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Pterocarpus erinaceus leaf extracts phytochemical composition and its effect on growth performance and intestinal microbial population of weaned rabbits

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Abstract
In Sumitra Research Institute, a total of 50 crossbred male rabbits with initial body weight of 486 ± 0.70 g and weaned at 28 days of age were individually housed in a specially constructed galvanized cage was used to examine the effect of Pterocarpus erinaceus leaf extracts (PELE) on the growth performance and intestinal microbial population of weaned rabbits. Rabbits were stratified based on their body weight and assigned to 5 groups with six animals with one animal per replicate in a completely randomized design. Experimental diet was adequate in all nutrients to meet the requirement of rabbits. Animals in group 1 was fed standard diet with 0 mL PELE while group 2, 3, 4 and 5 were fed standard diet with 0.2 mL, 0.4 mL, 0.6 mL and 0.8 mL/day respectively. Phytochemical evaluation of Pterocarpus erinaceus leaf extracts showed that it contained Flavonoids (112.61 mg/g CAE), terpenoids (87.52 mg/g CAE), phenols (106.39 mg/g GAE), alkaloids (91.53 mg/g ATE), tannins (40.88 mg/g TAE) and phytate (11.31 mg/g). Results on average daily weight gain in group 4 (1544.2 g) and 5 (1547.1 g) were similar ($p>0.05$) but significantly higher than those in group 1 (1197.8 g), group 2 (1311.28 g) and 3 (1383.9 g). Average daily feed intake were higher ($p<0.05$) in rabbits fed diet supplemented with PELE relative to group 1 (control). Best feed conversion ratio was recorded in group 4 (3.00) and 5 (3.00), intermediate in group 2 (3.28) and group 3 (3.20) and lower in group 1 (3.43). Microbial population of Escherichia coli and Salmonella sp were higher ($p<0.05$) in group 1 relative to the other groups. Conversely, Lactobacillus sp count were maximum in PELE supplemented diet relative to control (group 1). It was concluded that supplementation of PELE at 0.8 mL/day can optimize the performance of rabbits as well as suppressing the activities of pathogenic organisms without compromising the health status of animal.
Keywords: Pterocarpus erinaceus, phytochemicals, rabbits, antimicrobial, food safety, antibiotics

Introduction

Public concern over potential antimicrobial resistance risk related to human and animal health has driven interest to the development of natural alternatives to antibiotics (Caroline, 2020; Jenny, 2021). The use of plant extracts has been identified as potential replacers of antibiotics because they are non-toxic, eco-friendly, no drug resistance providing beneficial effects on rabbits, from antimicrobial, antioxidant, immune stimulatory, hepato-protective, antiviral, antifungal, anti-inflammatory properties amongst others (Sandra, 2020; Inge and Rainer, 2020). Most medicinal plants contain primary and secondary metabolites (phytochemicals) which make them useful as food due to their nutritional value or in the treatment of severe malaria, diarrhea, dysentery, sexually transmitted infection, skin disease, urethral discharge, gastro-intestinal infection and in dressings for chronic ulcers and wounds (Noufou et al. 2012).

Pterocarpus erinaceus, a medicinal plant belonging to the family Fabaceae and order fabales. The tree is deciduous, drought resistance, multipurpose, medium in size and can grow up to 12 – 15 meters tall (Heuzé et al., 2019). The plant is found in most part of West and Central Africa as well as some parts of Asia where over 80 species are distributed (Dery, 2023; Moyo et al., 2015). Their foliage and pods are of high nutritional value and are used for feeding livestock (Koffi et al., 2020). Akinyeye et al. (2010) reported that the leaf of Pterocarpus erinaceus contained dry matter (64.13 %), crude protein (22.14 %), ether extract (7.15 %), ash (9.66 %) and carbohydrates (25.17 %). Aqueous extracts from stem bark, leaves and roots of Pterocarpus erinaceus have shown in vitro antibacterial and antifungal activities against Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Candida albicans (Datta et al., 2019). Infusion of roots and Pterocarpus leaves have been traditionally used for the treatment of dysentery, gastro intestinal infection, blood shortage (anemia), skin infection, menstrual pains, sores and leprosy (Edwife et al., 2014).

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Alkaloids, flavonoids, tannins, terpenoids, saponins, phenols are some of the phytochemicals identified in Pterocarpus erinaceus leaves, stem bark and roots (Yusuf et al., 2020; Ahmed et al., 2017).

Previous studies have shown that the dietary supplementation of plant extracts revealed that it can enhance the activities of digestive enzymes, absorption of nutrients and efficiency of feed utilization of rabbits. For instance, Olafadehan et al. (2022) reported that Daniellia oliveri ethanolic extract at 6 mL/litre had a significant influence on average daily weight gain and blood parameters of broilers. Alagbe et al. (2023); Sandra (2022), also recorded that Piliostigma thonningii aqueous extracts at 8 mL/litre can positively influence the gastrointestinal tract morphology, physiology and most likely, stimulating or inhibiting particular metabolic pathways and optimize performance in rabbits.

Despite the extensive research on the effect of plant extracts on the performance of rabbits. Presently, there is insufficient information on the impact of Pterocarpus erinaceus leaf extract on the growth performance and intestinal microbial population of rabbits. This research is timely, because it will proffer solution to the increasing cases of multi drug resistance in livestock, promotes food safety and give a clue on the optimal intake levels for rabbits.

MATERIALS AND METHODS

Study area, sample collection, authentication and preparation

The study was conducted at the Sumitra Institute's Rabbit Unit, which is located between 23º 13' N and 72º 41' E. The experiment was carried out between January to February, 2021 was conducted in accordance with the guidelines and requirements of procedures that had been authorized by the research ethics council of India's Sumitra Research Institute.

Fresh leaves of Pterocarpus erinaceus were collected very early in the morning within the research institute premises in Gujarat. It was taken to the department of taxonomy in Sumitra Research Institute, Gujarat for identification. A voucher number UD/094AAA was assigned to the sample and the leaves were washed with running tap water and air dried in an open shade for 15 days until a constant weight was achieved. The dried leaves were grind using an electric blender and the powdered samples were transferred into a labelled transparent polythene bag and taken to the laboratory for further examination and extraction.

200 grams of grinded Pterocarpus erinaceus leaf was measured into a conical flask followed by the addition 1000 mL of ethanol until the powder was fully immersed. The mixture was stirred every 3 hours for 24 hours, then allowed to stay for another 2 days. The mixture was filtered and the filtrate was collected into a plastic labeled bottle and kept in the refrigerator at 4ºC before the commencement of the study.

Phytochemical examination of Pterocarpus erinaceus leaf extract (PELE)

Quantitative phytochemical evaluation of Pterocarpus erinaceus leaf was carried out using standard laboratory procedures with the following reagents: sodium hydroxide, sodium bicarbonate, folin-ciocalteau's reagent, aluminum chloride, sodium nitrate, sulphuric acid, bromocresol solution, ferric ammonium sulphate, amyl alcohol and ammonium thiocyanate solution.

Kits/equipment's used: test tubes, beaker, conical flask, water bath, thermometer and conical flask and Photolab® 7000 series UV-VIS spectrophotometer with a photometric accuracy of -0.003 E for E < 0.600; 0.5 % of values for 0.600 < E < 2.000 and coupled with a monochrometer with grating and step motor reference beam and tungsten halogen to be able to scan at a speed of 700 – 2000 nm/minutes, wavelength accuracy (± 1nm/0.5 nm) with 16 mm round, 10 mm, 20 mm, 50 mm rectangular cuvette with automatic detection. Result interpretation is aided by PC software photolab®, data spectral plus photolab®, colour, field case, checking tools for AQA.

Estimation of tannins and phenolic compounds in Pterocarpus erinaceus leaf extract (PELE)

0.5 mL of PELE was added to 1.5 mL of Folin-Ciocalteau reagent in a conical flask, the mixture was covered for 5 minutes and kept under room temperature followed by the addition of 1.0 mL sodium bicarbonate, 10 mL distilled water and stirred before it was introduced to Photolab® 7000 series UV-VIS spectrophotometer at an optical densities of 725 nm according to standard procedures described by EEE. Phenolic compound and tannins were expressed as gallic acid equivalent (GAE) and tannic acid equivalent (TAE) respectively.

Estimation of flavonoids and terpenoids

0.5 mL of PELE was added was added to 0.8 mL nitric oxide mixed together in a test tube, the mixture was incubated for 10 minutes followed by the addition of 0.5 mL of sodium hydroxide. Photolab® 7000 series UV-VIS spectrophotometer was adjusted at an optical density of 510 nm and 725 nm. The generated outcome was

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expressed in equivalents of catechin (mg/g) according to standard laboratory procedures outlined by Mahmoudi et al. (2016).

Determination of phytate concentrations (Anion exchange technique)

0.5 mL of PELE was diluted with 1.0 mL of distilled water followed by the addition of 0.5 mL ferric ammonium sulphate. The mixture was thoroughly mixed in a test tube, covered and cooled at room temperature for 10 minutes. Photolab® 7000 series UV-VIS spectrophotometer was adjusted at an optical density of 450 nm to determine the concentration of phytate in the test ingredient.

Determination of alkaloid concentration

0.5 mL of PELE was diluted with 2 mL of phosphate buffer and 1.5 mL of bromocresol green solution were mixed together in a test tube covered and allowed to stay for 30 minutes before it was injected into Photolab® 7000 series UV-VIS spectrophotometer and set at an optical density of 420 nm to determine the concentration of alkaloids in PELE and expressed as atropine equivalent. Other procedures were carried out according to the methods outline by Njoku and Chidi (2009).

Animal management and experimental design

In Sumitra Research Institute, a total of 50 crossbred male rabbits with initial body weight of 486 ± 0.70 g and weaned at 28 days of age were individually housed in a specially constructed galvanized cage measuring 40 cm by 35 cm by 25 cm in length, breath, height and 80 cm above the ground equipped with concrete feeder and automatic nipple drinkers in an open sided pen was disinfected with Aquaclean® two weeks before the commencement of the experiment. On arrival, rabbits were quarantined for 2 weeks, given prophylactic treatment (Ivermectin® administered subcutaneously at 0.3 mg/kg body weight and Sulphadimidine® at 0.1 mg/kg body weight) and fed standard feed (growers mash) adequate in all nutrients to meet the requirement of rabbits according to Nutritional Research Council in 1977. Rabbits were stratified based on their body weight and assigned to 5 groups with six animals with one animal per replicate in a completely randomized design. Rabbits were cared for following commercial management procedures, feed was provided twice a day (7:00 AM and 14:00 PM). Animals were also given free access to clean fresh water and the experiment lasted for 56 days.

Experimental procedures/methods

Rabbits in group one (G_1) was given standard feed without Pterocarpus erinaceus leaf extract (PELE) while those in group two (G_2), three (G_3), four (G_4) and five (G_5) were drenched daily with PELE at 0.2 mL, 0.4 mL, 0.6 mL and 0.8 mL respectively.

Measurements

Growth performance

Weight gain was estimated by subtracting the final body weight from the initial body weight of rabbits expressed in grams. Average daily weight gain and average daily feed intake was calculated by dividing final weight gain and total feed intake by the number of days for the trial expressed in grams. Feed conversion ratio was calculated by dividing the weight gain by feed intake. Mortality was recorded daily as it occurs among the various treatments.

Calculation

Weight gain (gram/rabbit) = final body weight minus initial body weight
Average daily weight gain (gram/rabbit) = weight gain / number of days for the trial
Average daily feed intake (gram/rabbit) = total feed intake / number of days for the trial

Intestinal microbial count (colony-forming unit {CFU}/mL)

At the end of the experiment intestinal content from 5 randomly selected rabbits per treatment. Content from each rabbits were collected into a sterile labeled sample bottle followed by the addition of a drop of peptone reagent for microbial evaluation of Escherichia coli, Lactobacillus sp and Salmonella sp. 7000 RMS microbial analyzer was adopted for the analysis, it was adjusted at a flow rate of 30 mL/minute, biological detection limit of 1 AFU (auto fluorescent units) and measurement range of 0 – 10,000 total counts/mL. Plate count results were generated via 2 – analog output channels: 4-20 mA standard equipped with user software with configurable output range.

Statistical analysis

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Data obtained were subjected to analysis of variance in a completely randomized design using Statistical Package for Social Sciences (SPSS version 21.0). Duncan multiple range test of the same software was used to test the difference among the means at $P \leq 0.05$ level of significance.

Using the model: $Yxy = \mu + \alpha x + \beta xy$, was used in this investigation, where Yxy = general response to variables; x = the overall mean; αx = effect of the xth treatment (1=5); and βxy = random error term for each estimate.

Table 1: Composition of experimental diet used (expressed in dry matter percentage)

Raw materials	Inclusion level (percentage)
Yellow corn	40.00
Rice bran	10.00
Palm kernel meal	15.00
Soya meal	23.00
Fish meal (Imported: 72 % crude protein)	2.00
Bone meal	6.00
Oyster shell	3.00
Mineral/Vitamin Premix (Growers)	0.25
Methionine	0.20
Lysine	0.20
Common salt	0.40
Total	100.00
Determined analysis	
Energy (Kcal/kg)	2660.8
Crude protein	18.51
Crude fibre	12.96
Ether extract	2.11
Ash	8.75

RESULTS

Table 2 reveals the phytochemical components of Pterocarpus erinaceus leaf extract. Seven phyto-constituents were identified namely: Flavonoids (112.61 mg/g CAE), terpenoids (87.52 mg/g CAE), phenols (106.39 mg/g GAE), alkaloids (91.53 mg/g ATE), tannins (40.88 mg/g TAE) and phytate (11.31 mg/g).

Table 2: Phytochemical components of Pterocarpus erinaceus leaf extract

Index	Unit	Concentration
Flavonoids	mg/g CAE	112.61
Terpenoids	mg/g CAE	87.52
Phenols	mg/g GAE	106.39

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Alkaloids	mg/g ATE	91.53
Tannins	mg/g TAE	40.88
Phytate	mg/g	11.31

mg/g: milligram per gram; CAE: catechin equivalent; TAE: tannic acid equivalent; GAE: gallic acid equivalent; ATE: atropine equivalent

The effects of Pterocarpus erinaceus leaf extract on the growth performance of weaner rabbits is shown in Table 3. The average daily weight gains of rabbits fed diet 4 (0.6 mL) and diet 5 (0.8 mL) were similar ($p>0.05$), similar trend was observed among animals fed diet 2 (0.2 mL) and diet 3 (0.4 mL) ($p>0.05$) but significantly higher ($p<0.05$) than diet 1 (0 mL). Average daily weight gain were higher in diet 4 and 5, intermediate in diet 2,3 and lower in diet 1 ($p<0.05$). Average daily weight gain of rabbits fed Pterocarpus erinaceus leaf extract significantly ($p<0.05$) improved against the control. Conversely, average daily feed intake in rabbits fed diet 2, 3, 4 and 5 were similar ($p>0.05$) but significantly higher than diet 1 as a result, feed conversion ratio was better for Pterocarpus erinaceus leaf extract supplemented rabbit's relative to the control (diet 1). No mortality was recorded throughout the experimental period.

Table 3: Effect of Pterocarpus erinaceus leaf extract on the growth performance of weaner rabbits

dex	[1]Diet 1	[2]Diet 2	[3]Diet 3	[4]Diet 4	[5]Diet 5	[6]SEM
tial body weight (g/rab)	488.12	488.82	487.50	486.00	486.85	0.01
nal body weight (g/rab)	1685.9^{c}	1800.1^{b}	1871.4^{b}	2030.2^{a}	2033.9^{a}	39.61
verage weight gain (g/rab)	1197.8^{c}	1311.28^{b}	1383.9^{b}	1544.2^{a}	1547.1^{a}	28.50
verage daily weight gain (g/rab)	21.39^{c}	23.42^{b}	24.71^{b}	27.58^{a}	27.63^{a}	0.03
tal feed intake (g/rab)	4109.3^{b}	4300.1^{a}	4330.9^{a}	4461.9^{a}	4465.8^{a}	61.20
verage daily feed intake (g/rab)	73.38^{b}	76.79^{a}	77.34^{a}	79.68^{a}	79.75^{a}	0.12
ed conversion ratio	3.43^{c}	3.28^{b}	3.20^{b}	3.00^{a}	3.00^{a}	0.02
ortality (%)	-	-	-	-	-	

[1]Standard feed without Pterocarpus erinaceus leaf extract (PELE); [2]Standard feed with 0.2 mL PELE/day; [3]Standard feed with 0.4 mL PELE/day; [4]Standard feed with 0.6 mL PELE/day; [5]Standard feed with 0.8 mL PELE/day; [6]Standard error of mean; [a,b,c]Means with different superscripts along row are significantly ($P<0.05$) different

Table 3 presents the effect of Pterocarpus erinaceus leaf extract on the intestinal microbial population of weaner rabbits. Lactobacillus sp count of rabbits fed diet 4 (0.6 mL) were similar to those in diet 5 (0.8 mL) ($p>0.05$). Conversely, rabbits fed diet 1 (0 mL) had higher population of Salmonella sp and Escherichia coli relative to the other treatments ($p<0.05$).

Table 3: Effect of Pterocarpus erinaceus leaf extract on the intestinal microbial population of weaner rabbits

dex (Cfu/mL)	[1]Diet 1	[2]Diet 2	[3]Diet 3	[4]Diet 4	[5]Diet 5	[6]SEM
ctobacillus sp	5.74^{c}	6.06^{b}	6.17^{b}	6.98^{a}	7.04^{a}	0.35
lmonella sp	4.02^{a}	2.71^{b}	2.60^{b}	2.43^{b}	2.40^{b}	0.11
cherichia coli	6.39^{a}	4.66^{b}	4.50^{b}	4.42^{b}	4.40^{b}	0.28

[1]Standard feed without Pterocarpus erinaceus leaf extract (PELE); [2]Standard feed with 0.2 mL PELE/day; [3]Standard feed with 0.4 mL PELE/day; [4]Standard feed with 0.6 mL PELE/day; [5]Standard feed with 0.8 mL PELE/day; [6]Standard error of mean; [a,b,c]Means with different superscripts along row are significantly ($P<0.05$) different

DISCUSSION

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The presence of phytochemicals in Pterocarpus erinaceus leaf extract implies that it has several potential health benefits such as: antimicrobial, hypoglycemic, antioxidant, neuroprotective, antifungal, hepatoprotective, anti-inflammatory, antiproliferative, anti-androgenic, activities amongst others (Singh et al., 2022; Anuore et al., 2023). The result on the phyto-constituents in Pterocarpus erinaceus leaf extract is in consonance with the report of Gabriel and Onigbanjo (2010). Terpenoids are known to have antimicrobial, anti-inflammatory, anti-viral, antioxidant and cardio-protective properties, which can help to reduce the risk of heart disease (Alagbe et al., 2022; Alagbe, 2024; Shittu et al., 2021). Additionally, terpenoids can help in boosting the immune system, improves digestion and prevent the emergence of free radicals (El-Hawary and Rabeh, 2014; Adewale et al., 2021). Flavonoids are a type pf polyphenol which possess antioxidant properties which may help to protect cell from damage caused by free radicals (Evans et al., 2002; Alagbe, 2022). They have also known to have cardiovascular benefit, they can help to prevent blood clots, reduce inflammation, and improve the health of blood vessels (Edeoga et al., 2005). Alkaloids have a wide range of pharmacological functions including – anti-arrhythmic, anti-malarial, vasodilatory, and stimulant activities (Aniszewski, 2007; Davies, 2002). Phenols are compounds with antioxidant, antimicrobial, anti-inflammatory and neuro-protective properties (Aniszewsk, 2007; Brielmann et al., 2006). Most of the tannins in medicinal plants possess anti-bacterial, antiseptic, anti-carcinogenic and astringent properties (Brielmann et al., 2006; Alagbe, 2022).

Outcome on growth performance of rabbits fed diet supplemented with Pterocarpus erinaceus leaf extract shows that it has positive effects on average daily weight gain, average daily feed intake and feed conversion ratio particularly among animals fed 0.6 mL (diet 4) and 0.8 mL (diet 5). A report by Anuore et al. (2023); Sandra (2021), suggests that dietary supplementation of medicinal plants in animals increases the activities of enzymes on the feed ingredients, breaking them down to nutrients and making them available to the body. Alagbe (2023) also reported that plant extracts have flavoring and sensorial stimulation, antimicrobial and antioxidant properties. This explains why rabbits fed Pterocarpus erinaceus leaf extract consumed more feed which was digested, absorbed and used by animal metabolism. Results obtained are consistent with the reports of Ismail et al. (2019) when Boswellia serrata was fed to growing rabbits at 1.00 g/kg.

Results on intestinal microbial population reveals that Pterocarpus erinaceus leaf extract favours the production of Lactobacillus sp and disrupts the proliferation of Escherichia coli as well as Salmonella sp among the groups. Pterocarpus erinaceus leaf extract's mode of action is based on the principle of competitive exclusion, including outcompeting undesirable bacteria in terms of nutrients and space, as well as producing potent antimicrobial metabolites with a strong affinity for undesirable bacteria such as Escherichia coli and Salmonella sp. As a result, the rabbits gut microbiota is positively impacted, whilst gut integrity is maintained to improved feed utilization. The above result is in agreement with the findings of Kiczorowska et al. (2016) when Boswellia serrata was supplemented in the diet of broilers. This result is also consistent with the reports of Emami et al. (2020) when probiotics was supplemented to the diet of rabbits at 2 g/kg. All this result suggests that medicinal plants and their extracts have antibacterial properties and can prevent dysbiosis without compromising the health of the animal.

CONCLUSION

In conclusion, supplementing Pterocarpus erinaceus leaf extract (PELE) up to 0.8 mL/day significantly improved average daily weight gain and feed intake of rabbits against the control (diet 1). PELE has also proven to be capable of reducing microbial pressure in the gut thereby preventing nutrient competition due to the presence of phytochemicals. This translates to a better feed conversion ratio in rabbits without compromising their performance and health status.

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