

DIETARY SUPPLEMENTATION OF OCOTEA ACIPHYLLA ESSENTIAL OIL: EFFECT ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF WEANED RABBITS

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Abstract: A 60-day experiment was carried out at Sumitra Research Institute in Gujarat to investigate the effect of dietary supplementation with *Ocotea aciphylla* essential oil on weaned rabbit growth performance and nutrient digestibility. The experiment used 50 weaned cross-bred male rabbits. The animals were acclimatized for two weeks and fed a basal diet that was adequate in all nutrients based on their requirements. Then, their body weights were equalized and randomly assigned to five treatments, each with ten rabbits. Every treatment has ten replicates, with one animal per replicate. Animals in treatment 1 were fed basal diet without *Ocotea aciphylla* oil, treatment 2, 3, 4 and 5 were fed same diet supplemented with *Ocotea aciphylla* oil at 0.1 mL, 0.2 mL, 0.3 mL and 0.4 mL/kg diet. The main chemicals found in *Ocotea aciphylla* oil were α -pinene (22.60%), α -cymene (12.41%), α -terpinene (10.45%), myrcene (7.53%), α -thujene (6.05%), and limonene (5.10%). The other chemicals obtained were less than 5.00%. The treatment had a significant ($P < 0.05$) effect on the average daily weight increase and feed intake values, which ranged from 19.86 to 26.40 g and 100.3 to 106.9 g, respectively. Dietary supplements improved FCR compared to control, with just 1.20% mortality among rabbits on diet 1 ($P < 0.05$). The nutrient digestibility of dry matter (68.14 - 77.81%), crude protein (59.07 - 68.19%), crude fiber (35.62 - 40.41%), ether extract (47.35 - 50.73%), and nitrogen free extracts (49.18 - 59.94%) varied considerably ($P < 0.05$) across treatments. Finally, *Ocotea aciphylla* essential oil can be added to the food of weaned rabbits at a dose of up to 0.4 mL/kg without impairing animal performance.

Keywords: *Ocotea aciphylla*; Essential oil; Rabbits; Phyto-constituents; Performance; Nutrients

1 INTRODUCTION

The European Union and other advanced countries imposed an untimely ban on the use of antibiotics in animal nutrition in 2006, citing the continuous rise in cases of antimicrobial resistance caused by continuous sub-therapeutic supplementation of antibiotic growth promoters in livestock feeding; Jan and Esther [1]. As a result, alternative feed additives are gaining popularity among scientists and feed makers. Essential oils derived from medicinal plants are one of the viable options because they are effective, environmentally friendly, and require no withdrawal period [2,3]. Essential oils contain an infinite number of bioactive substances with various pharmacological characteristics [4,5]. It has antimicrobial, antifungal, immune-stimulatory, antioxidant, hepato-protective, antibacterial, antiviral, hypolipidemic, cytotoxic, anti-cancer, anti-diuretic, and anti-inflammatory activities, among others [6,7,8].

Ocotea aciphylla is a medicinal plant from the Lauraceae family. It is widely spread in South America, specifically Brazil, Columbia, Peru, Ecuador, Venezuela, South Africa, Zimbabwe, Nigeria, and various parts of Asia, notably India [9]. The tree can reach a height of 35 meters and produces little hermaphrodite flowers that are 3 to 4 mm long [10]. The plant has been shown to contain a variety of bioactive compounds, including terpenoids, flavonoids, phenols, and alkaloids, which can inhibit the activities of pathogenic organisms such as *Escherichia coli*, *Pseudomonas spp.*, *Salmonella spp.*, and *Aspergillus spp.* [11]. In traditional medicine, it has been utilized for the treatment of a range of illnesses, including ulcers, dermatitis, bacterial infections, liver disorders such as hepatitis, jaundice, and cirrhosis, intestinal parasite elimination, skin disease, malaria, and gastrointestinal disease [12,13].

Previous research has demonstrated that essential oils have a good impact on rabbit growth outcomes [14,15], nutrient utilization [16,17], blood parameters [18, 19]. However, the findings obtained are not consistent due to factors such as storage conditions, species, plant age, processing method used, and geographical location, among others [20]. Essential oils contain biologically active compounds that have antibacterial effects [21]. It is worth investigating the synergistic effect of

these substances on animal performance. This discovery will help to increase global tendencies to minimize antibiotics and alleviate growing concerns about bird health and safeguarding the environment.

2 MATERIALS AND METHODS

2.1 Research Area

The experiment was conducted from April to June 2022 at the Sumitra Research Institute's Rabbit department in Gujarat, India. The institute is situated between 23° 13' North and 72° 41' East.

2.2 Collection and Extraction of Essential Oils from *Ocotea aciphylla* Leaves

Fresh leaves of *Ocotea aciphylla* were harvested from Orathur village in Kancheepuram district, India, and delivered to Sumitra Research Institute's taxonomy department for identification and verification, where they were assigned the voucher number ED/2022A/08. The leaves were cleaned, air dried for 6 days to preserve the plant's medicinal components, then cut. The essential oil from the cut leaves was extracted using the steam distillation method previously reported by Alagbe JO [14]. In brief, 200g of chopped *Ocotea aciphylla* leaves were placed in a round bottom flask with 500 mL water and heated to 70 °C for 15 minutes. The steam generated passes through the condenser and allowed to cool. *Ocotea aciphylla* is collected via a calibrated receiver and transported to the laboratory for further investigation.

2.3 Management of Experimental Animal and Design

This study was carried out in accordance with animal protocol requirements approved by the Research and Ethics Committee of the Department of Animal Nutrition and Biochemistry at Sumitra Research Institute in Gujarat, India. A total of 50 cross-bred male rabbits (Flemish giant x Dutch) with an initial body weight of 516.2 ± 0.22 kg were weaned at 35 days. The rabbits were fed a basal diet that was appropriate in all nutrients required for rabbits [14]. During the fourteen-day acclimatization phase, the animals were treated against parasites with Ivercare® tablets (1 tab per 10 kg body weight). Following the adjustment phase, rabbit weights were equalized, housed singly, and assigned to five treatments with ten repetitions of one animal each in a completely randomized design. The experimental cage, measuring 1 m × 1.5 m × 1.0 meters (length × breadth × height), has manual concrete feeders and drinks. Animals had full access to food and clean water, and the trial lasted 60 days under careful management. Animals in treatment one was fed basal diet without *Ocotea aciphylla* oil (control); treatments two, three, four, and five are the same diet supplemented with *Ocotea aciphylla* oil at 0.1, 0.2, 0.3, and 0.4 mL per kg, respectively. Rabbits were fed three times a day between the hours of 7:00 H, 12:00 H, and 16:00 H.

3 ASSESSMENTS

Feed consumption was calculated by subtracting the leftover from what was supplied to the animals (expressed in grams). Average daily feed intake = total feed intake divided by the number of days in the trial. Average daily weight growth equals total weight gain divided by the number of days in the experiment. Feed conversion ratio is total feed divided by weight growth.

3.1 Nutrient Digestibility Assessment

At the end of the trial, five rabbits were randomly chosen from each treatment and placed in a metabolic cage. The trial lasted seven days, including two days of acclimatization and five days of fecal collection. Each rabbit received a specified amount of feed mixed with chromium oxide every day for five days. Fecal droppings were collected daily and dried in an oven at 70°C for 24 hours before being delivered to the laboratory for analysis and evaluation.

3.2 Bioactive Profiling of Phyto-Constituents by Gc/MS

The laboratory procedures recently published by Alagbe JO [14] were used for the determination of phyto-constituents in *Ocotea aciphylla* oil. Quantification of bioactive compounds was carried out using GC-MS 6800 gas chromatography and mass spectrometer with electronic pressure flow. 2 mL of oil sample is injected into the machine and adjusted to an inlet temperature of 450°C, heating rate (up to 120°C), pressure range 100 psi while the mass spectrometer unit was maintained at a filament emission current 0-350 µA, ionization temperature (100 – 350 °C), stability ± 0.10 amu/48 hours and scan rate of 10,000 amu/seconds.

3.3 Proximate Analysis of Experimental Diet

Analysis of experimental diet was carried out using Foss near infra-red automated feed analyzer NIRS™ DS2600 with reflectance or transmittance. The machine has a technical specifications of wavelength range of 400 – 2500 nm, optical bandwidth (8.75 ± 0.1 nm), spectral resolution (0.5 nm), wavelength accuracy (< 0.05 nm) and absorbance range up to 2 AU.

4 STATISTICAL ANALYSIS

Data obtained were analyzed by one-way analysis of variance using SPSS statistical software (Version 25.0 for Windows, SPSS, Inc., Chicago, IL, USA). Differences between treatment means was separated using Duncan's test. Significant differences were declared at $P < 0.05$.

Table 1 Ingredient and Chemical Composition of Basal Diet

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Yellow corn	40.0	40.0	40.0	40.0	40.0
Rice bran	20.0	20.0	20.0	20.0	20.0
Soya bean meal	23.0	23.0	23.0	23.0	23.0
Groundnut meal	11.0	11.0	11.0	11.0	11.0
Limestone	2.00	2.00	2.00	2.00	2.00
Di-calcium phosphate	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15	0.15
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40	0.40
<i>Ocotea aciphylla</i> oil	0.00	0.10	0.20	0.30	0.40
Total	100.0	100.0	100.0	100.0	100.0
Determined analysis					
Metabolisable energy (kcal/kg)	2601.8	2601.8	2601.8	2601.8	2601.8
Crude protein	16.21	16.21	16.21	16.21	16.21
Crude fibre	13.44	13.44	13.44	13.44	13.44
Ether extract	6.09	6.09	6.09	6.09	6.09
Calcium	1.46	1.46	1.46	1.46	1.46
Phosphorus	0.65	0.65	0.65	0.65	0.65

2.5 kg Mineral/Vitamins contains: Retinol 10 000 000 IU, Cholecalciferol 3 000 000 UI, Tocopherol 2500 IU, Phylloquinon 4000 mg, Thiamin 5000 mg, Riboflavin 500 mg, Pyridoxin 2500 mg, Cyanocobalamin 5 mg, Folic acid 10 000 mg, Niacin 2000 mg, Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100mg

Bioactive compounds identified in *Ocotea aciphylla* oil by GC-MS is presented in Table 2. Results revealed the presence of 33 bioactive compounds with their percentage peak areas. The most prominent compounds were, α -pinene (22.60 %), α -cymene (12.41 %), α -terpinene (10.45 %), myrcene (7.53 %), α -thujene (6.05 %) and limonene (5.10 %) other compounds obtained were less than 5.00 %. The result obtained in this study is in agreement with the reports of Chaverri C and Ciccio JF[22]. However, on the contrary Adrian AM, et al.[23] recorded a lower α -pinene, α -terpinene and concentrations of 21.2 % and 5.20 % respectively. These variations can be linked to differences in species, geographical location, age of plant, harvesting and processing method [24]. Bioactive compounds recorded in Table 2, possesses several pharmacological properties viz; antimicrobial, antifungal, gastro-protective, immune-stimulatory, hepato-protective, anti-inflammatory, antioxidant, cytotoxic, antiviral, hypolipidemic amongst others [5,6]. For instance, α -pinene, limonene and α -terpinene are reported to have antimicrobial activity, antioxidant, cytotoxic activity, anti-mutagenic and anti-carcinogenic and anti-inflammatory activities [25]. α -springene, thymol, eugenol, α -hexyl salicylate, β -selinene, α -cedrene, α -copaene and caryophyllene have been recognized to possess antidiabetic [26] antioxidant [27], antimicrobial [28], anti-inflammatory [29], anti-allergic and anti-carcinogenic activities [30].

Table 2 Bioactive Compounds Identified in *Ocotea Aciphylla* Oil by GC-MS

Chemical components	Reaction time (Minutes)	% peak area
Myrcene	6.55	7.53
Linalool	7.10	2.16
Spathulenol	8.55	0.64
Camphene	8.91	1.17
Bisabolene	9.17	1.00
α -Pinene	9.44	22.6
α -Terpinene	9.61	10.45
Limonene	10.62	5.10
α -Hexyl salicylate	11.22	0.01
α -Springene	13.61	1.46
Geranyl acetate	13.73	0.08
Benzyl ether	14.05	0.02
Isooctanol	14.22	1.44
Di-butyl phthalate	14.95	0.03
Thymol	15.69	4.21
Carvacrol	17.63	3.03
Eugenol	18.08	2.18
β -Selinene	18.21	0.40
α -Cedrene	18.46	0.22
α -Copaene	20.04	0.85
Caryophyllene	22.15	0.70
α -Terpineol	22.70	0.04
Terpinen-4-ol	22.93	1.82
Camphor	23.17	2.93
α -Cymene	24.00	12.41
Limonene	24.86	3.10
α -Thujene	24.91	6.05
1-Nonene, 4,6,8 –trimethyl	26.03	0.06
1,14-Tetradecanediol	27.10	2.05
Hexanedioic acid	28.54	1.41
2-Bromonane	28.96	0.02
1-Hexanol, 2-ethyl -2-propyl	29.52	0.14

Table 3 shows the effect of *Ocotea aciphylla* oil on rabbit growth performance after weaning. Diet 5 (0.4 mL *Ocotea aciphylla* oil/kg diet) had the greatest final body weight (1710.5 - 2100.8 g), weight gain (1192.1 - 1583.8 g), and average daily weight gain (19.86 - 26.40 g) ($P < 0.05$), followed by diets 2 (0.1 mL *Ocotea aciphylla* oil/kg diet), 3 (0.2 mL *Ocotea aciphylla* oil/kg diet), 4 (0.3 mL *Ocotea aciphylla* oil/kg diet), and 1 (without *Ocotea aciphylla* oil). In contrast, rabbits fed *Ocotea aciphylla* oil had higher total feed intake (6022.6 - 6418.8 g) and average daily feed intake (100.3 - 106.9 g) compared to diet 1 ($P < 0.05$). Higher weight gain recorded in diet 5 suggests that supplementing *Ocotea aciphylla* oil at 0.4 mL/kg diet was capable of increasing pancreatic enzyme production and bile secretion in the intestinal tract compared to the other groups. Though rabbits fed diet 1 recorded the lowest body weight compared to the other groups suggesting that an important part of the nutritional action of *Ocotea aciphylla* oil is the stimulation of bile mucus and digestive enzymes [31, 32]. The result obtained is in agreement with the reports of Ayodele SO, et al., and Oloruntola, OD, et al. [33,34] when phytochemicals were supplemented in the diets of weaned rabbits. Feed intake significantly ($P < 0.05$) increase among rabbits fed *Ocotea aciphylla* oil relative to the other group. This result suggests that supplementing *Ocotea aciphylla* oil in the diets of rabbits influences the flavor thus improving palatability and feed conversion ratio. This is in concordance with the reports of

Oloruntola OD, et al. [35] who observed a significant difference in weaned rabbits fed *Gliricidia* leaf meal. Mortality was recorded only among rabbits fed diet 1 (1.20 %), this result indicates that *Ocotea aciphylla* oil contains bioactive compounds capable of creating a balance in microbial gut flora thus preventing infections with pathogens via secretion of antimicrobial substances [36,37].

Table 3 Effect of *Ocotea Aciphylla* Oil on the Growth Performance of Weaned Rabbits

Variables	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Initial body weight (g)	518.4	516.2	517.1	516.4	517	0.03
Final body weight (g)	1710.5 ^c	1812.3 ^b	1811.9 ^b	1810.7 ^b	2100.8 ^a	18.42
Weight gain (g)	1192.1 ^c	1296.1 ^b	1294.8 ^b	1294.3 ^b	1583.8 ^a	13.45
Average daily weight gain (g)	19.86 ^c	21.60 ^b	21.58 ^b	21.57 ^b	26.40 ^a	0.02
Total feed intake (g)	6022.6 ^b	6400.9 ^a	6400.6 ^a	6415.2 ^a	6418.8 ^a	47.22
Average daily weight intake (g)	100.3 ^b	106.6 ^a	106.6 ^a	106.9 ^a	106.9 ^a	0.09
Feed conversion ratio	5.05 ^a	4.94 ^b	4.94 ^b	4.95 ^b	4.05 ^c	0.03
Mortality (%)	1.20 ^a					0.01

Means within a row with different letters are significantly different ($P < 0.05$); diet 1: basal diet without *Ocotea aciphylla* oil (control); diet 2: basal diet with 0.1 mL *Ocotea aciphylla* oil/kg; diet 3: basal diet with 0.2 mL *Ocotea aciphylla* oil/kg; diet 4: basal diet with 0.3 mL *Ocotea aciphylla* oil/kg; diet 5: basal diet with 0.4 mL *Ocotea aciphylla* oil/kg; diet 5: basal diet with 0.5 mL *Ocotea aciphylla* oil/kg; SEM: standard error of mean

Effect of *Ocotea aciphylla* oil on nutrient digestibility of weaned rabbit is displayed in Table 4. Dry matter digestibility value ranged from 68.14 – 80.91 %, crude protein (59.07 – 70.92 %), crude fibre (35.62 – 48.77 %), ether extract (47.35 – 55.82 %) and nitrogen free extract (49.14 – 69.12 %). Rabbits fed diet 5 had the highest dry matter, crude protein, crude fiber, ether extract, and nitrogen free extract values, followed by diets 2, 3, and 4, and lowest by diet 1 ($P < 0.05$). The results revealed that rabbits fed *Ocotea aciphylla* oil had improved nutrient utilization than rabbits on diet 1. Furthermore, the antimicrobial properties of *Ocotea aciphylla* oil due to the presence of their bioactive compounds (Table 2) lowers microbial multiplication in the intestine and reduce the competition of the microflora with the host for nutrients [35,38]. Results obtained in this study is in concordance with the reports of Abd El-Hady, AM[38]. However, on the contrary Celia C, et al. and Alagbe OJ, et al.[39,40] observed a non-significant difference ($P > 0.05$) in weaned rabbits fed diet supplemented with digestarom herbal formulation. These discrepancies can be attributed to differences in the origin of essential oil and the inclusions level in the diet of animals [40,41].

Table 4 Effect of *Ocotea Aciphylla* Oil on Nutrient Digestibility of Weaned Rabbit

Parameters (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Dry matter	68.14 ^c	76.05 ^b	76.12 ^b	77.81 ^b	80.91 ^a	2.16
Crude protein	59.07 ^c	68.02 ^b	68.11 ^b	68.19 ^b	70.92 ^a	1.61
Crude fibre	35.62 ^c	40.08 ^b	40.27 ^b	40.41 ^b	48.77 ^a	0.09
Ether extract	47.35 ^c	50.61 ^b	50.67 ^b	50.73 ^b	55.82 ^a	1.21
Nitrogen free extract	49.14 ^c	58.80 ^b	58.85 ^b	59.94 ^b	69.12 ^a	1.25

Means within a row with different letters are significantly different ($P < 0.05$); diet 1: basal diet without *Ocotea aciphylla* oil (control); diet 2: basal diet with 0.1 mL *Ocotea aciphylla* oil/kg; diet 3: basal diet with 0.2 mL *Ocotea aciphylla* oil/kg; diet 4: basal diet with 0.3 mL *Ocotea aciphylla* oil/kg; diet 5: basal diet with 0.4 mL *Ocotea aciphylla* oil/kg; diet 5: basal diet with 0.5 mL *Ocotea aciphylla* oil/kg; SEM: standard error of mean

5 CONCLUSION

In conclusion, *Ocotea aciphylla* oil is abundant in numerous phyto-constituents or bioactive compounds with medicinal properties. Most of these bioactive compounds are generally regarded as safe, eco-friendly and has no withdrawal period. The outcome of this study showed dietary supplementation of *Ocotea aciphylla* oil up to 0.4 mL per kg diet improves the weight gain and feed conversion ratio of rabbits without compromising their health status, the antimicrobial properties in *Ocotea aciphylla* oil was also able to prevent dysbiosis and accelerates nutrient utilization.

COMPETING INTERESTS

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

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