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Essential oils from *Theobroma cacao* leaf supplementation in the diet of weaned rabbits: effects on growth performance

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Abstract

A total of 50- 5 weeks old weaned rabbits (male) of mixed breed with an initial body weight 586.1 ± 0.41 g were grouped into five, each consisting of ten rabbits each. Each animal served as a replicate. Basal diet was formulated to meet all the nutrient requirements for rabbits and a completely randomized design was adopted. Rabbits in group 1 received basal diet without essential oil from *Theobroma cacao* leaf which served as control; while group 2, 3, 4 and 5 were fed same diet with *Theobroma cacao* leaf oil at 200 mg, 400 mg, 600 mg and 800 mg per kg. Animals had unlimited access to feed and clean water throughout the 60 days experimental period. Bioactive profiling of essential oils from *Theobroma cacao* leaf showed that β-bisabolol (13.39 %), α-curcumene (10.54 %), methyl nonanoate (9.62 %) and α-patchoulene (9.11 %) were the most prominent bioactive compounds while α-longipinene (0.01 %), α-bisabolene (0.01 %), β-ionone (0.01 %) and β-cymene (0.02 %) were amongst the minor compounds. Body weight gain values which varied from 12.37 - 20.16 g were higher among rabbits fed diet 5 (800 mg *Theobroma cacao leaf oil* /kg diet), intermediate in group 2, 3 and 4 and lower in group 1 ($p<0.05$). Total feed intake (4900.4 - 6011.1 g) were higher among rabbits fed *Theobroma cacao leaf* oil relative control. Conversely, mortality was recorded only among rabbits given control diet ($p<0.05$). It was concluded that *Theobroma cacao leaf* oil is rich in bioactive compounds with therapeutic properties and can be supplemented up to 800 mg/kg in the diet of rabbits without causing any deleterious effect on their performance.

Introduction

Theobroma cacao L. belongs to the family Malvaceae containing more than 20 species widely distributed globally in American, African, and Asian continents, and other countries involved in cocoa production, marketing, and consumption (Bertolde et al., 2014). The leaves of T. cacao are large, ovate - oblong and about 4 - 20 cm wide while the seeds are usually inside a pod which is 15–30 cm long and 8–10 cm wide, ripening yellow to orange depending on the specie (Sharma et al., 2012). The plant was found to be rich in phytochemicals metabolites like triterpenoids flavonoids, alkaloids, phenols, saponins and tannins in the leaf, stem bark and seed (Zainal et al., 2014; Nwokonkwo and Okeke, 2014; Ogunmefun et al., 2013). These phytochemicals have medicinal properties like: antioxidant, antifungal, anti-viral, immune-stimulatory, antimicrobial, anti-bacterial, gastro-protective, cytotoxic, anti-ulcer amongst others (Izuka and Mbagwu, 2013; Pereira-Caro et al., 2013).

Theobroma cacao is used in folklore in the treatment low blood sugar, fatigue, kidney malfunction and for the treatment of ulcer and tumor (Sharma et al., 2012). They have also been reported to inhibit the activities of *Escherichia coli, Pseudomonas spp, Salmonella spp, Staphyllococcus spp* and other pathogenic organisms (Jayeola et al., 2011). Aqueous extracts from the stem bark can be used for the treatment of anaemia, body pains and malaria (Gbadamosi et al., 2012; Adeyi, 2010). It can also be mixed with black soap and used for the treatment of various forms of skin infections (Rzeppa et al., 2011; Quiñones et al., 2011). The plant is reported as an excellent source of vitamins, irons, flavours to diets and have also been established to promote health by acting against oxidative stress related diseases such as; diabetics, cancer and coronary heart diseases (Contreras et al., 2009; Othman et al., 2007). Recent research on extracts from the leaves and stem bark of T. cacao have also demonstrated cancer cell growth

inhibition. It appears to boost the production of key signaling molecules called Th1-type cytokines, which help regulate the immune system (Othman et al., 2007).

The use of essential oils in the diet of animals have recently been gaining increasing attention in the feed industry due to their beneficial effects on palatability, feed intake and performance (Oloruntola et al., 2016; Alagbe et al., 2020). For instance, Fayiz et al. (2024) reported that the application of pumpkin oil as a new feed additive in broilers influenced its final body weight and suppressed the activities of pathogenic organisms. Reda et al. (2020); Alagbe et al. (2023) reported that dietary supplementation of essential oils in the diet of animals can increase Lactobacilli and decreased the caecal counts of *Salmonella*, *coliforms*, and *E. coli* as well as enhances immune response parameters including immunoglobulin A and G. However, there is little or no information on the supplementation of *Theobroma cacao* leaf essential oil in the diet of rabbits. This research will help to evaluate the efficacy of bioactive compounds in the test ingredient as well as reveal the optimum level for rabbits.

Materials and methods

Description of experimental area and ethical approval

Rabbit unit of Sumitra Research Institute, Gujarat located between 28º 20' N and 75º 30' East India was used for experiment. All experimental guidelines and procedures were approved by the ethics committee of Animal Production department of the Institute (PC/2088F/0L).

Collection and extraction of essential oil from *Theobroma cacao* leaf

Mature leaves of *Theobroma cacao* was collected within Sumitra Research Institute's environment and sent to taxonomy department of the same institute for proper authentication before it was assigned an identification number (GH/009CV). Extraction of essential oil from *Theobroma cocao* leaf was carried out using steam distillation technique with Clevenger apparatus. Extracted oil was sent to the laboratory for further analysis.

Bioactive profiling of essential oil from *Theobroma cacao* leaf

Bioactive profiling of essential oil from *Theobroma cacao* leaf was carried out using Lauret gas chromatography - mass spectrometry (Model FG/008, Netherlands). Identification of each bioactive compound was carried out by comparing their mass spectra with those of reference compounds from the Library of National Institute of Standard and Technology (NIST, 2011) database.

Animal management, experimental treatments and design

A total of 50- 5 weeks old weaned rabbits (male) of mixed breed with an initial body weight 586.1 ± 0.41 g were used for this study. Rabbits were transferred early in the morning to the rabbit section of Sumitra Research Institute, Gujarat. They were grouped into five, each consisting of ten rabbits each. Each animal served as a replicate and housed individually in already disinfected battery cages of dimension 75 cm x 50cm x 40 cm equipped with manual feeders and drinkers made with concrete. Animals fed basal diet which was adequate in all necessary nutrients according to NRC (1977) and quarantined for 14 days. Thereafter, they were treated against parasites using Ivermectin plus® administered subcutaneously following the manufacturer's recommendation and also given water soluble vitamins. After the completion of adjustment period, rabbits were stratified based on their body weight. Those in group 1 (control) received basal diet only without *Theobroma cacao* leaf oil, those in group 2, 3, 4 and 5 were fed basal diet supplemented with *Theobroma cacao* leaf oil at 200 mg, 400 mg, 600 mg and 800 mg per kg diet. A completely randomized design was adopted. Rabbits had unrestricted access to fresh clean water and feed, all necessary management practices were strictly carried out and the experiment lasted for 60 days.

Growth performance parameters

Feed consumption or intake in grams was determined as the difference between the feed offered and refused.

Average weight gain was determined by subtracting the average initial weight of birds from their final body weight.

Average daily weight gain was calculated as average weight gain divided by 60 days (period of experiment).

Average daily feed intake was estimated by dividing the total feed intake by 60 days (period of experiment)

Feed conversion ratio (feed consumed to produce a unit of gain) was computed as the ratio of average feed consumption to average body weight gain

Mortality rate was recorded as it occurs in each treatment

Proximate composition of experimental diet

Analysis of feed was done using commercial kit, Near Infra-Red automated analyzer (Model NIRS^{TM} DS1800, Netherlands) feed analyzer with the following technical specifications; wavelength range (1100 to 1650 nm), ambient temperature (5 - 40 ℃), ambient humidity (less than 93 % RH), and results was generated at an analysis time of less than 1 minutes.

Analysis of data

Data collected on growth performance was subjected to one-way ANOVA 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.

Experimental results

Composition and nutrient levels of basal diet fed to weaner rabbits for 60 days (Table 1) showed that it contained crude protein (16.09 %), ether extract (13.87 %), ether extract (3.31 %), calcium (1.66 %), phosphorus (0.72 %) and metabolizable energy (2653.6 Kcal/kg).

Table 1: Composition and nutrient levels of basal diet fed to weaner rabbits for 60 days

Items/Ingredients	Levels (%)
Maize	48.00
Soya bean meal	30.00
Rice bran	16.00
Calcium carbonate	2.00
Di-calcium phosphate	4.00
Lysine	0.20
DL-Methionine	0.25
*Premix	0.25
Salt	0.30
Total	100.00
Determined nutrient levels (%)	
Crude protein	16.09
Crude fibre	13.87
Ether extract	3.31
Calcium	1.66
Phosphorus	0.72
Energy (metabolizable) (Kcal/kg)	2653.6

Mineral-vitamin premix, Each 2.5 kg sachets contains; Thiamine, 8000 mg, riboflavin, 12,000 mg, pyridoxine, 5000 mg, cyanocobalamine, 5000 mg, niacin, 20,000 mg, D-panthotenate, 10,000 mg, folic acid, 500 mg, biotin, 2000 mg, cholecalciferol, 3,000,000 iu., tocopherol acetate, 25,000 iu., ascorbic acid, 62,000 mg, manganese, 56mg, iron, 70,200 mg, 300 mg, iodine, 200 mg, selenium, 85 mg, choline chloride, 46,000 mg

Bioactive profiling of essential oils from *Theobroma cacao* leaf (Table 2) showed that β-bisabolol (13.39 %), α-curcumene (10.54 %), methyl nonanoate (9.62 %) and α-patchoulene (9.11 %) were the most prominent bioactive compounds while α-longipinene (0.01 %), α-bisabolene (0.01 %), β-ionone (0.01 %) and β-cymene (0.02 %) were amongst the minor compounds.

Table 2: Bioactive profiling of essential oils from *Theobroma cacao* leaf

S/N	Compounds	Retention time (Minutes)	% Area
1	β-Caryophyllene	2.33	1.12
2	α-Copaene	3.16	0.63
3	β-Cymene	3.30	0.02
4	1,8-Cineole	5.62	0.04
5	Methyl nonanoate	6.91	9.62
6	α-Longipinene	8.04	0.01
7	β-Himachalene	8.73	0.02
8	α-Cedrene	9.09	0.25
9	Acoradiene	10.40	0.13
10	α-Patchoulene	10.64	9.11

11	α-Curcumene	10.98	10.54
12	α-Selinene	11.56	1.56
13	β-Caryophyllene oxide	11.90	0.40
14	α-Bisabolene	12.55	0.05
15	β-Ionone	13.64	0.01
16	Eremophilene	14.37	0.16
17	Tetradecanoic Acid	15.96	0.19
18	Isopropyltetradecanoate	16.03	0.27
19	β-Bisabolol	17.11	13.29
20	2-Methylundecanal	17.84	0.44
21	hexadecanoic acid	18.09	30.81
22	octadecanoic acid	19.23	19.55
Total			98.82

Growth performance of weaned rabbits fed diet supplemented with essential oils from *Theobroma cacao* leaf (Table 3). Body weight gain of rabbits fed diet 2 (200 mg essential oils from *Theobroma cacao* leaf per kg diet) and diet 3 (400 mg essential oils from *Theobroma cacao* leaf per kg diet) were similar ($p>0.05$) to those which received diet 4 (600 mg essential oils from *Theobroma cacao* leaf per kg diet) but significantly lower ($p<0.05$) than those fed with diet 5 (800 mg essential oils from *Theobroma cacao* leaf per kg diet). Body weight gain values were higher ($p<0.05$) in diet 5, intermediate in diet 2, 3, 4 and lower in diet 1. Total feed intake were higher ($p<0.05$) in rabbits fed *Theobroma cacao* leaf essential oil compared to diet. Conversely, feed conversion ratio was maximum among rabbits that received diet 1, optimum among rabbits given diet 2, 3 and 4 and minimum in diet 5 ($p<0.05$). The only mortality recorded (1.00 %) was only among rabbits fed diet 1 ($p<0.05$).

Table 3: Growth performance of weaned rabbits fed diet supplemented with essential oils from *Theobroma cacao* leaf

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Number of rabbits	10.00	10.00	10.00	10.00	10.00	-
Duration of experiment in days	60.00	60.00	60.00	60.00	60.00	-
Initial body weight (g/rabbit)	586.5	586.2	586.3	586.1	586	0.06
Final body weight (g/rabbit)	1700.2^{c}	2019.8^{b}	2021.5^{b}	2026.1^{b}	2400.3^{a}	46.21
Body weight gain (g/rabbit)	1113.7^{c}	1433.6^{b}	1435.2^{b}	1440.0^{b}	1814.3^{a}	25.88
Daily body weight gain (g/rabbit)	12.37^{c}	15.93^{b}	15.95^{b}	14.80^{b}	20.16^{a}	0.05
Total feed intake (g/rabbit)	4900.4^{b}	6000.6^{a}	6010.8^{a}	6010.9^{a}	6011.1^{a}	139.4
Daily feed intake (g/rabbit)	51.67^{b}	66.67^{a}	66.78a	66.78^{a}	66.79^{a}	0.17
Feed conversion ratio	5.91^{a}	4.18^{b}	4.18^{b}	4.17^{b}	3.40^{c}	0.02
Mortality (%)	1.00	-	-	-	-	0.01

SEM – standard error of mean; 1: basal diet without essential oils from *Theobroma cacao* leaf; diet 2: basal diet supplemented with 200 mg essential oils from *Theobroma cacao* leaf per kg diet; diet 3: basal diet supplemented with 400 mg essential oils from *Theobroma cacao* leaf per kg diet; diet 4: basal diet supplemented with 600 mg essential oils from *Theobroma cacao* leaf per kg diet; diet 5: basal diet supplemented with 800 mg essential oils from *Theobroma cacao* leaf per kg diet.

Discussion

Body weight gain value (113.7 g - 1814.3 g) recorded in this experiment with the dietary supplementation of essential oils from *Theobroma cacao* leaf was similar to the result of a study by Adewale et al. (2021) who observed that body weight gain of rabbits fed diet supplemented with *Rauvolfia vomitoria* root extract varied from 1156.2 - 1900.4 g. The result was higher than those reported by Alagbe (2023) and John (2024a) discovered that body weight gain of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* essential oil and *Clerodendron splendens* leaf extract varied from 1004.2 - 1100.3 g and 1000 - 1134.1 g respectively. Rabbits fed diet *Theobroma cacao* leaf essential oil had an improved body weight gain and feed conversion ratio relative to diet 1, this outcome suggests that the bioactive compounds in the test ingredient as presented in Table 2 could enhance absorption of nutrient and digestion leading to better feed efficiency and increased weight gain (Muritala et al., 2022; Shittu et al., 2021; John 2024d). For instance, β-caryophyllene, α-copaene, β-cymene, α-longipinene, β-himachalene and α-cedrene have been reported to have anti-inflammatory, anti-oxidant, gastro-protective and antimicrobial activities (Ojediran et al., 2024; Agoramoorthy et al., 2007; Gawali and Jadhav, 2011). 2-Methylundecanal isopropyltetradecanoate and octadecanoic acid have

been reported to possess antimicrobial properties (Daniel et al., 2023; John, 2024b). According to Alagbe et al. (2023); Alagbe et al. (2024), α-patchoulene, α-curcumene, α-selinene, α-bisabolene, tetradecanoic acid and eremophilene have been suggested to possess bactericidal properties and can inhibit non-beneficial organisms in the gut of rabbits. The above pharmacological properties in *Theobroma cacao* leaf essential oil explains the reason why mortality was not recorded among rabbits fed diet 2, 3, 4 and 5 respectively. Total feed consumption in this experiment was highest among rabbits supplemented with *Theobroma cacao* leaf essential oil, which suggests that it can influence the palatability (appearance, taste, texture, temperature and odour) of feed (John, 2024c). Total feed consumption range (4900.4 - 6011.1 g) recorded in this experiment with the dietary supplementation of *Theobroma cacao* leaf essential oil is similar to the results of a study by Oloruntola et al. (2016) who found out that the total feed intake of rabbits fed diet supplemented with *Alchornea cordifolia* leaf meal varied from 5663.2 - 6500.1 g. This result was lower than those presented by Olabanji *et al*. (2007) who found out that rabbits fed diet supplemented with *Tithonia diversifolia* leaf meal feed intake varied from 4599.3 - 5006.2 g.

Conclusion

In conclusion, essential oils from *Theobroma cacao* leaf contains numerous bioactive compounds with therapeutic properties like, anti-inflammatory, antioxidant, antimicrobial, immune-stimulatory, anti-ulcer, antibiabetic, cytotoxic, gastro-protective amongst others. These oils are efficient and can be supplemented in the diet of rabbits up to 800 mg/kg without causing any deleterious effect on the health status of animals.

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