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Investigating the effect of dietary supplementation of Ficus exasperata oil on the growth performance, apparent digestibility and nitrogen utilization of weaner rabbits

Abstract

This research was carried out to investigate the effect of dietary supplementation of Ficus exasperata oil on the growth performance, apparent digestibility and nitrogen utilization of weaner rabbits. Forty healthy weaned male rabbits of mixed breed with a mean body weight of 507 g (± 1.80 g) and an average age of 4 months were randomly assigned into four treatments (n=10 rabbits/treatment). Experimental treatments included: 1- control diet (without essential oil), 2- basal diet supplemented with 250 mg Ficus exasperata oil kg DM per day, 3- basal diet supplemented with 500 mg Ficus exasperata oil kg DM per day and 4- basal diet supplemented with 750 mg Ficus exasperata oil/ kg DM daily. Basal diet was compounded to meet the standard nutritional requirements for rabbits according to NRC recommendation. The experimental period was 60 days excluding 14 days' acclimatization period. A completely randomized design was adopted and animals received fresh water ad libitum. Ficus exasperata oil supplementation significantly increased ($p<0.05$) average daily weight gain, average daily feed intake as well as feed conversion ratio. Similarly, dietary supplementation of Ficus exasperata oil enhanced ($p< 0.05$) the apparent digestibilities of dry matter, organic matter, ether extract, crude protein and crude fibre. Ficus exasperata oil supplementation decreased faecal and urinary nitrogen and increased the concentrations ($p < 0.05$) of nitrogen intake and nitrogen retention. In conclusion, Ficus exasperata oil dietary supplementation at 750 mg/kg DM daily improved growth performance, nutrient digestibility and nitrogen retention of weaner rabbits without causing any deleterious effect on their health status.

Keywords

Ficus exasperata oil, growth, nutrient retention, nutrient utilization, rabbits

Research Article

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Introduction

Antibiotic residues in animal products are considered as potential health hazard for human consumption due to developing multi-drug resistance [1, 15]. In recent years, the use of essential oils from medicinal plants has emerged as one of the most sustainable strategy to enhance animal performance particularly in reducing the use of antibiotics [2]. This is because plant extracts are generally regarded as safe, effective, do not produce environmental pollution and has no withdrawal period [15, 22]. Herbs like Ficus exasperate have several potentials and could be used as alternative to antibiotics due to their abundant phyto-components [3].

Ficus exasperate commonly known as sand paper fig is a deciduous small plant belonging to the family Moraceae and order Urticales [3]. The plant is widely distributed in Africa, Australia, America and some parts of Asia including India [4]. Phytochemical studies on the various parts of the

plant have revealed the presence of flavonoids, saponins, tannins, phenolics, glycosides, saponins, carbonyl compounds and alcohols [4]. In recent work [25], Ficus exasperate essential oil have been found to contained; α-Pinene (2.2 %), p-Cymene (11.4 %), 1, 8-cineole (13.8 %), β-Caryophyllene (3.80 %), α-Ionone (2.90 %), Neryl acetone (4.2 %), β-Ionone (7.50 %), Caryophyllene oxide (5.4%), 6,10,14-Trimethyl-2-pentadecanone (7.00 %), 9-Octadecenoic acid (2.90 %), Cyclooctasulfur (6.30 %) and phytol (13.70 %). These compounds have numerous pharmacological activities such as, anti-inflammatory, antioxidant, anti-tumor, antidiabetic, hypolipidemic, hypoglycemic, anti-arthritic, anticonvulsant, immuno-modulatory, gastro-protective, antiplatelet, cytotoxicity, angiogenic, antineoplasic, antiviral, sedative, muscle relaxant, cytotoxic, antiviral, insecticidal, cardiotonic, analgesic, inhibition of lipid peroxidation, fungicidal, antiprotozoal, antimalarial, and anti-rheumatic, antifertility, antinociceptive, amongst others [6,8].

Aqueous and ethanolic extract from the leaves, stem and roots of Ficus exasperate have been reported to inhibit the activities of Escherichia coli, Staphyllococcus spp, Salmonella spp, Klebsiellia spp, Aspergillus niger, Candida albicans, Penicillium notatum and Rhizopus stolon [7,9]. It has also been used to traditionally treat fever, catarrh, allergic eruptions, somniferous, nervousness, cough, diabetes, asthma, bronchitis, prostrate disease, tumor, gastro-intestinal disorder, skin infections, snake bite amongst others [10].

It has been well-documented [22] that dietary inclusion of essential oils could improve rabbit performance by increasing digestive enzyme secretion, lowering the number of harmful bacteria in the digestive tract, modulating intestinal morphology functions, and positively affecting productivity, blood metabolites, and immunological and antioxidant status of animals. For instance, extracts from herbs such as Rauvolfia Vomitoria extract at 3 mL per litre has been demonstrated to promote growth and scavenge the activities of free radicals due to the active ingredients (phenols, tannins, alkaloids, saponins and flavonoids) of these herbs possessing antioxidants effect [23]. However, there is little or no information on the dietary supplementation of Ficus exasperate on the performance of rabbits. Evaluating its effect on rabbits will help to establish an optimum level, promote sustainability and help to address the increasing cases of antimicrobial resistance.

Materials and methods

Study Area

The study was carried out at the Rabbit section, Gandhi College of Agriculture located at Rajasthan, India. Experiment animals were taken care of according to the Indian Council guidelines of Animal Care. Laboratory analysis on the chemical composition of experimental diet, fecal droppings and chemical composition of Ficus exasperata oil was carried out at Sumitra Research Institute, Gujarat India using standard procedures according to Association of Analytical Chemist [11].

Ficus exasperata oil and Gas Chromato graphy- Mass Spectrometry analysis

Ficus exasperata oil was sourced from Singh Unique Company in Rajasthan, India. The oil was extraction by hydrodistillation method according to the package insert. 200 mL of purchased Ficus exasperata oil was sent to the Biochemistry Department, Sumitra Research Institute in Gujarat for proper identification of their bioactive components by Gas Chromatography – Mass Spectrometry (GC-MS). GC-MS analysis was done using Thermo Specific TSQ Quadrupole GC-MS system (Belgium). The kit was equipped with fused silica capillary column (35 m x 0.30 mm, film thickness 0.20 µm). Analytical conditions of the GC-unit were: Oven temperature: 65 □, with 2 minutes' initial hold, and then to 250 □ at 6 □/min, with final hold time of 10 minutes; helium was used as carrier gas at a flow rate of 1 mL/min. The MS chamber was maintained at a EI mode with ionization voltage 70 eV and ion source temperature, 220 □ to maintain precision in results. Percentage composition of each constituent was calculated by integration of the GC peak areas. Retention indices were determined with reference to a homologous series of normal alkanes analyzed under the same conditions.

Rabbits and management

Forty healthy weaned male rabbits of mixed breed with a

mean body weight of 507 g (± 1.80 g) and an average age of 4 months were purchased from a reputable breeding farm in Rajasthan, India. Animals were reared in a specially constructed battery cage which was properly disinfected two weeks before the commencement of the study. On arrival, rabbits were quarantined for 14 days and dewormed with Worm cap (Albendazole 2.5 % Amit Pharmaceutical Company, Gujarat) and fed basal diet which meet the standard nutritional requirements for rabbits according to NRC recommendation [12]. After the acclimatization period, rabbits were weighed and randomly allocated to four dietary treatments based on live weights (n=10 rabbits/ treatment). Experimental treatments included: 1- control diet (without essential oil), 2- basal diet supplemented with 250 mg Ficus exasperata oil kg DM per day, 3- basal diet supplemented with 500 mg Ficus exasperata oil kg DM per day and 4- basal diet supplemented with 750 mg Ficus exasperata oil/ kg DM per day. Animals were fed thrice daily and offered equal amount of feed at 7:30, 12:30 and 17:30. To ensure that animals take a full dose of Ficus exasperata oil, supplement, the oil was mixed in 200 g total diet DM to each rabbits before the first feeding. Fresh water was supplied unrestricted throughout the period of the experiment. A completely randomized design was adopted and the duration of the experiment is 60 days. Composition of experimental diet and chemical composition of Ficus exasperata oil is presented in Table 1 and Table 2 respectively.

Animals were weighed weekly during the experimental period using digital sensitive scale, feed intake was estimated as the difference between the feed offered and refusal from the previous day feeding. Average body weight gain was calculated as the difference between the average initial body weight and the average final body weight. Average daily weight gain and average daily feed intake was calculated as body weight gain and total feed intake divided by the experimental period respectively. Feed conversion ratio was calculated by dividing average daily feed intake by average daily weight gain.

Nutrient apparent digestibility and nitrogen utilization trial

At the end of the experiment, five rabbits were randomly selected per treatment for the seven days' digestibility trial. Animals were kept in a metabolic cage (to separate their fecal dropping from urine) and allowed to acclimatize for two days while the remaining five days is for data collection. Internal indigestibility marker was mixed with feed supplied to each animals, fecal droppings and urine from each animal were collected separately every morning before feeding and were weighed and recorded daily for five days. Each day's total faecal output was weighed and about 10% of it was saved, oven dried at 60□ for 48 hours and coefficients of apparent digestion was calculated. The urine output also measured daily for each rabbit and kept in an airtight sample bottle containing a few drops of concentrated H2SO4 used to prevent volatilization loss of nitrogen. About 10% of the daily urinary output was saved over the 5 days' collection period for each rabbit. Proximate composition of faecal droppings and experimental diet was carried out according to the procedure outlined by [13]

Apparent nutrient digestibility (%) =

$$\frac{\text{Nutrient intake (DM) - Nutrient in faeces (DM)}}{\text{Nutrient Intake (DM)}} \times 100$$

Nitrogen utilisation was estimated as:

N absorbed = Nitrogen intake - Faecal N

N balance or retained (%) =

Nitrogen intake – faecal N – urinary N

$$\text{N retention (as the \% on N absorbed)} = \frac{\text{N absorbed} \times 100}{\text{N intake}}$$

N retention (expressed as the % of N intake) =

$$\frac{\text{N retained} \times 100}{\text{N intake}}$$

Concentrations (% DM) of organic matter (OM) = 100 – ash

Statistical Analysis

All data collected were subjected to a one-way analysis of variance (ANOVA). Means showing significant differences were separated using Duncan's Multiple Range Test. The SPSS (version 21) statistical package was used for all statistical analysis.

S/N	Compounds	Retention time (min)	Percentage Area
1	β-caryophyllene	6.18	12.95
2	δ-cadinene	6.57	9.35
3	hexadecanoic acid	7.01	15.32
4	β-Linalol	7.22	1.46
5	Methyl Nonanoate	7.69	0.6
6	Cis-7-Decen-1-ol	8.08	1.73
7	m-Xylene	8.55	8.75
8	D-Limonene	9.08	0.81
9	4,6-Dimethyldodecane	9.26	0.17
10	Cyclododecyl Ethanone	9.71	0.33
11	Cis-9-Tetradecenol	10.04	0.49
12	Cis-9,17-Octadecadiene	10.63	0.61
13	Squalene	10.92	1.33
14	α-Terpinolene	11.36	1.78
15	Trans-3(10)-Caren-2-ol	11.55	0.12
16	1-Octen-3-ol	12.09	0.04
17	Bicyclo [10,6,0]Octade-ca-1(12),15- diene	12.56	0.11
18	α-Bergamotene	12.79	2.57
19	Phytol	13.02	27.07
20	Isopropyl Myristate	13.86	0.66
21	Humulane-1,6-dien-3-ol	14.05	1.24
22	Epiglobulol	14.65	0.02
23	α-Glyceryl Linolenate	14.87	0.71
	Total	-	88.22
	Number of compounds	-	23
	Monoterpenes	-	1.85
	Sesquiterpenes	-	59.20
	Diterpenes	-	13.76
	Triterpenes	-	3.91
	Non-terpenes	-	9.50

Table 1. Compounds identified in Ficus exasperata oil by GC-MS

Components	Quantity (% DM)
Maize (9.0 % CP)	30.00
Corn barn (11% CP)	20.00
Palm kernel meal	20.00
Groundnut cake (39 % CP)	13.50
Soya bean meal (48 % CP)	12.00
Bone meal	
Mineral/Vitamin Premix	2.00
Bone meal	4.00
Limestone	2.00
Salt	1.00
Total	100.0
Chemical composition (% DM)	
Dry matter	92.00
Organic matter	92.50
Crude protein	14.70
Ether extract	3.80
Ash	7.50
Energy (Kcal/kg)	2691.8
2.5 kg Growers premix contained: 15,000 I.U. vitamin A; 8000 mg vitamin B1; 3000 I.U. vitamin D3; 60.0 mg vitamin E; 15 mg Choline; 0.96 mg Cobalt; 2.00 mg I; 50 manganese Mn; 0.50 mg Selenium; 250 mg.	

Table 2. Ingredient and chemical composition of basal diet (% DM)

Variables	T1	T2	T3	T4	SEM
Average initial body weight (g/rabbit)	508.7	507.6	507.0	506.9	0.08
Average final body weight (g/rabbit)	1997.8c	2200.6b	2298.1b	2355.8a	41.29
Average body weight gain (g/rabbit)	1489.1c	1693.0b	1791.1b	1848.9a	20.85
Average daily weight gain (g/rabbit)	24.81c	28.22b	29.85b	39.26a	0.06
Average total feed intake (g/rabbit)	6200.1c	6500.3b	6511.1b	6800.2a	95.07
Average daily feed intake (g/rabbit)	103.34c	108.34b	108.51b	113.3a	1.22
Feed conversion ratio	4.20a	4.00b	4.00b	3.10c	0.02
a,b,c: Means within a row with different superscripts are significantly different ($p<0.05$); T1: basal diet without oil; T2 T3 and T4: basal diet with supplemented with 250 mg, 500 mg and 750 mg Ficus exasperata oil per kg DM feed daily					

Table 3. Growth performance of weaned rabbits fed diet supplemented with Ficus exasperata oil

Variables (%)	T1	T2	T3	T4	SEM
Dry matter	73.66c	80.92b	81.40b	86.95a	0.19
Organic matter	79.43c	81.87b	81.92b	85.67a	0.10
Crude protein	68.40c	72.15b	72.55b	78.94a	0.07
Crude fibre	59.95b	63.74a	65.61a	67.09a	0.05
Ether extract	48.17c	55.91b	59.08b	60.80a	0.02
a,b,c: Means within a row with different superscripts are significantly different ($p<0.05$); T1: basal diet without oil; T2 T3 and T4: basal diet with supplemented with 250 mg, 500 mg and 750 mg Ficus exasperata oil per kg DM feed daily					

Table 4. Apparent digestibility of weaned rabbits fed diet supplemented with Ficus exasperata oil

Variables (%)	T1	T2	T3	T4	SEM
Nitrogen Intake	9.55c	12.76b	12.85b	15.77a	0.02
Faecal Nitrogen	6.90a	4.05b	3.96b	2.95c	0.01
Urinary Nitrogen	2.59a	1.28b	1.21b	1.05c	0.01
Nitrogen retention	43.89c	50.02b	50.10b	56.17a	0.09
a,b,c: Means within a row with different superscripts are significantly different ($p<0.05$); T1: basal diet without oil; T2 T3 and T4: basal diet with supplemented with 250 mg, 500 mg and 750 mg Ficus exasperata oil per kg DM feed daily					

Table 5. Apparent digestibility of weaned rabbits fed diet supplemented with Ficus exasperata oil

Results and Discussion

In Table 1 compounds identified in Ficus exasperata oil by GC-MS reveals the prevalence of phytol (27.07 %), hexadecanoic acid (15.32 %), β-caryophyllene (12.95 %), δ-cadinene (9.32 %), xylene (8.75 %) as major bioactive compounds. Methyl Nonanoate (0.61 %), D-Limonene (0.81 %), Cis-9-Tetradecenol (0.49 %), 4,6-Dimethyldodecane (0.17 %), 1-Octen-3-ol (0.04 %) and Bicyclo [10,6,0] Octadeca-1(12),15- diene (0.11 %) amongst others were part of the minor compounds. Furthermore, on analysis of the class of compounds present, the essential oils from Ficus exasperata leaf were dominated by sesquiterpenes (59.20 %) followed by diterpenes (13.76 %), non-terpenes (9.50 %), triterpenes (3.91 %) and monoterpenes (1.85 %). All these compounds have numerous therapeutic activities, anti-inflammatory, anti-tumor [14], antioxidant, antifungal, immuno-modulatory [15], gastro-protective [16], cardio-protective, anti-helminthic [17], antiviral [18], antidiabetic [19], anti-cancer, antidiarrheal [20], hypo-lipidemic, hepato-protective [21] and dermato-protective [22]. It can also be used in folk medication in the treatment of cough, gastro-intestinal disorder, sexually transmitted infections, skin infection, snake bite, wound, pyrexia, malaria, ulcer amongst others [23, 24]. Result obtained in this study is in agreement with the reports of [25].

Growth performance of weaned rabbits fed diet supplemented with Ficus exasperata oil (Table 3).

Average daily weight gain was lower in treatment 1 [T1] (24.81 g) than in T2 (28.22 g), T3(29.85)

and T4 (39.26 g) ($p<0.05$). This result suggests that Ficus exasperata oil has an inhibitory effect against pathogenic organisms exerting beneficial effects on the gastrointestinal tract by increasing the activities of digestive enzymes translating to better weight gain especially when it is supplemented in the diet of rabbits between 250 – 750 mg/kg DM daily. The result obtained is in agreement with the reports of [26] who supplemented Moringa oleifera and Rosmarius officinalis leaves was supplemented in the diet of growing rabbits. [27] recorded a higher body weight in weaner rabbits fed diet supplemented with 200 mg of Allium sativum and Curcuma longa oil. Average daily feed intake in T2 (108.34 g), T3 (108.51 g) and T4 (113.30 g) were similar ($p>0.05$) but significantly higher than those in T1 (103.34 g) ($p<0.05$). Feed conversion ratio value which varied from 3.10 – 4.20 were influenced by the treatment ($p<0.05$). Rabbits fed diet supplemented with Ficus exasperata oil had a better feed conversion ratio compared to the control (T1). Result obtained indicates that Ficus exasperata oil has the capacity to increase feed intake by improving palatability of diets possibly due to enhanced favour and odor [23]. This outcome confirms the earlier report of [28] who recorded a higher feed intake in finishing pigs fed different levels of Saururus chinensis extracts. This is contrary to the report of [29], disparity in result could be as a result of dosage used, chemical composition of oil, duration of the experiment as well as extraction procedure adopted [17].

Table 4 reveals the apparent digestibility of weaned rabbits fed diet supplemented with Ficus exasperata oil. Dry matter, organic matter, crude protein and ether extract values follow similar pattern and ranged from 73.66 – 86.95 %, 79.43 – 85.67 %, 68.40 – 78.94 % and 48.17 – 60.80 % in that order. In this parameters, rabbits fed T2, T3 and T4 were similar ($p>0.05$) but significantly higher than those in T1 ($p<0.05$). Conversely, crude fibre value was lower in T1 (59.95 %) than T2 (63.74 %), T3 (65.61

%) and T4 (67.09 %). This outcome suggests that Ficus exasperata oil has the potential to improve villus height to crypt depth and muscular layer of the jejunum and ileum thereby enhancing intestinal absorption capacity [15]. The dietary supplementation of Ficus exasperata oil could also enhance nutrient absorption due to the stimulation of bile, saliva to facilitate the activities of enzymes in the gastro intestinal tract and enhance their health status [17]. This result is in consonance with the reports of [29] who recorded an improvement in apparent digestibility of dry matter, organic matter and crude protein of rabbits fed diet supplemented with rocket seed oil and onion seed oil.

Apparent digestibility of weaned rabbits fed diet supplemented with Ficus exasperata oil (Table 5). Nitrogen intake was higher in T4 (15.77 %), intermediate in T2, T3 (12.76 %, 12.85 %) and lower in T1 (9.55 %). Faecal nitrogen and urinary nitrogen values follow similar trend, as faecal nitrogen was higher in T1 (6.90 %) than T2 (4.05 %), T3 (3.96 %) and T4 (2.95 %) ($p<0.05$). Urinary nitrogen was lower in T4 (1.05 %), intermediate in T2 (1.28 %), T3 (1.21 %) and lower in T1 (2.59 %) ($p<0.05$). This outcome suggests Ficus exasperata oil possess antimicrobial properties which made animals in T2, T3 and T4 have lesser loss of nitrogen needed for growth, this justifies their higher body weight gain. Similarly, nitrogen retention was higher in T4 (56.17 %) than in T3 (50.10 %), T2 (50.02 %) and T1 (43.89 %) ($p<0.05$). Rabbits to fed diet supplemented with Ficus exasperata oil retained more nitrogen to produce superior body weight gain. The result obtained in this study is in consonance with the reports of [30,31] when phytogenics was supplemented in the diet of grass cutters.

Conclusion

In conclusion, Ficus exasperata oil is rich in bioactive compounds with therapeutic properties, such as: anti-inflammatory, antioxidant, anti-tumor, immuno-stimulatory, hepato-protective, gastro-protective, cardio-protective, amongst others. Supplementing the oil up to 750 mg/kg DM daily gave a positive result on the growth performance, apparent nutrient digestibility as well as nutrient utilization of rabbits without causing any deleterious effect on the health status.

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