IMPACT OF DIETARY SUPPLEMENTATION OF PERSEA AMERICANA STEM BARK ON THE GROWTH PERFORMANCE AND SOME HAEMATOLOGICAL INDICES OF WEANER RABBIT

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Abstract: This experiment was carried out to ascertain the impact of dietary supplementation of *Persea americana* stem bark on the growth performance and some haematological indices of weaner rabbit. A total of 5- weeks, 60 cross bred male rabbits randomly distributed into 5 groups each with 20 rabbits. Each group was further sub-divided in to five groups each with 2 rabbits of comparable group weight in a 60 day experimental period adopting a completely randomized design. Basal diet was formulated to meet all the nutrient requirements for growing rabbits. Animals in group 1 (G¹) was fed basal diet without *Persea americana* stem bark powder, G², G³, G⁴ and G⁵ received basal diet supplemented with *Persea americana* stem bark powder, G², G³, G⁴ and G⁵ received basal diet supplemented with *Persea americana* stem bark powder at 50 g, 100 g, 150 g and 200 g per kilogram diet respectively. Rabbits had unrestricted access to clean water and feed. Phytochemical screening of *Persea americana* stem bark powder revealed the presence of phenol and flavonoids as major phyto-compounds followed by terpenoids, tannins, alkaloids, steroids and saponins. Average daily weight gain, average daily feed intake, feed conversion ratio were significantly (P<0.05) influenced by the treatment. Rabbits that received *Persea americana* stem bark powder had a higher average body weight, best feed conversion ratio and had no mortality compared to the control. Similarly, total erythrocyte, leucocyte, hematocrit, haemoglobin and lymphocytes had higher (P<0.05) values relative to the control. However, all recorded values were within the baseline ranges for healthy growing rabbits. In conclusion, *Persea americana* stem bark powder can be added to weaner rabbit diets up to 200g/kg without affecting their growth performance and blood profile.

Keywords: Food safety; Persea americana; Phyto-constituents; Performance; Stem bark

1 INTRODUCTION

The use of medicinal or herbal plants in animal nutrition is increasingly gaining attention due to rising cases of antibiotic resistance, strong global tendencies to reduce antibiotic growth promoters, raising concerns about animal health and environmental protection as well as food safety [1,2]. The dietary supplementation of herbal plants optimises the performance of animals and has no withdrawal period [3,4]. Among the potential herbal plants with numerous potential is *Persea americana* stem bark.

Persea americana belongs to the family Lauraceae and is commonly called Avocado. The plant is medium sized, erect and deciduous tree ranging from 15-20 m in height [5] with trunks 30 cm (12 inches) or more in diameter. They are over 20 species of pear that are widely distributed in Africa, America, Asia amongst others [6]. Different parts of the plant are used in folk medicine for the treatment of several ailments such as hypertension, diabetes and inflammation [7]. The plant are good sources of the B-complex vitamins and also contain vitamin C [8]. The fruit is a pome, juicier than the apple, and varying from apple-shaped to teardrop-shaped. The leaves are oval and simple and, unlike those of the apple, smooth and glossy while the stem bark is dark brown in colour [6].

Proximate analysis of *Persea americana* fruit suggests that it contains more of fat and energy; seed had more of fat, protein and energy while the leaf had more of protein, fibre, and ash [9]. The presence of fatty acids (linoleic, oleic, palmitic, stearic, linolenic, capric and myristic acids), polyphenols (catechin, isocatechin, protocyanidin, flavonoids, tannins and proanthocyanidin monomerics), saponins, glucosides (-perseit,- α -manoheptit, D-monoheptulose, persiteol), sterols (β sitosterol, campesterol, stigmasterol, cholesterol), the amino acid carnitine and two glucosides of abscicic acid has been reported for *Persea americana* seeds [10,11]. Phytochemical screening of *Persea americana* leaves, seeds and stem bark showed the presence of tannins, steroids, alkaloids, phenols, flavonoids which have been reported to function as antimicrobial, anti-fungal, immune-stimulatory, cytotoxic, hepato-protective, hypolipidemic, antioxidant, antiviral, amongst others [8,12]. Methanolic and ethanolic extract from the leaves, stem bark and roots of *Persea americana* have been reported to demonstrate significant antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Salmonella aureus, Staphylococcus spp, Proteus spp* and *Klebsiella spp* [13,14].

Recent studies has shown that phyto-constituents in medicinal plants can positively influence the growth performance [15], immune response [16], intestinal microbial count [17], nutrient digestibility of animals [18]. However, there is a dearth of

reports on the dietary supplementation of *Persea americana* stem bark on the performance of rabbits. This research is timely because it will help to verify the efficacy of supplementing *Persea americana* stem bark in rabbits diets, boost food safety and livestock production.

2 MATERIALS AND METHODS

2.1 Experimental Location and Ethical Approval

This experiment was carried out at the Rabbitary section of Sumitra Research Institute, Gujarat located between 28° 20' N and 75° 30' East India between the months of Feburary to April, 2024. All experimental guidelines and procedures were approved by the ethics committee of Animal Production and Health at Sumitra Research Institute, Gujarat (GD/2024B/044). The study lasted for 60 days.

2.2 Preparation of Persea Americana Stem Bark Powder

Persea americana stem bark was harvested fresh within the premises of Sumitra Research Institute, Gujarat. It was collected from different strands of *Persea americana* tree and sent to the biological science department of the institute for proper identification by a certified taxonomist. Thereafter, it was assigned an identification number (ASD/008/2024F), air dried under shade for 18 days and grinded into powder with an electric blender and packed into polythene bag before it was sent to the Institute's laboratory for qualitative analysis of phytochemicals or phyto-constituents in the sample.

2.3 Reagents Required for Phytochemical Screening of Persea Americana Stem Bark Powder

Concentrated tetraoxosulphate (VI) acid, Iron (III) chloride, sodium hydroxide, iodine solution, chloroform, diluted ammonia, glacial acetic acid.

2.4 Qualitative Screening of Phytochemicals in Persea Americana Stem Bark Powder

Qualitative screening of phytochemicals in *Persea americana* stem bark powder was carried out according to the procedures described by Harborne [19].

3 EXPERIMENTAL TESTING

3.1 Test for Tannins

1 gram of *Persea americana* stem bark powder was added to 2 mL of Iron (III) chloride aqueous solution in a test tube and shaken for 1 minutes. A visual check for the development of a blue-green colour suggests the presence of tannins.

3.2 Test for Saponins

1 gram of *Persea americana* stem bark powder was mixed with two millilitres of distilled water and the mixture was shaken thoroughly for 30 seconds. The formation of foam suggested the presence of Saponins.

3.3 Test for Steroids

1 gram of *Persea americana* stem bark powder was mixed with 1 ml of chloroform in a test tube. Thereafter, 1 ml of concentrated tetraoxosulphate (VI) acid was added and mixed for 1 min. A visual check observing the development of a red colouration in the chloroform layer suggested the presence of steroids.

3.4 Test for Alkaloids

1 gram of *Persea americana* stem bark powder was mixed to 1 ml of 10 % potassium iodide followed by the addition of 2 drops of iodine solution and kept for 1 min. A visual check for the development of a red or brown colouration suggested the presence of alkaloids.

3.5 Test for Flavonoids

1 gram of *Persea americana* stem bark powder was mixed with 4 ml of diluted ammonia followed by the addition of 1 mL of concentrated tetraoxosulphate (VI) acid. A visual check for the development of a yellow colouration indicated the presence of flavonoids.

3.6 Test for Terpenoids

1 gram of *Persea americana* stem bark powder is mixed to 2 ml of concentrated tetraoxosulphate (VI) acid. The mixture was heated to 70° C for 2 min. A visual check for the development of a grey colouration suggested the presence of terpenoids.

3.7 Test for Cardiac Glycosides

1 gram of *Persea americana* stem bark powder was added to 4 ml of chloroform and 3 ml of glacial acetic acid followed by Iron (III) chloride and 1 ml of concentrated tetraoxosulphate (VI) acid. A visual check for the development of a brown colouration suggested the presence of cardiac glycosides.

3.8 Test for Quinones

1 gram of *Persea americana* stem bark powder was added to 3 mL sodium hydroxide in a test tube. The mixture was shaken for 1 minutes and a visual check observing the development of a red colouration suggested the presence of quinones.

4 MANAGEMENT OF EXPERIMENTAL ANIMALS AND DESIGN

A total of 5- weeks, 60 cross bred male rabbits were purchased from a commercial breeding farms in Gujarat. On arrival to the rabbitary unit of Sumitra Institute, Gujarat, rabbits were weighed individually and randomly distributed into 5 groups each with 20 rabbits. Each group was further sub-divided in to five groups each with 2 rabbits of comparable group weight. An already disinfected battery cage with dimension (350 cm by 250 cm) was placed in a cleaned semi-housed pens. Cages were equipped with manual feeder and nipple drinkers. Rabbits were quarantined for 14 days, treated with Ivermac tablet (against endo and ecto parasites) and fed basal diet (Table 1) which is adequate in all nutrient required by rabbits as recommended by NRC [20]. A completely randomized design method was adopted and rabbits in group 1 (G¹) was fed basal diet without *Persea americana* stem bark powder, G², G³, G⁴ and G⁵ received basal diet supplemented with Persea americana stem bark powder at 50 g, 100 g, 150 g and 200 g per kilogram diet respectively. Experiment lasted for sixty days and animals were fed trice daily 7:30 H, 12:00 H and 16:00 H and given unrestricted access to clean water. The formulated feeds (basal diet) were offered and the left over were collected and weighed using a digital scale to determine feed consumption of rabbits. After the initial weight, weekly weights were taken. These records were used to monitor and determine the growth performance parameters.

5 ESTIMATION ON GROWTH PERFORMANCE

Average body weight gain = average initial body weight - average final body weight Average daily body weight gain = Average body weight gain/60 days Average daily feed intake = average total feed intake/60 days Feed conversion ratio = Average daily feed intake/ average daily weight gain

6 BLOOD COLLECTION AND ANALYSIS

At the end of the 60th day of the study, six rabbits were randomly selected from each treatment for haematological studies. Blood was taken from their jugular vein with 2 mL hypodermic needle and syringe into a labeled sterile bottles containing anticoagulant. Bleeding was done in the morning and collected samples were placed in a flask with ice to preserve the sample before analysis. Haematological examination was carried out using Automated Sysmex blood analyzer (Model ASD/2208C, Netherlands). All analytical procedures were carried out according to the manufacturer's guidelines.

6.1 Analysis of Experimental Diet

Experimental diet was analyzed according to the methods outlined by Analysis of Analytical Chemist procedure [21].

6.2 Analysis of Data

Data collected on growth performance and haematology evaluation were subjected to one-way Analysis of Variance using Statistical Package for Social Sciences (version 25). The differences among the treatment means were determined (P<0.05) by Duncan multiple range test of the same statistical package.

7 EXPERIMENTAL RESULTS

Components	Experimental Diet Fed to Rabbit for 60 Days Period Content (%)				
Corn	55.00				
Rice bran	10.00				
Soya meal	25.00				
Fish meal	2.00				
Stone powder (Calcium carbonate)	2.00				
Bone meal	4.00				
Lysine	0.22				
Methionine	0.22				
*Premix	0.25				
Toxin binder (Agrobar)®	0.01				
Common salt	0.30				
Total	100.0				
Determined analysis					
Crude protein (%)	17.66				
Crude fibre (%)	13.04				
Crude fat (%)	4.41				
Calcium (%)	1.83				
Phosphorus (%)	0.57				
Metabolizable energy (MJ/kg)	11.20				

 Table 1 Gross Composition of Experimental Diet Fed to Rabbit for 60 Days Period

Qualitative phytochemical screening of *Persea americana* stem bark powder (Table 2) showed that flavonoids, steroids and phenols are the predominant compound in the sample followed by tannins, alkaloids, saponins and terpenoids. Quinones and glycosides were absent in the sample.

Compounds	Results			
Alkaloids	++			
Flavonoids	+++			
Saponins	++			
Steroids	++			
Phenols	+++			
Tannins	++			
Quinones	-			
Glycosides	-			
Terpenoids	++			

 Table 2 Qualitative Phytochemical Screening of Persea americana Stem Bark Powder

++ Moderately present; +++ highly present; -absent

As described in Table 3, average daily weight gain and average daily feed intake was increased (p<0.05) in *Persea americana* stem bark powder supplemented treatment compared with the control (G¹). The dietary supplementation of *Persea americana* stem bark powder decreased feed conversion ratio (p<0.01) compared with the control. Mortality in *Persea americana* stem bark powder supplemented group was 0 while 1.78 % was recorded in the control group. Average daily weight gain value varied from 18.45 to 22.69 g, average daily feed intake (90.06 - 100.2 g), feed conversion ratio (4.84 to 4.47) and percentage mortality (0.00 - 1.78 %).

Table 5 Effect of Persea	americana Stem	Dark Powder	on the Grown	n Periormance	of weahed R	addits
Variables	G^1	G ²	G ³	G ⁴	G ⁵	SEM
Number of animals	20.0	20.0	20.0	20.0	20.0	-
Duration of study	60.0	60.0	60.0	60.0	60.0	-
Average initial body weight (g)	663.8	665.1	664.8	665.2	664.2	0.42
Average final body weight (g)	1771.3 ^b	2007.5ª	2016.8ª	2023.7ª	2025.8ª	1.95
Average body weight gain (g)	1107.5 ^b	1342.4ª	1352.0ª	1358.5ª	1361.6ª	1.03
Average daily weight gain (g)	18.45 ^b	22.37ª	22.53ª	22.64ª	22.69ª	0.02
Average total feed intake (g)	5403.6 ^b	6000.7ª	6008.7ª	6009.1ª	6010.3ª	9.87
Average daily feed intake (g)	90.06 ^b	100.0ª	100.1ª	100.2ª	100.1ª	0.04
Feed conversion ratio	4.87ª	4.47 ^b	4.44 ^b	4.42 ^b	4.41 ^b	0.01
Mortality (%)	1.78ª	0	0	0	0	0.01
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Table 3 Effect of Persea americana Stem Bark Powder on the Growth Performance of Weaned Rabbits

Means in the same row with different superscript letters are significantly different (p<0.05); G¹ (basal diet without *Persea americana* stem bark powder); G² (basal diet with 50 g *Persea americana* stem bark powder per kg diet); G³ (basal diet with 100 g *Persea americana* stem bark powder per kg diet); G⁴ (basal diet with 150 g *Persea americana* stem bark powder per kg diet); G⁵ (basal diet with 200 g *Persea americana* stem bark powder per kg diet); G⁵ (basal diet with 200 g *Persea americana* stem bark powder per kg diet).

As described in Table 4, Haemoglobin, total erythrocytes, leucocytes, hematocrit and lymphocytes were enhanced (p<0.05) when *Persea americana* stem bark powder was supplemented in the diet of rabbits. Values obtained were higher (p<0.05) in G^2 , G^3 , G^4 and G^5 and lower in G^1 (control). Conversely, heterophil count were higher (p<0.05) in control (G1) relative to the other group. Hematocrit values varied from 25.67 to 31.91 %, total erythrocytes [(4.86 to 7.69 (×10¹²/L)], total leucocytes [(9.75 to 13.94 (×10¹²/L)], haemoglobin [(97.11 to 113.1 g/L)], lymphocytes [(8.89 to 12.92 (×10⁹/L)] and heterophils [(10.07 to 7.02 (×10⁹/L)].

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Variables	G^1	G^2	G ³	G^4	G^5	SEM
Haemoglobin (g/L)	97.11 ^b	106.8ª	110.7ª	111.5ª	113.1ª	0.08
Total erythrocytes (×10 ¹² /L)	4.86 ^b	7.11ª	7.32ª	7.58ª	7.69ª	0.01
Total leucocytes (×10 ⁹ /L)	9.75 ^b	12.34ª	13.08ª	13.11ª	13.94ª	0.02
Hematocrit (%)	25.67 ^b	30.74ª	31.54ª	31.85ª	31.91ª	0.02
Lymphocytes (×10 ⁹ /L)	8.89 ^b	12.11ª	12.56ª	12.71ª	12.92ª	0.03
Heterophils (×10 ⁹ /L)	10.07ª	8.51 ^b	7.22°	7.16°	7.02°	0.10

Table 4 Effect of Persea americana Stem Bark Powder on the Haematological Parameters of Weaned Rabbits

Means in the same row with different superscript letters are significantly different (p<0.05); G¹ (basal diet without *Persea americana* stem bark powder); G² (basal diet with 50 g *Persea americana* stem bark powder per kg diet); G³ (basal diet with 100 g *Persea americana* stem bark powder per kg diet); G⁴ (basal diet with 150 g *Persea americana* stem bark powder per kg diet); G⁵ (basal diet with 200 g *Persea americana* stem bark powder per kg diet); G⁵ (basal diet with 200 g *Persea americana* stem bark powder per kg diet).

8 DISCUSSION

The result obtained in this study confirms the outcome of an earlier studies by Arukwe et al. [9], who reported that samples of *Persea americana* seeds, leaves, fruits, roots and stem bark contains phenols, saponins, tannins, steroids, alkaloids and flavonoids as major phyto-constituents. The presence of these phytochemicals in *Persea americana* stem bark powder enables it to exhibit a wide spectrum of pharmacological activities such as, anti-inflammatory, antfungal, antimiccrobial, pain relief (anagelsics), anti-bacterial, anti-inflammation, immune-stimulatory, anti-ulcer, anti-diabetic, cytotoxic, hypolipidemic, amongst others [22,23]. Medicinal plants or herbs containing phytochemicals have been scientifically proven to be effective in treating various ailments like, skin infections, tooth ache, fever, stomach disorder, rheumatism, arthritis, sexually transmitted infections and many others [18,24,25]. Flavonoids and terpenoids are reported to exhibit anticancer, anti-inflammatory, anticonvulsant, cardio-protective and anti-ulcer activities [26,27]. Phenols are involved in antimicrobial activities and are capable of scavenging free radicals, thus preventing diseases or infection in the body [28,29]. Tannins have been reported to have antimycobacterial and anti-diarrhoea activity [3]. According to Ojediran et al. [28],

climate, location, harvest, stage, storage conditions, processing and extraction methods could influence the concentration of phyto-constituents in herbal plants. A synergy in the activities of these phyto-constituents presented in Table 2, could have a positive influence on growth, feed consumption, immune parameters amongst others [30,31].

Average weight gain were highest in rabbits fed diet supplemented with Persea americana stem bark powder relative to those in the control. This outcome suggests that Persea americana stem bark powder possess the ability to stimulate appetite, saliva secretion, intestinal mucus production, bile acid secretion, and activity of digestive enzymes such as trypsin and amylase to improve the digestibility of nutrients in the gut of rabbits [26,32]. Weight gain recorded in this study was similar to 1836.5 - 2076.33 g recorded for weaner rabbits fed diet supplemented with pawpaw leaf meal [33]. John recorded a lower average body weight of 1600.6 - 1887.1 g in rabbits fed diet supplemented with Clerodendron splendens leaf extract[27]. The variation in results could be attributed to concentrations of phyto-constituents in the sample as well as level of supplementation [34]. Similarly, average total feed intake were similar (p>0.05) among rabbits fed G² (50 g Persea americana stem bark powder per kg diet), G³ (100 g Persea americana stem bark powder per kg diet), G⁴ (150 g Persea americana stem bark powder per kg diet) and G⁵ (200 g Persea americana stem bark powder per kg diet) but significantly higher than those in G¹ (control). This result indicates that supplementation of Persea americana stem bark powder influenced the taste, texture, appearance and smell of feed making it appealing to rabbits. Average daily feed intake recorded in this study was similar to the range of 91.22 - 103.1 g reported by Adedire et al. [35] when rabbits were fed crop residues but lower than 68.14 - 75.06 g recorded by Adeyemo and Akanmu [36]; Alagbe et al. [37] who supplemented neem leaves in the diet of growing rabbits. Feed conversion ratio decreased as the level of Persea americana stem bark powder increased in the diet, this explains why Persea americana stem bark powder recorded a higher average total weight gain compared with the control. This result suggests that phytochemicals especially flavonoids, phenols and terpenoids exerts beneficial effects on gut morphology by increasing the proliferation of beneficial bacteria and inhibiting the activities of pathogens giving room for efficient nutrient utilization [2]. According to Muritala et al. [38], supplementation of phytogenics in the diet of animals suppressed the activities of Escherichia coli, Pseudomonas spp, Salmonella spp, Staphylococcus spp amongst others. This is the main reason why mortality was not recorded in groups supplemented with Persea americana stem bark powder. Result obtained in this study is in agreement with the findings of Servin and Coconnier [39]; Valenzuela-Grijalva et al. [40].

Haematological studies can be used in disease diagnosis and for therapeutic and feed stress monitoring [41,42]. The haemoglobin, total erythrocytes, total leucocytes and hematocrit values were within the normal ranges 90.00 - 150.0 g/L, [(4.00 - 8.00 (×10¹²/L)], [(7.00 - 16.00 (×10⁹/L)] and 25.00 - 36.00 % cited by Özkan et al. [43]; Singh et al. [44]. Haemoglobin, total erythrocytes, total leucocytes and hematocrit were higher in rabbits fed diet supplemented with *Persea americana* stem bark powder compared with the control. This result suggests that the absence of dehydration, chronic inflammation, bone marrow damage and vitamin deficiency in the blood [2,45]. Higher hematocrit values also indicates optimum carrying capacity of oxygen in the blood (Issac et al., 2013). Lymphocyte and heterophil values obtained in this study were within the baseline values [(6.88 - 14.00 (×10⁹/L)] and [(5.00 - 12.00 (×10⁹/L)] cited by Chineke et al. [46]; Abdel-Azeem et al. [47]. High lymphocytes is closely associated with increased antibody formation to fight against disease and other infection in the body [34,48]. Lower heterophil value was observed with rabbits with *Persea americana* stem bark powder which possess antioxidant properties to prevent against stress and other infections.

9 CONCLUSION

Persea americana stem bark powder, when supplemented in the diet of rabbits may potentially act in support of modulating growth performance. *Persea americana* stem bark powder at 200 g/kg have been shown to have the potential functionality to eliminate activities of pathogenic organisms and improve the proliferation of beneficial organisms in the gut, which may lead to lower stress and improved performance.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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