

Influence of Chromolaena Odorata Extract on the Growth Performance and Fatty Acid Composition of Weaner Rabbits

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ABSTRACT

Growth performance and fatty acid composition of Weaner rabbits. Sixty-four weaned white New Zealand rabbits of mixed sex (30 males and 30 females) 7 weeks old with an average initial body weight of 650 ± 0.65 g were used in a completely randomized design (4 rabbits (2 males + 2 females) each cage \times 4 replicates). The treatment consisted of the control (treatment 1): basal diet without Chromolaena odorata extract, treatment 2, 3 and 4 were given basal diet with Chromolaena odorata extract at 3 mL, 6 mL and 9 mL/rabbit daily respectively. The duration of the experiment is 60 days, feed and fresh water were given ad libitum throughout the period of experiment. Analysis of bioactive compounds in Chromolaena odorata extract by GC-MS showed that 2,21 - Methylenebis (6-tertbutyl-4- ethyl) Phenol (20.70 %), 1,8-Cineole (12.31 %), α -Bergamotene (10.02 %), Trans- α -Bisabolene Epoxide (9.55 %), 2-Butyl octanol (8.87 %), Methyl Nonanoate (7.52 %), 2,3-Tridecene (6.55 %), -Caryophyllene (6.33 %) and Humulane-1,6-dien-3-ol (5.44 %) were the major compounds. Dietary supplementation of Chromolaena odorata extract enhanced ($p < 0.05$) average daily weight gain, average daily feed consumption as well as feed conversion ratio. Chromolaena odorata extract supplementation increased ($p < 0.05$) the concentration of unsaturated fatty acid (mono-saturated fatty acid and polyunsaturated fatty acid) and decreased saturated fatty acid concentrations among the groups. In conclusion, Chromolaena odorata extract contains several compounds with therapeutic properties which can improve the growth performance and unsaturated fatty acid of weaner rabbit's meat without causing any negative effect on the performance of animals

INTRODUCTION

Medicinal plants have been utilized for decades for both humans and animals thus their efficacy and safety is ensured because they contain phyto-constituents with medicinal properties (John, 2024b). These compounds are environmentally friendly and have no withdrawal period (Alagbe, 2022). Currently, there has been rising cases in the incidence of antimicrobial resistance due to the misuse of antibiotics. Therefore, there is ongoing research concerning the mechanism of action of these herbal plants as well as their optimal level (Muritala et al., 2022; Omokore and Alagbe, 2019). Phyto-constituents from herbs have also displayed positive effect on animal feed intake resulting in better growth performance and improved feed conversion ratio (John, 2024c). Among the potential herbal plant with therapeutic relevance is *Chromolaena odorata*. The plant has a long history of use in traditional medicine. However, its potential antimicrobial properties remain under explored.

Plants of the genus *Chromolaena odorata* belong to the family Asteraceae and are found mainly in America, Europe and so parts of Asian. A phytochemical evaluation conducted by (Naidoo et al., 2011) on the leaves of *Chromolaena odorata* indicates the presence of glycosides, phenolic compounds, tannins, alkaloids, saponins and flavonoids which have been suggested to possess antimicrobial, anti-androgenic, cytotoxic, antidiabetic, anti-tumor, anti-inflammatory (Danlami et al., 2013; Stanley et al., 2014), anti-cancer, gastro-protective, cardio-protective, anti-helminthic, antidiarrheal, immunomodulatory, antioxidant, analgesics, antiepileptic, anxiolytic, anti-protozoal, anti-trypanosomal, anti-fungal, anti-hypertensive amongst others (Akinmoladun et al., 2007). Biologically active phyto-constituents such as 1,3-diethyl-5-methyl-Benzene, 1,2,3,4-tetrahydro- Naphthalene, α -Terpineol, cyclopentylmethyl - Cyclohexane, Tridecane, Decahydro-1,1,4a,5,6- penta-methylnaphthalene, 8-Isopropyl-1,5- dimethyltricyclo, dec-4-en-3-one, 1,6-dimethyl- Naphthalene, 5-Hydroxy-4',7-dimethoxyflavanone and 5,6,7,4'-Tetramethoxyflavanone have been isolated from leaf extracts of *Chromolaena odorata* (Okigbo and Ajalie, 2005). (Vaisakh and Pandey, 2012) noted that essential oil from *Chromolaena odorata* leaves possess insecticidal, antimicrobial and anti-inflammatory properties.

Extracts made from only the leaves and roots of the plant have been used traditionally for the treatment of cold, whooping cough, asthma, bronchitis, diarrhoea, dysentery, Fever, pain, skin infection, snake bite, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiratory disorders, and digestive troubles, muscle pain, depression, anaemia, insanity and other mental disorders (Chakraborty et al., 2011). Extracts from the leaves can also interfere with the proliferation of pathogens such as *Escherichia coli*, *Aspergillus flavus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus sp* (Vital et al., 2009; Kigigha and Zige, 2013).

Previous research by notable authors (Enayat et al. (2021); Ojo and Adetoyi (2017); Zotte et al. (2016); El-Badawi et al. (2014); Sun et al. (2018) have shown that dietary supplementation of herbs can influence apparent digestibilities of nutrients, stimulate the production of digestive enzymes and improve the

balance of gut microflora, increase the concentrations of total volatile fatty acids, acetate, propionate and valerate, decrease the concentrations of total lipids, cholesterol in the serum and organoleptic properties of meat. However, there is a dearth of information on the effect of *Chromolaena odorata* on the performance of weaner rabbits. This research will help to promote a sustainable and antibiotic free livestock production.

LITERATURE REVIEW

Site of the Experiment

The research was carried out at the Rabbit Unit, Ganhi College of Agriculture, Rajasthan situated in the North-Western part of India which lies between longitude 23° 03' to 30° 12' North and latitude 69° 30' and 78° 17' East.

Collection of Plant Materials and Extraction

Fresh *Chromolaena odorata* leaves were collected from Sumitra Research Institute, Gujarat and sent to the Taxonomy department of Gandhi College of Agriculture, Rajasthan India for proper authentication by a certified Crop Scientist (Mrs. Amit), assigned a voucher number TD-08A/2024 and deposited at the Herbarium of the Department of Crop Science, Gandhi College of Agriculture, Rajasthan. The collected samples were washed with running tap water followed by distilled water to remove all dirt's and air dried for 12 days, grounded into powdered using mortar and pestle before it was transferred into an air tight container prior to extraction. 200 g of grounded *Chromolaena odorata* powder was soaked in 1-liter ethanol solution, stirred intermittently and kept under room temperature for 2 days. The mixtures were then filtered using Whatman filter paper and the filtrate was kept in a refrigerator at 4 °C until analysis.

GC-MS Analysis of Bioactive Components in Chromolaena Odorata Extract

Analysis of bioactive components in *Chromolaena odorata* extract was done using an Agilent Model 7530C Gas Chromatography (GC) interfaced to an Agilent 7200 GC/MS Triple Quad (Model AA-08D, Netherlands). The temperature program used for the GC was 300 °C, total flow of 16.000 mL/min. Oven was initially programmed at 30 °C for 2 minutes then ramped at 16 °C/min to 300 °C (10 min). The Mass spectrometry compartment was operated in EI mode with ionization voltage 60 eV and ion source temperature of 250°C. The bioactive components of *Chromolaena odorata* extract were identified on the basis of their retention indices. Identification confirmation was carried out by comparing of their mass spectra with published spectra and those of reference compounds from the Library of National Institute of Standard and Technology (NIST, 2011) database.

Rabbits and Management

Sixty-four weaned white New Zealand rabbits of mixed sex (30 males and 30 females) 7 weeks old with an average initial body weight of 650 ± 0.65 g was purchased from a breeding farm in Rajasthan. The animals were cared for according to the procedure stated by Indian Institute of Animal Production. On arrival, animals were taken to already disinfected battery cages (100 cm length × 75 cm width × 60 cm height) kept in a well ventilated open sided pen. The animals

were quarantined for 14 days and treated against endo and ecto -parasites with Albendazole plus® (Pultra Veterinary Pharmaceutical, Gujarat, India). Rabbits were fed basal diet (Table 1) formulated according to the requirement of rabbits approved by Nutritional Research Council (2012). Thereafter, they were randomly divided into four treatments of 16 animals each stratified for sex and weight (4 rabbits (2 males + 2 females) each cage × 4 replicates).

Experimental diet was analyzed according to method outlined by AOAC (2016) and cages were supplied with concrete feeders and nipple drinkers. Dietary treatment includes; treatment 1: basal diet without *Chromolaena odorata*, treatment 2, 3 and 4 received basal diet with 3 mL, 6 mL and 9 mL *Chromolaena odorata* extract /daily respectively. To ensure that rabbits takes a full dose of the extract, each animal was drenched with COE using calibrated plastic straw before morning feeding. A completely randomized experimental design was used and the experiment lasted for 60 days excluding the 2 weeks' acclimatization period. Rabbits were supplied feed and fresh clean water ad libitum. Live weights of the rabbits were taken using a digital sensitive scale at the commencement of the experiment and they were weighed on a fortnight basis afterwards. The weight gain from each group was calculated by subtracting the initial weight from the final weight. Average daily weight gain was calculated by subtracting the live weight at the beginning of the fortnight from the live weight at the end of the fortnight and dividing by the number of the days in the fortnight. Feed conversion ratio was estimated by dividing feed intake by the weight gain.

Fatty Acid Composition of Meat Sample

At the end of the experiment meat samples were collected from the breast muscle of four randomly selected rabbits. Animals were feed starved 12 hours before slaughtering before collecting meat samples in sample bottles and placed in an ice before transferring samples to the laboratory for analysis. Fatty acid composition of meat samples was carried out according to methods recently published by Anjum et al. (2013). Briefly, meat sample was injected into gas chromatograph Triple Tripod® (Model GCH-006H, Japan) in splitless mode and subjected to a temperature of 300 °C. Purge flow to spilt vent was 20 mL/min at 1.05 min with a total flow of 15.000 mL/min. The fatty acid methylated esters were recognized by comparing their retention times with those of their standards. Fatty acid relative percent was quantified as a percentage of the total analyzed fatty acid in the sample. Adjustment in the kit was done according to recommendations of the manufacturers to obtain precision in results.

METHODOLOGY

Statistical Analysis

Data collected on growth performance and fatty acid were analyzed by using the general linear model (GLM) procedures of SAS (2004), by employing one-way analysis of variance (ANOVA). Significant differences between treatment's means were separated by Duncan's multiple range test (Duncan, 1955)

The following statistical model was used: $Y_{ij} = \mu + T_i + e_{ij}$ Where: Y_{ij} = the observation of ij ; μ = overall mean; T_i = effects of treatments, ($i = 1, 2, 3$ and 4) and e_{ij} = experimental random error.

Table 1. Ingredient and Chemical Composition of the Basal Diet (G/Kg Of DM)

Ingredients	g/kg DM
Yellow Corn	372.0
Wheat bran	330.0
Soya bean meal	150.0
Groundnut meal	100.0
Limestone	10.00
Bone meal	20.00
Mineral and Vitamin mixture	5.00
Lysine	5.00
Methionine	5.00
Salt	3.00
Total	1000.0
Chemical composition (g/kg DM)	
Dry matter	930.1
Organic matter	875.0
Crude protein	15.10
Ether extract	20.00
Crude fibre	150.0
Ash	125.0
Digestible energy (MJ/kg)	12.04

Each 2.5 kg Mineral + Vitamin Premix contained: vit. A 10,000 IU; vit. D3 2500 IU; vit. E 80 mg; vit. B1 4.0 mg; vit. B2 5.5 mg; vit. B6 3.00 mg; vit. B12 0.06 mg; vit. K 5.5 mg; niacin 35 mg; folic acid 9.6 mg; pantothenic acid 25 mg; choline 1500 mg; Mn 100 mg; Cu 3.5 mg; Fe 80 mg; Zn 70 mg.

Table 2. Compounds Identified in Chromolaena Odorata Extract By GC-MS

Compounds	Retention time (min)	Percentage Area
2-Butyl octanol	4.11	8.87
α -Bergamotene	6.07	10.02
11-Dodecen-2-one	6.11	0.07
2,3-Tridecene	6.85	6.55
4,6-Dimethyldodecane	7.09	0.11
Methyl Nonanoate	8.08	7.52
Trans- α -Bisabolene Epoxide	8.23	9.55
2,21 -Methylenebis(6-tertbutyl-4-ethyl)Phenol	8.66	20.70
2-Dimethylsilyloxytetradecane	9.41	1.55
Humulane-1,6-dien-3-ol	9.58	1.10
Cis-4,15-Octadecadien-1-ol acetate	10.02	0.09
2,2-dimethyl- Cyclohexanol	10.55	0.53
Humulane-1,6-dien-3-ol	11.82	5.44

Cis-9-Tetradecenol	12.09	0.17
Neophytadiene	12.42	0.34
2- Chloropropionic acid	12.88	0.19
1,8-Cineole	13.07	12.31
8-Cyclohexadecen-1-one	13.51	1.59
Cis-7,10-Hexadecadienal	13.80	0.21
Bicyclo [10,6,0]Octadeca-1(12),15- diene	14.04	0.46
1,2,15,16-Diepoxylhexadecane	14.60	0.51
11-Tridecyn-1-o	14.94	0.10
γ -Elemene	15.05	0.62
2-Dimethylsilyloxytetradecane	16.07	0.31
α -Terpinolene	16.33	1.90
Ethylbenzene	18.59	0.12
Acoradiene	19.03	0.18
Isophytol	19.47	0.22
γ -Palmito acetone	19.90	0.39
2-Butyl octanol	20.50	0.58
β -Caryophyllene	21.80	6.33
β -Linalol	23.77	0.12
Total	-	98.75 %

Table 3. Growth Performance of Weaner Rabbits as Affected By Dietary Supplementation of Chromolaena Odorata Extract (COE)

Parameters	T1 (0 % COE)	T2 +3 mL COE/day	T3 + 6 mL COE/day	T4 + 9 mL COE/day	SEM
Initial body weight (g)	650.61	650.65	649.88	650.11	0.03
Final body weight (g)	1922.8 ^c	2118.2 ^b	2120.6 ^b	2305.8 ^a	67.12
Body weight gain (g)	1272.2 ^c	1467.6 ^b	1470.7 ^b	1655.7 ^a	28.07
Average daily weight gain (g)	21.20 ^c	24.46 ^b	24.51 ^b	27.59 ^a	0.94
Total feed consumption (g)	5220 ^c	5880 ^b	6000 ^a	6000 ^a	125.2
Average daily feed consumption (g)	87.00 ^c	98.00 ^b	100.0 ^a	100.0 ^a	2.87
Feed conversion ratio	4.10 ^a	4.00 ^b	4.00 ^b	3.70 ^c	0.06

a,b,c: Means within a row with different superscripts are significantly different ($p < 0.05$); Treatment 1: basal diet without Chromolaena odorata extract; Treatment 2, 3 and 4: basal diet +3 mL, 6 mL and 9 mL Chromolaena odorata extract/day respectively; SEM: Standard error of mean

Table 4. Fatty Acid Composition of Meat as Affected by Dietary Supplementation of Chromolaena Odorata Extract

Components	T1 + 0 % without COE	T2 + 3 mL COE/day	T3 + 6 mL COE/day	T4 + 9 mL COE/ day	SEM
C4:0	1.92 ^a	1.75 ^b	1.72 ^b	1.70 ^b	0.02
C6:0	3.88 ^a	3.51 ^b	3.48 ^b	3.42 ^b	0.13
C8:0	0.96 ^a	0.73 ^b	0.71 ^b	0.68 ^b	0.42
C9:0	8.33 ^a	7.82 ^b	7.74 ^b	7.72 ^b	0.05
C10:0	5.41 ^a	5.37 ^b	5.32 ^b	5.33 ^b	0.15
C12:0	4.88 ^a	4.71 ^b	4.69 ^b	4.67 ^b	0.03
C13:0	2.95 ^a	2.05 ^b	2.01 ^b	1.94 ^b	0.12
C14:0	10.55 ^a	9.11 ^b	9.05 ^b	8.89 ^c	0.09
C15:0	8.92	8.73	8.51	8.44	0.11
C16:0	18.92 ^a	16.33 ^b	15.61 ^b	14.46 ^c	1.60
C17:0	0.60 ^c	0.69 ^b	0.71 ^a	0.72 ^a	0.01
C18:0	1.66 ^b	2.45 ^a	2.51 ^a	2.56 ^a	0.02
C18:1n-9	11.57 ^b	13.06 ^a	13.87 ^a	13.92 ^a	1.51
C18:2	0.79 ^b	1.21 ^a	1.25 ^a	1.34 ^a	0.01
C18:2n-6	1.75 ^b	2.05 ^a	2.11 ^a	2.18 ^a	0.02
C18:3n-6	2.88 ^b	3.25 ^a	3.46 ^a	3.61 ^a	0.03
C18:3n-4	4.77 ^b	5.93 ^a	6.06 ^a	6.11 ^a	0.05
C18:3n-3	3.00 ^b	3.94 ^a	4.05 ^a	4.12 ^a	0.15
C20:0	2.06 ^b	2.85 ^a	2.97 ^a	3.01 ^a	0.04
C22:0	4.02 ^b	5.56 ^a	5.95 ^a	6.08 ^a	0.18
SFA	67.50 ^a	60.80 ^b	59.55 ^c	57.97 ^c	0.45
UFA	32.50 ^c	40.30 ^b	42.23 ^a	42.93 ^a	1.29
MUFA	25.11 ^b	30.05 ^a	32.11 ^a	32.04 ^a	0.92
PUFA	7.39 ^b	10.25 ^a	10.12 ^a	10.89 ^a	0.02

a,b,c: Means within a row with different superscripts are significantly different ($p < 0.05$); Treatment 1: basal diet without Chromolaena odorata extract; Treatment 2, 3 and 4: basal diet +3 mL, 6 mL and 9 mL Chromolaena odorata extract/day respectively; SFA: Saturated fatty acid; UFA: Unsaturated fatty acid; MUFA: Monosaturated fatty acid; PUFA: Polyunsaturated fatty acid; SEM: Standard error of mean

RESULTS AND DISCUSSION

The GC-MS analysis of Chromolaena odorata extract is presented in Table 2. 31 bioactive compounds were identified representing 98.75 %. 2,21 - Methylenebis (6-tertbutyl-4- ethyl) Phenol (20.70 %), 1,8-Cineole (12.31 %), α -Bergamotene (10.02 %), Trans- α -Bisabolene Epoxide (9.55 %), 2-Butyl octanol (8.87 %), Methyl Nonanoate (7.52 %), 2,3-Tridecene (6.55 %), -Caryophyllene (6.33 %) and Humulane-1,6-dien-3-ol (5.44 %) were predominant amongst the

identified compounds in *Chromolaena odorata* extract while 11-Dodecen-2-one (0.07 %), Cis-4,15-Octadecadien-1-ol acetate (0.09 %), 11-Tridecyn-1-o (0.10 %), 4,6-Dimethyldodecane (0.11 %), Ethylbenzene (0.12 %), β -Linalol (0.12 %), 2-Chloropropionic acid (0.19 %) amongst others were found in low concentration. These compounds have several medicinal properties including, anti-inflammatory (Subavathy and Thilaga, 2015; Simlai and Roy, 2012), antifungal, anti-helminthic (Ojediran et al., 2024a), immuno-modulatory (Oluwafemi et al., 2022), cytotoxic, gastro-protective (Musa et al., 2020; Adewale et al., 2021), cardio-protective (John, 2024a; Singh et al., 2021), antiviral (Hernandez and Alagbe, 2025; Mamza et al., 2012), anti-tumor (Singh et al., 2022; John, 2024b), antidiabetic (John, 2024c; Ojediran et al., 2024b), anti-cancer, analgesics (Hernandez and Alagbe, 2025; Namkeleja et al., 2014), antiviral (Alagbe, 2022; Manilal et al., 2011), antimicrobial, amongst others. Result obtained in this study is in consonance with the reports of Danlami et al. (2013) but contrary to the reports of Keshab et al. (2012). The variation in results could be attributed to variation in age of medicinal plants, geographical location, harvesting time as well as processing method (Singh et al., 2022). Bioactive compounds have been generally regarded as safe, eco-friendly and has no withdrawal period (Ojediran et al., 2024b).

Table 3 represents the growth performance of weaner rabbits as affected by dietary supplementation of *Chromolaena odorata* extract. Dietary supplementation with *Chromolaena odorata* extract increased ($p < 0.05$) average daily weight gain compared with the control. Dietary supplementation with *Chromolaena odorata* extract decreased ($p < 0.05$) feed conversion ratio compared with the control. The outcome in this experiment showed that *Chromolaena odorata* extract have therapeutic properties and it is capable of providing essential nutrients, increasing feed palatability, improving growth performance and feed utilization in rabbits (Muritala et al., 2022; Shittu et al., 2024). The dietary supplementation of *Chromolaena odorata* extract was also capable of modulating intestinal enzymes and inhibiting the activities of pathogens in the gut thus translating to better weight gain (Daniel et al., 2023). These result is in agreement with the reports of Czech et al. (2009) who observed that dietary supplementation of garlic bulbs at 0.80 g/kg stimulates growth performance and exerts beneficial actions in the gastro intestinal tract by increasing the activities of digestive enzymes of pigs. Burno et al. (2013) observed a non-significant difference in the weight of birds fed diet supplemented with 2000 ppm *Mentha piperita* extract. Variation in these outcomes could be linked to composition of bioactive compounds, dosage administered as well as duration of the experiment (Musa et al., 2020).

Fatty acid composition of meat as affected by dietary supplementation of *Chromolaena odorata* extract is presented in Table 4. Dietary supplementation with *Chromolaena odorata* extract increased ($p < 0.05$) the concentrations of mono-unsaturated fatty acid and polyunsaturated fatty acid compared with the control. Moreover, a lower concentration of saturated fatty acid ($p < 0.05$) was observed in the *Chromolaena odorata* extract group. Results obtained in this study suggests that *Chromolaena odorata* extract contains natural antioxidants which contributes to the oxidative stability of meat (Alagbe et al., 2022). High

concentration of polyunsaturated fatty acid can also improve the shelf life of meat, organoleptic properties and also prevent against cardiovascular disease (Alagbe et al., 2022). Result obtained is in agreement with the report of Ghazaghi et al. (2014); Guray et al. (2011) when green tea extract was fed to broiler chicks.

CONCLUSIONS AND RECOMMENDATIONS

Chromolaena odorata extract contains numerous bioactive compounds of therapeutic relevance. Feeding rabbits with *Chromolaena odorata* extract up to 9 mL/day throughout the experimental period had no deleterious effect on their performance. It will also help to address the increasing rate of antibiotic resistance, promote sustainable animal production and food safety. Therefore, high concentration is recommended.

FURTHER STUDY

This research still has limitations, so further research is needed on the topic of Influence of *Chromolaena Odorata* Extract on the Growth Performance and Fatty Acid Composition of Weaner Rabbits in order to perfect this research and increase insight for readers.

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