semi-arid and arid regions of tropical Africa. It is a multi-purpose tree known for its hard and durable saplings, nitrogen fixation ability and condiments, pharmaceutical and medicinal properties and feed stuff for animals. The pods, pulps and leaves are highly relished by ruminants. It is opined that all parts of *Prosopis* spp. are used for curing various ailments[6]. It is also noted that the phytochemicals of *P. african*a such as flavonoids, tannins, alkaloids, phenols demonstrate potentials in various bio-functions, such as analgesic, anthelmintic, antibiotic, antiemetic, microbial, antioxidant, antimalarial, antiprotozoal, antipustule and antiulcer activities enhancement of Hb, Kb, ATPases, oral disinfection and probiotic and nutritional effects, as well as, in other biopharmaceutical applications, such as binding abilities for tablet production[5]. Currently, there is scarcity of information on the toxicological effect of *P. africana* pulp intake on the growth performance and blood indices of male rabbits. This study was done, therefore, to investigate the effect of *P. africana* pulp supplemented diets on growth performance and blood characteristics of rabbits.

MATERIALS AND METHODS

The research was carried out at the Rabbitry, Department of Animal Production Technology, Federal College of Agriculture Ishiagu (FCAI), Ivo Local Government Area of Ebonyi State, Nigeria, between September-December, 2017. FCAI is located at about 3 km away from Ishiagu main town. The college is situated at latitude 5°6' N and longitude 7° 31'E with average rainfall of 1000-1600 mm and a prevailing air temperature condition of 27-28°C relative humidity of about 88%, respectively.

The iron wood (*Prosopis africana*) pods were sourced from Cross River State in Okpoma Ochochi LGA. The *Prosopis africana* pods were crushed to remove the seeds and the pulps were then milled to a required size and used to formulate the experimental diets. Four experimental designated as T_1, T_2, T_3 and T_4, respectively as shown in Table 1.

Twenty four of about 5-6 week old, rabbits were sourced from Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria. The experimental animals were acclimatized for 21 days before the commencement of the study in accordance with the permission and stipulated guidelines of the Federal College of Agriculture (FCA) Ishiagu, Animal Ethics Committee. During the adaptation period, each animal was vaccinated against prevalent diseases. They were also dewormed using kepromec (Ivermectin) at the rate of 0.1 mL/rabbit sub-cutaneously and given acaricide bath using Roys' Amitraz 20 at the rate of 1 mL in 2 L water prior to the experiment. After that, they were randomized into 4 experimental treatments with 6 animals per group. Each rabbit was housed in a standard hutch measuring 120 by 150 cm and raised 120 cm above the ground level. The four treatment groups were assigned the 4 diets in a Completely Randomized Design (CRD). Each rabbit received an assigned diet for 50 days.

Data were collected daily for feed intake and weekly for weight gain using weighing scale of 30 kg capacity and 0.001 kg sensitivity. The feed intake of each rabbit was determined by the difference between the feed supplied and the left over in the feeding trough the next day. While body weight changes were measured on a weekly basis and the difference between initial and final weight of the rabbits constituted the live body weight gain. Feed conversion ratio was calculated.

Blood samples (5 mL) were drawn from each animal on the last day of the study. The rabbits were bled through the

Table 1: Percentage composition of the experimental diet

	Dietary levels			
Ingredients	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)
Maize	45.00	45.00	45.00	45.00
Soybean meal	18.00	18.00	18.00	18.00
Wheat offal	12.00	10.00	6.00	2.00
Palm kernel cake	19.00	11.00	5.00	0.00
Prosopis africana pod pulp	0.00	10.00	20.00	30.00
Blood meal	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25
Lysine	o.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

Table 2: Proximate composition of the experimental diets and *Prosopis africana* pod pulp

	Dietary levels				
Parameters (%)	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)	PAP (%)
Dry Matter (DM)	90.10	90.35	90.80	90.80	86.30
Crude Protein (CP)	17.97	17.52	16.74	16.33	13.83
Crude Fibre (CF)	14.45	15.40	15.53	15.85	17.36
Ether Extract (EE)	3.25	3.40	3.25	2.90	1.35
Ash	7.30	7.78	7.95	8.22	6.28
Nitrogen Free Extract (NFE)	47.13	46.25	47.33	47.50	47.48
ME (kcal kg^{-1})	2554.75	2520.95	2504.35	2480.55	2260.60

PAP: *Prosopis africana* pulp, ME: Metabolizable energy

ear marginal vein. The samples were separated into 2 lots and used for biochemical and haematological studies. An initial 2.5 mL was collected from each sample in labelled sterile universal bottle containing 1.0 mg mL^{-1} ethylenediamine-tetraacetic acid and used for haematological analysis. Another 2.5 mL was collected over anticoagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within 3 h of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean Cells Haemoglobin (MCH), MCV and Mean Cell Haemoglobin Concentrations (MCHC) were calculated.

All feeds and experimental material (PAP) were analyzed for proximate compositions using the method of AOAC[7] and Metabolizable Energy (ME) calculated using the formula:

$$ME = (3.5\times\text{Crude protein})+(8.5\times\text{Crude fat})+(3.5\times\text{Nitrogen free extract})\times 10$$

Statistical analysis: The results were analyzed using the Statistical Package for Social Science Window 17.0. One-way analysis of variance (ANOVA) was employed to determine the means and standard error. Significant differences between the treatment means were separated using the Duncan multiple new range test[8].

RESULTS AND DISCUSSION

The proximate composition of the experimental diets and *Prosopis africana* Pulp (PAP) results are presented in Table 2. The Dry Matter (DM) of the treatment groups (T_2, T_3 and T_4) 90.35-90.80% compared favorably with 90.10 of the control (T_1) diets. The DM also showed a particular sequence increasing with increasing levels of *Prosopis africana* pulp (PAP). Perhaps the range of 90.10-90.80% in this study is lower than range of 93.98-95.06% reported by Jiwuba *et al.*[2] for weaner rabbits fed *Moringa oleifera* leaf meal but higher than 82.00-86.00% reported in study for rabbits fed diets containing garlic bulb meal[9]. The difference in the DM maybe attributed to the differences in the test ingredients, level of dryness of the experimental diets and the processing methods used. The Crude Protein (CP) range of 16.33-17.97% obtained in this present study is in agreement with the CP requirement of growing rabbits as recommended by NRC[10], Fielding[11] and Lebas[12]. The Crude Fibre (CF) range of 14.45-15.85% reported in this study is in agreement with the recommended range of 14-18 and 14-16% reported for growing rabbits[13,14]. The relatively high ash content of the experimental diets is an indication that the diets were not lacking in minerals and that the mineral needs of the animals were met. The ash contents increased with increasing levels of the test ingredient and the highest value was reported in T_4. This may be attributed to high levels of minerals that abound in *Prosopis africana* pulp. The energy values reported in this study fell within the recommendation of 2500.00, 2.400-2.800 kcal kg^{-1} for growing rabbits[10,15]. Jiwuba[4] stated that rabbits tend to adjust their feed intake as a function of their dietary energy concentration. The results of the proximate analysis of the PAP revealed 86.30% DM, 13.83% CP, 17.36% CF, 6.28% ash, 1.35% Ether Extract (EE), 47.48% NFE and 2260.60 kcal kg^{-1} metabolisable energy. The DM content of 86.30% reported in this study compared favorably with 86.00% reported in previous studies by Pond *et al.*[16] for *Prosopis Africana* pulp. The crude protein value reported in this study is however higher than 10.00% recorded for *Prosopis africana* pulp[15]. Metabolizable energy and CF obtained in this study is lower than 2734.64 kcal kg^{-1} and 23% recorded for metabolizable energy and CF respectively for *Prosopis africana* pulp[15]. The differences in the proximate composition of the PAP maybe attributed to location of the study, season, processing methods and soil fertility.

The phytochemical composition of *Prosopis africana* pulp is presented in Table 3. The results indicated the presence of flavonoid, glycoside, tannins, saponins, steroid, phenols and alkaloids in various proportions. Researchers attributed the ethno-veterinary and pharmaceutical properties of different plants to the presence of phytochemical constituents like saponins, tannins, alkaloids, alkenylphenols, glycol alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters[17]. In earlier study, alkaloids, flavonoids and phenolic compounds act as the most important bioactive substances of *Prosopis* spp.[18]. Flavonoids have the highest percentage in this study and these compounds are natural products known for their beneficial effects on health and enhancing palatability. Flavonoids are recently considered highly important in medicinal, nutraceutical, pharmaceutical and cosmetic industries. This may be attributed to its anti-mutagenic, anti-oxidative, anti-inflammatory and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function[19]. Alkaloids, a natural occurring organic compound from *Prosopis* spp. has inherent ability in capturing free oxygen radicals. Alkaloids from *P. africana* are used as analgesics, anti-malarial agents and broad spectrum of antifungal activities against fungi, such as; *Fusarium*, *Drechslera* and *Alternaria*. Alkaloids, flavonoids and tannins from iron tree have therapeutic effect against ulcer and wounds. Phenols from Iron tree have shown strong anti-inflammatory, antitumor, anti-HIV, anti-infective, vasodilatory, anti-ulcerogenic analgesic and immunostimulant activities[20]. Tannins have antioxidants and anti-microbial properties that aid the wound attribute of *Prosopis africana* by preventing and keeping the wound area from being damaged by free radicals and inhibiting the growth of pathogenic bacteria in the wound. Saponins have numerous therapeutic properties. They have been reported to have anti-protozoan, hypocholesterolemic, anti-carcinogenic, anti-oxidant immunostimulatory, anti-microbial, molluscicidal, antifungal and anti-inflammatory properties[21]. Steroids have diverse biological functions. Steroids reportedly have cholesterol-lowering properties, cancer prevention, antimutagenic, anxiolytic, analgesic, sedative, anticonvulsant and anesthetic properties[22,23]. Glycosides have been efficient in treatment and reducing heart related diseases, tumor prevention and inhibition of rhinovirus.

Table 3: Phytochemical composition of *Prosopis africana* pulp

Parameters	Composition
Flavonoid	3.07
Glycoside	2.24
Tannins	2.75
Saponins	1.54
Steroids	0.97
Phenols	2.42
Alkaloids	2.87

The growth performance of rabbits fed diets containing *Prosopis africana* pulp is shown in Table 4.

Table 4: Growth performance of rabbit's fed *Prosopis africana* pod pulp

	Dietary levels				
Parameters	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)	SEM
Initial weight (g)	633.00	615.10	600.20	622.30	7.73
Final weight gain (g)	1850.00	2033.21	1950.54	1933.72	9.04
Total weight gain (g)	1217.00^c	1418.11^a	1350.34^b	1311.42^b	8.62
Average weight gain (g/day)	24.34^c	28.36^a	27.01^b	26.23^b	0.53
Total feed intake	4230.01	3890.49	3730.32	3699.56	12.79
Average feed intake	84.60^a	77.81^b	74.61^c	73.99^c	1.34
Feed conversion ratio	3.48^a	2.74^b	2.76^b	2.82^b	0.71

^{a,b,c}Means on the same row with different superscript are significant ($p<0.05$), SEM: Standard error of mean

Table 5: Haematology of rabbits fed dietary levels *Prosopis africana* pod pulp

	Dietary levels				
Parameters	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)	SEM
Packed cell volume (%)	39.20	42.00	37.55	38.25	6.18
Haemoglobin (g L^{-1})	105.30^{b}	134.00^{a}	102.50^{b}	118.50^{ab}	18.66
Red blood cells ($\times 10^{12}$ L^{-1})	5.60^{b}	7.01^{a}	4.76^{c}	4.57^{c}	0.98
Mean cell volume (fL)	60.35	62.10	53.75	60.10	11.44
Mean cell haemoglobin (pg)	20.82	20.60	20.70^{b}	21.00	3.90
Mean cell haemoglobin concentration (%)	25.20^{ab}	32.60^{a}	25.50^{b}	36.50^{a}	5.46
White blood cells ($\times 10^{9}$ L^{-1})	7.03	7.50	7.00	7.15	1.04
Lymphocytes (%)	51.36^{b}	60.75^{a}	57.31^{a}	51.39^{b}	10.44
Neutrophils (%)	34.23^{b}	41.02^{a}	35.45^{b}	32.91^{c}	6.72
Platelet ($\times 10^{9}$ L^{-1})	478.50	368.50	355.00	394.00	54.80

a,b,cMeans on the same row with different superscript are significant (p<0.05), SEM: Standard error of mean

The diets had significant (p<0.05) effect on total weight gain, average weight gain, average daily feed intake and Feed Conversion Ratio (FCR). Rabbits fed treatment diets (T_2, T_3 and T_4) were significantly (p<0.05) better for total weight gain and daily weight gain than the rabbits on the control diet (T_1). The improved body weight gain may be attributed to the growth promoting ability of phytogenic plant materials. The improved (p<0.05) weight gain reported in this study agreed with the previous findings for rabbits fed 10 and 20% levels of PAP containing diets[16]. *Prosopis africana* have been reported to have many medicinal values which may have supported the health of the rabbits, thus resulted to increased body weight gain[5,24]. In addition, rabbits on higher fibre diets have been reported to gain better weight (p<0.05) than those on low fibre diets[4,25]. Hence it is noted that adequate supply of dietary fibre reduces digestive problems, promotes intestinal motility and enhances growth in rabbits[4]. Average daily feed intake was (p<0.05) higher in T_1. This opposes the results of previous researches for rabbits fed diets containing phytogenic plant parts and higher dietary fibre[4,9]. The improved (p<0.05) feed conversion ratio obtained for the animals in the treatment groups when compared to those on the control group entails that the rabbits utilized the feeds better at the treatment groups.

The haematological characteristics of rabbits fed diets containing *Prosopis africana* pulp is presented in Table 5. Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), White Blood Cell (WBC) and platelet showed no significant (p>0.05) difference across the treatment groups. Haemoglobin, Red Blood Cell (RBC), Mean Cell Haemoglobin Concentration (MCHC), lymphocytes and neutrophils differed significantly (p<0.05) across the treatments. PCV although similar (p>0.05) were within 37-47% reported for apparently rabbits, thus indicating tolerable or no anti-nutrients in the diets as PCV is used as index of feed toxicity[26]. The haemoglobin of the bucks ranged from 102.50-134 g L^{-1} for T_3 and T_2, respectively. The haemoglobin values of the rabbits fed *P. africana* in their diets were within the normal range of 80-150 g L^{-1} for rabbits reported by Poole[27], an indication of PAP in supporting high oxygen carrying capacity of blood in the animals[2]. The RBC values ranged from 4.57-7.01$\times 10^{12}$ L^{-1} for T_4 (30%) and T_2 (10%), respectively. The RBC range of values recorded for the rabbits fell within the values of 4.0-7.0$\times 10^{12}$ L^{-1} is reported for apparently healthy rabbits[27,28]. This may indicate that the utilization of the experimental diets ensured effective transport of haemoglobin through the red blood cells of the rabbits. This further gave a clear indication of adequate oxygen transportation within the tissues of the rabbits for oxidation of digested feeds for the release of energy for efficient body functioning. The non-significant (p>0.05) values for MCV and MCH obtained in this study agreed with the results of Jiwuba *et al.*[2]. The MCHC values of 25.20-36.50 reported is this study were within the normal range 27-37% reported by RAR[29]. The within normal physiological range for the RBC indices reported in this study gave clear indication that the animals were not anaemic. The range of values 7.03-7.50$\times 10^{9}$ L^{-1} reported in this present study for WBC are within 4.90-10.52$\times 10^{9}$ L^{-1} reported by Jiwuba *et al.*[3] for rabbits fed Gmelina leaf meal containing diets and fell within the normal physiological range of 4.0-13 and 6.3-10.1$\times 10^{9}$ L^{-1} for healthy rabbits, respectively[26,30]. This indicated that there were no microbial infections or presence of foreign bodies or parasites in the circulatory system of the experimental rabbits[2] which further indicates that the feeding of PAP in the diets of rabbits do not affect the immune system. In addition, the values of lymphocytes 51.36-60.75% obtained in this study are within the normal range of 30-85% for apparently healthy rabbits, reported in previous studies, respectively[28,31]. Similarly, the neutrophil value of 32.91-41.02% obtained in this study is within the normal range of 20-75% for apparently healthy rabbits reported, respectively[28,31]. These gave clear indications

Table 6: Serum chemistry of rabbits fed dietary levels *Prosopis africana* pod pulp

	Dietary levels				
Parameters	T_1 (0%)	T_2 (10%)	T_3 (20%)	T_4 (30%)	SEM
Total protein (mg dL^{-1})	6.91	8.36	7.33	6.69	0.53
Albumin (mg dL^{-1})	3.77	4.22	3.78	3.79	0.24
Globulin (mg dL^{-1})	3.14	4.14	3.56	2.91	0.30
Urea (mmol L^{-1})	4.92	4.62	5.25	4.80	0.95
Creatinine (mg dL^{-1})	0.87	0.92	0.92	0.97	0.03
Sodium (mmol L^{-1})	143.49[a]	137.50[ab]	135.27[ab]	131.21[b]	1.88
Potassium (mmol L^{-1})	6.15[a]	4.93[b]	4.60[b]	4.47[b]	0.26
Chloride (mmol L^{-1})	105.72	96.39	99.94	92.86	2.26
Cholesterol (mg dL^{-1})	59.56[a]	42.84[b]	32.60[c]	32.13[c]	1.31

[a,b,c]Means on the same row with different superscript are significant (p<0.05), SEM: Standard error of mean

that PAP is not deleterious to the rabbits at 30% level of inclusion. The platelets range values recorded for rabbits were within the normal range of 250-661×10^9 L^{-1} for apparently healthy rabbits reported by Thrall *et al.*[26]. Thus this indicated the wound healing abilities of *P. africana* by preventing bleeding as the values tend to increase with increasing levels of PAP.

Table 6 shows serum biochemical indices of rabbits fed *Prosopis africana* pulp in their diets. Total protein, albumin, globulin, urea, creatinine and chloride showed no significant (p>0.05). The cholesterol levels for the rabbits ranged between 32.13-59.56 mg dL^{-1} on T_4 diets showing lower cholesterol levels as against the T_1 group with the highest cholesterol value. The serum cholesterol reported in this study fell with normal physiological range of 5.3-71.0 mg dL^{-1} for apparently healthy rabbits reported by Kaneko *et al.*[32]. The progressive lower cholesterol values obtained in this study with increasing levels of PAP indicated the ability of *P. africana* in the treatment and management of heart diseases associated with high cholesterol in the blood. The blood potassium (K) levels for the rabbits fed *Prosopis africana* pulp in their diets ranged between 4.47 and 6.15 mmol L^{-1} fell within the reference range of 3.5-7.0 mmol L^{-1} for rabbits as reported by Suckow *et al.*[33] for apparently healthy rabbit. Normal physiological range obtained for the rabbits in this present study suggested the ability of the diets to maintained normal functioning of the body through maintaining cellular tonicity, fluid balance and pH, regulation of metabolic processes, neural and muscular functions as well as maintaining blood volume. The serum sodium values for the rabbits obtained in this study ranged between 131.21-143.49 mmol L^{-1} for apparently healthy rabbits fell within the normal physiological range of 131-155 and 125-150 mmol L^{-1} was reported respectively[33,34]. The normal physiological range obtained for the rabbits in this present study suggested the diets were able to maintain cellular tonicity fluid balance and pH, regulate metabolic processes, as well as, involved in regulation of neural and muscular function. In addition, serum potassium and sodium are electrolytes used as index of renal functions, hence the normal physiological range obtained for these electrolytes, implies that the normal body function, fluid and blood volume of the rabbits were maintained.

CONCLUSION

The results showed that inclusion of *Prosopis africana* pulp up to 30% levels produced no deleterious effect on body weight gain, feed intake, feed conversion, haematology and serum biochemical indices of rabbits. This may be attributed to the presence of biological compounds that abound in *Prosopis africana* pulp.

SIGNIFICANCE STATEMENT

This study discovered that Iron tree (*Prosopis africana*) pulp is rich in phytochemicals, other essential nutrients and thus could be a constituent of rabbit feeding system. This finding will help animal scientists uncover the critical areas of *Prosopis africana* that many scientists have not explored. Thus, incorporation of *Prosopis africana* pulp in diets of rabbits will ensure a regular supply of animal protein.

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