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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

To investigate the impact of Pterocarpus erinaceus leaf extracts (PELE) on the growth performance and intestinal microbial population of weaned rabbits, fifty crossbred male rabbits weighing an initial 486 ± 0.70 g and weaned at twenty-eight days of age were individually housed in a specially constructed galvanized cage at Sumitra Research Institute. In a fully randomized design, rabbits were divided into five groups consisting of six animals each, one animal per replication, and were stratified according to body weight. The amount of nutrients in the experimental food was sufficient to suit the needs of the rabbits. Animals in groups 2, 3, 4, and 5 were fed standard diets with 0.2 mL, 0.4 mL, 0.6 mL, and 0.8 mL/day, respectively, whereas animals in group 1 were provided standard diets with 0 mL PELE. 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). Groups 4 (1544.2 g) and 5 (1547.1 g) showed comparable ($p>0.05$) average daily weight increase results, although they were substantially greater than those of groups 1 (1197.8 g), 2 (1311.28 g), and 3 (1383.9 g). In comparison to group 1 (control), the average daily feed intake was greater ($p<0.05$) in rabbits fed a diet supplemented with PELE. Groups 4 and 5 had the best feed conversion ratios (3.00 and 3.00), group 2 (3.28) and group 3 (3.20) had intermediate ratios, while group 1 (3.43) had lower ratios. Group 1 had a significantly greater ($p<0.05$) microbial population of Salmonella sp and Escherichia coli than the other groups. In contrast, the PELE supplemented food showed the highest Lactobacillus sp count compared to the control group 1. It was determined that adding 0.8 mL of PELE per day to rabbit diets can enhance their performance while also inhibiting the growth of harmful organisms without endangering the animals' health.

Keywords

Pterocarpus erinaceus, Phytochemicals, Rabbits, Antimicrobial, Food Safety, Antibiotics

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1. Introduction

The development of natural antibiotic substitutes has attracted attention due to public concern over the risks of antimicrobial resistance to human and animal health. 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. Due to their nutritional value, the majority of medicinal plants can be used as food or as treatments for conditions like severe malaria, diarrhea, dysentery, STDs, skin diseases, urethral discharge, gastrointestinal infections, and chronic wounds and ulcers. This is because these plants contain primary and secondary metabolites, or phytochemicals, which make them useful [1-17].

The medicinal plant Pterocarpus erinaceus is a member of the Fabaceae family and order of fabales. The tree is deciduous, drought resistance, multipurpose, medium in size and can grow up to 12 – 15 meters tall. The plant is scattered throughout 80 species and may be found in much of West and Central Africa, certain portions of Asia, and other places. Raising cattle is done using their very nutritious leaves and pods. Akinyeye [38] 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 %) [6-28]. Pterocarpus erinaceus stem bark, leaves, and roots have aqueous extracts that have demonstrated in vitro antibacterial and antifungal activity against Bacillus subtilis, Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. According to Edwife, infusions of Pterocarpus roots and leaves have long been used to treat leprosy, diarrhea, gastrointestinal infections, blood shortages (anemia), skin infections, menstrual discomfort, and ulcers. Alkaloids, flavonoids, tannins, terpenoids, saponins, phenols are some of the phytochemicals identified in Pterocarpus erinaceus leaves, stem bark and roots [5-19].

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 [27] 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 [23], Sandra also observed that 8 mL/litre of Piliostigma thonningii aqueous extracts can im-

prove the morphology and physiology of the gastrointestinal system in rabbits, and most likely, stimulate or inhibit certain metabolic pathways to maximize performance [9-33].

Despite a wealth of data on how plant extracts affect rabbit performance. The effects of Pterocarpus erinaceus leaf extract on the intestinal microbial population and growth performance of rabbits are not well understood at this time. This research is important because it will help address the rising number of livestock cases of multidrug resistance, improve food safety, and provide guidance on the ideal consumption amounts for rabbits [30-40].

2. Materials and Methods

2.1. Study Area, Sample Collection, Authentication and Preparation

The research was carried out in the Rabbit Unit of the Sumitra Institute, situated between 23o 13' N and 72o 41' E. The experiment, which took place in January and February of 2021, followed the rules and specifications of protocols approved by the research ethics committee of India's Sumitra Research Institute.

Early in the morning, fresh Pterocarpus erinaceus leaves were gathered on the Gujarati research institute's grounds. It was brought to the Sumitra Research Institute's taxonomy section in Gujarat so that it might be identified. The sample was given the voucher number UD/094AAA. The leaves were air dried in an open shade for 15 days until a consistent weight was reached. They were then cleaned under running tap water. After the dried leaves were ground with an electric blender, the powdered samples were placed in a clear polythene bag with a label and brought to the lab for further analysis and extraction.

A conical flask was filled with 200 grams of ground Pterocarpus erinaceus leaf, and 1000 milliliters of ethanol were added until the powder was completely submerged. After being left for two days, the mixture was mixed every three hours for a total of twenty-four hours. Before the research started, the mixture was filtered, and the filtrate was collected into a plastic container with a label, and it was stored in the refrigerator at 4^{o}C.

2.1.1. 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.

The following kits and equipment were used: test tubes, beaker, conical flask, water bath, thermometer, 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, 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 (± 1 nm/0.5 nm) with 16 mm round, 10 mm, 20 mm, and 50 mm rectangular cuvette with automatic detection.. PC applications such as Photolab®, Data Spectral Plus Photolab®, Color, Field Case, and AQA Checking Tools facilitate the understanding of results.

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

In a conical flask, 0.5 mL of PELE and 1.5 mL of Folin-Ciocalteau reagent were combined. The mixture was covered and allowed to stand at room temperature for five minutes. Next, 1.0 mL sodium bicarbonate and 10 mL distilled water were added, and the mixture was stirred. Finally, it was added to a Photolab® 7000 series UV-VIS spectrophotometer at an optical density of 725 nm, following standard protocols outlined by EEE. Gallic acid equivalent (GAE) and tannic acid equivalent (TAE) were used to represent phenolic compounds and tannins, respectively.

2.1.3. Estimation of Flavonoids and Terpenoids

In a test tube, 0.5 mL of PELE and 0.8 mL of nitric oxide were combined. The mixture was then incubated for 10 minutes before adding 0.5 mL of sodium hydroxide. The optical density of the Photolab® 7000 series UV-VIS spectrophotometer was set between 510 and 725 nm. As per the usual laboratory protocols described by Mahmoudi et al. (2016), the resulting output was represented in equivalents of catechin (mg/g).

2.1.4. Determination of Phytate Concentrations (Anion Exchange Technique)

After diluting 0.5 mL of PELE with 1.0 mL of distilled water, 0.5 mL of ferric ammonium sulphate was added. In a test tube, the liquid was well combined, covered, and allowed to cool for ten minutes at room temperature. At find the amount of phytate in the test component, the optical density of the Photolab® 7000 series UV-VIS spectrophotometer was set at 450 nm.

2.1.5. Determination of Alkaloid Concentration

In order to calculate the concentration of alkaloids in PELE and express it as atropine equivalent, 0.5 mL of PELE was diluted with 2 mL of phosphate buffer and 1.5 mL of bromocresol green solution. The mixture was combined in a test tube, covered, and left for 30 minutes. After that, it was injected into a Photolab® 7000 series UV-VIS spectrophotometer and set at an optical density of 420 nm. Additional procedures were conducted in compliance with the guidelines provided by Njoku and Chidi (2009).

2.2. Animal Management and Experimental Design

At the Sumitra Research Institute, fifty male crossbred rabbits weighing 486 ± 0.70 g at birth who were weaned after 28 days were kept individually in a specially made galvanized cage that measured 40 cm by 35 cm by 25 cm in length, breath, height, and 80 cm above the ground. The cage was furnished with an automatic nipple drinker and a concrete feeder, and it was disinfected with Aquaclean two weeks prior to the experiment's start. After receiving prophylactic treatment (Ivermectin® administered subcutaneously at 0.3 mg/kg body weight and Sulphadimidine at 0.1 mg/kg body weight) and standard feed (growers mash) sufficient in all nutrients to meet rabbits' requirements, the rabbits were quarantined for two weeks upon arrival, as per the guidelines set forth by the Nutritional Research Council in 1977. In a fully randomized design, rabbits were divided into five groups consisting of six animals each, one animal per replication, and were stratified according to body weight. 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 [30-42].

2.3. Experimental Procedures/Methods

Rabbits in groups one (G1), two (G2), three (G3), four (G4), and five (G5) received normal feed without Pterocarpus erinaceus leaf extract (PELE), whereas PELE was soaked daily in 0.2 mL, 0.4 mL, 0.6 mL, and 0.8 mL amounts for each group.

2.3.1. Measurements

2.3.1.1. Growth Performance

To determine weight increase, the ultimate body weight of the rabbits was subtracted from their beginning body weight, given in grams. By dividing the final weight growth and total feed intake by the number of trial days, reported in grams, av-

erage daily weight gain and average daily feed intake were computed. Weight increase was divided by feed intake to get the feed conversion ratio. Every day, the number of deaths among the different therapies was documented.

2.3.1.2. Calculation

Final body weight less beginning body weight equals weight increase (gram/rabbit).
The formula to get the average daily weight growth (gram/rabbit) is weight gain / trial days.
Total feed intake / number of trial days equals the average daily feed intake (gram/rabbit).

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

Intestinal content from five randomly chosen rabbits per treatment at the conclusion of the trial. Salmonella sp., Lactobacillus sp., and Escherichia coli were tested microbiologically by adding a drop of peptone reagent to the contents of each rabbit that had been collected into a sterile, labeled sample container. The analysis used a 7000 RMS microbial analyzer, which was set to have a flow rate of 30 mL/minute, a biological detection limit of 1 AFU (auto fluorescence units), and a measurement range of 0 – 10,000 total counts/mL. The two analog output channels—4–20 mA standard—that were outfitted with user software and an adjustable output range were used to create the plate count data.

2.3.1.4. Statistical Analysis

A totally randomized design of analysis of variance was applied to the collected data using the Statistical Package for Social Sciences (SPSS version 21.0). The same software's Duncan multiple range test was performed to assess the significance of the mean difference at the $P \leq 0.05$ level.

This study utilized the following model: $Yxy = \mu + \alpha x + \beta xy$, where x is the overall mean, αx is the influence of the xth treatment (1=5), and βxy is the 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

3. Results and Discussion

3.1. 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
Alkaloids	mg/g ATE	91.53
Tannins	mg/g TAE	40.88
Phytate	mg/g	11.31

The terms "mg/g," "milligram per gram," "catechin equivalent," "tannic acid equivalent," "galic acid equivalent," and "ateropine equivalent" are used.

Table 3 displays the impact of Pterocarpus erinaceus leaf extract on the weaner rabbits' growth performance. Rabbits fed diets 4 (0.6 mL) and 5 (0.8 mL) showed similar average daily weight gains ($p>0.05$), as did animals fed diets 2 (0.2 mL) and 3 (0.4 mL) ($p>0.05$), but diet 1 (0 mL) showed considerably larger weight gains ($p<0.05$). Diets 4 and 5 had larger average daily weight gains, diets 2, 3, and 1 had lower average daily weight gains ($p<0.05$). When compared to the control group, the average daily weight gain of rabbits given Pterocarpus erinaceus leaf extract improved considerably ($p<0.05$). However, the average daily feed intake in the rabbits fed diets 2, 3, 4, and 5 was considerably greater than diet 1 ($p>0.05$). As a consequence, the feed conversion ratio for the rabbits supplemented with Pterocarpus erinaceus leaf extract was superior to that of the diet 1 control group. During the whole trial time, no deaths were reported.

Table 3. Effect of Pterocarpus erinaceus Leaf Extract on the Growth Performance of Weaner Rabbits.

Index	[1]Diet 1	[2]Diet 2	[3]Diet 3	[4]Diet 4	[5]Diet 5	[6]SEM
Initial body weight (g/rab)	488.12	488.82	487.50	486.00	486.85	0.01
Final body weight (g/rab)	1685.9^{c}	1800.1^{b}	1871.4^{b}	2030.2^{a}	2033.9^{a}	39.61

Average weight gain (g/rab)	1197.8[c]	1311.28[b]	1383.9[b]	1544.2[a]	1547.1[a]	28.50
Average daily weight gain (g/rab)	21.39[c]	23.42[b]	24.71[b]	27.58[a]	27.63[a]	0.03
Total feed intake (g/rab)	4109.3[b]	4300.1[a]	4330.9[a]	4461.9[a]	4465.8[a]	61.20
Average daily feed intake (g/rab)	73.38[b]	76.79[a]	77.34[a]	79.68[a]	79.75[a]	0.12
Feed conversion ratio	3.43[c]	3.28[b]	3.20[b]	3.00[a]	3.00[a]	0.02
Mortality (%)	-	-	-	-	-	

[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 4 demonstrates the impact of Pterocarpus erinaceus leaf extract on the weaner rabbits' intestinal microbial community. Lactobacillus sp. counts in rabbits given diet 4 (0.6 mL) and diet 5 (0.8 mL) were comparable (p>0.05). In contrast, the population of Salmonella sp. and Escherichia coli in rabbits fed diet 1 (0 mL) was larger than in the other treatments (p<0.05).

Table 4. Effect of Pterocarpus erinaceus Leaf Extract on the Intestinal Microbial Population of Weaner Rabbits.

Index (Cfu/mL)	[1]Diet 1	[2]Diet 2	[3]Diet 3	[4]Diet 4	[5]Diet 5	[6]SEM
Lactobacillus sp	5.74[c]	6.06[b]	6.17[b]	6.98[a]	7.04[a]	0.35
Salmonella sp	4.02[a]	2.71[b]	2.60[b]	2.43[b]	2.40[b]	0.11
Escherichia 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.

3.2. Discussion

The presence of phytochemicals in Pterocarpus erinaceus leaf extract implies that it has several potential health benefits such as: antimicrobial, hypoglycemic, antioxidant, neuro-protective, antifungal, hepatoprotective, anti-inflammatory, anti-proliferative, anti-androgenic, activities amongst others [26] (Singh et al., 2022). The result on the phyto-constituents in Pterocarpus erinaceus leaf extract is in consonance with the report of Gabriel and Onigbanjo (2010) [16]. According to studies by Alagabe et al. [27, 29] Shittu et al. [34] terpenoids are known to possess antibacterial, anti-inflammatory, antiviral, antioxidant, and cardio-protective qualities that may help lower the risk of heart disease. Additionally, terpenoids can help in boosting the immune system, improve digestion and prevent the emergence of free radicals El-Hawary and Rabeh [4], Adewale et al. [33]. A kind of polyphenol known as flavonoids has antioxidant qualities that may aid in defending cells against harm from free radicals [5,27]. Additionally, they have been shown to offer cardiovascular benefits, including the ability to lower inflammation, prevent blood clots, and enhance blood vessel health [6]. Alkaloids possess a diverse array of pharmacological properties, such as stimulant, vasodilatory, anti-malarial, and anti-arrhythmic effects [11, 7]. Antioxidant, antibacterial, anti-inflammatory, and neuroprotective qualities are characteristics of phenolic compounds [11,9]. Alagbe and Brielmann et al. [9,27] state that the majority of tannins found in medicinal plants have astringent, antibacterial, antiseptic, and anti-carcinogenic qualities.

Pterocarpus erinaceus leaf extract has been found to have good effects on average daily weight gain, average daily feed intake, and feed conversion ratio in rabbits fed diets supplemented with it. This is especially true for animals fed 0.6 mL (diet 4) and 0.8 mL (diet 5). The results of the study illustrate this point. According to a study by Anuore et al. [21-23] and Sandra

12], feeding medicinal plants to animals may boost the activities of the enzymes in the feed ingredients, allowing the body to absorb and metabolize them as nutrients. Plant extracts include flavorful, sensory-stimulating, antibacterial, and antioxidant qualities, according to Alagbe [19-25]. This explains why the amount of feed that was broken down, absorbed, and used by animal metabolism was higher in rabbits given Pterocarpus erinaceus leaf extract. The outcomes observed when Boswellia serrata was fed to developing rabbits at a rate of 1.00 g/kg are in line with the results of Ismail et al. [17].

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. The mechanism of action of Pterocarpus erinaceus leaf extract is founded on the idea of competitive exclusion, which includes outcompeting harmful bacteria for nutrients and available space as well as generating strong antimicrobial metabolites that have a strong affinity for harmful bacteria like Salmonella sp. and Escherichia coli. Consequently, the gut microbiota of the rabbits is positively influenced, and their gut integrity is preserved for optimal feed consumption. The aforementioned outcome is consistent with the research conducted by Kiczorowska et al. after broiler diets treated with Boswellia serrata [17]. This outcome is also in line with studies by Emami et al. [8], who added probiotics to rabbits' diets at a rate of 2 g/kg. This entire finding implies that medicinal herbs and their extracts possess antibacterial qualities and can stop dysbiosis without endangering the animal's health.

4. Conclusions and Future Scope

Finally, compared to the control (diet 1), adding Pterocarpus erinaceus leaf extract (PELE) up to 0.8 mL/day greatly increased the average daily weight gain and feed intake of the rabbits. Because phytochemicals are present in PELE, it has also demonstrated the ability to lower microbial pressure in the gut and reduce nutritional competition. This means that rabbits will have a higher feed conversion ratio without sacrificing their health or performance.

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