Research in: Agricultural & Veterinary Sciences
Vol.7, No.3, 2023, pp.139-148

Jomard Publishing

INVESTIGATING THE EFFECTS OF DIETARY SUPPLEMENTATION OF EUCALYPTUS CAMALDULENSIS ESSENTIAL OIL (ECEO) ON THE GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND CAECAL FERMENTATION OF WEANED RABBITS

J.O. Alagbe*

Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, India

Abstract. The aim of this experiment was to investigate the effects of dietary supplementation of Eucalyptus camaldulensis essential oil (ECEO) on the growth performance, nutrient digestibility and caecal fermentation of weaned rabbits. Sixty clinically healthy weaned cross breed male rabbits (Chinchilla × Newzealand white) of about 4 weeks with initial body weight (BW) of 430.81 ± 0.81 g were used for the study. Rabbits were stratified based on their body weight and randomly distributed into four treatment groups of fifteen rabbits each in a completely randomized design. Treatment 1 (T1): Basal diet without Eucalyptus camaldulensis essential oil (ECEO); T2: basal diet with 200 mg/kg ECEO; T3: basal diet with 400 mg/kg ECEO; T4: basal diet with 600 mg/kg ECEO. Average daily weight gain were higher (P<0.05) in T2, T3 and T4 relative to T1. There was no effect of treatments in the average daily feed intake and mortality rate (P>0.05) while feed conversion ratio were greater in T2, T3 and T4 than in T1 (P<0.05). Dry matter, crude protein, crude fibre, nitrogen free extracts, organic matter and ether extract digestibility were significantly (P<0.05) influenced by the treatments. Improved nutrient absorption were improved in rabbits fed diet supplemented with ECEO compared to control group. Volatile fatty acid, acetate, butyrate, propionate and ammonia nitrogen were significantly (P<0.05) different among the treatments. Results revealed that ECEO can be supplemented up to 600 mg/kg without posing no harm on rabbit's body physiology and health status.

Keywords: *Eucalyptus camaldulensis, essential oil, phytogenics, phytochemicals, rabbits.*

****Corresponding Author:*** *J.O. Alagbe, Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat 360002, India, e-mail: dralagbe@outlook.com*

Received: *8 September 2023;* ***Accepted:*** *20 October 2023;* ***Published:*** *8 December 2023.*

1. Introduction

Reducing the use of antibiotics in animal agriculture is one of the key goals, as a result of customer demand. A large majority of them continue to be employed as antibiotic growth promoters in addition to their therapeutic function (Singh *et al*., 2021). In order to avoid and reduce antibiotic resistance and to support the sustainability of livestock production, lessening the use of antibiotics, especially antibiotic growth boosters, is crucial (Singh *et al*., 2022). A comprehensive strategy should always be the goal in this situation. This covers feed nutrient intake, gut flora and integrity, farm management, and farm health. A novel mixture of plant-based natural extracts provides livestock animals with an alternative to growth promoters and supports improved performance (Adewale *et al*., 2021).

Essential oils in particular have been shown to be phytogenics, with no harmful residue, drug resistance, environmentally friendly, or deleterious side effects when used in the proper dosages (Muritala *et al*., 2023). Various plant parts, including flowers, buds, seeds, bark, stems, roots, fruits and pulp, contain essential oils (Helpo, 2021; Singh, 2021). Essential oils are not connected to any adverse side effects, such as residues and bacterial resistance that are connected to the use of antibiotic growth promoters (Inge & Rainer, 2021). They have a variety of qualities, including flavoring, anti-microbial, antioxidant, antiviral, antidepressant, immunological modulating, and physiological ones, among others, that are crucial for their performance-enhancing benefits on rabbits (Ines, 2022).

According to Kone *et al*. (2016), essential oils have a number of beneficial effects on rabbit efficiency, animal health, and feed consumption. According to Miguel (2010), mortality decreased in growing rabbits given a diet supplemented with 0.5% garlic oil. This supported the findings of Bozin *et al*. (2006), who noted that rabbits fed 0.2% lavender oil gained more weight. In light of rising feed use, there is growing demand for researching new, risk-free antimicrobial compounds that can prevent diseases while also enhancing wellbeing/food safety and health.

This study was designed to investigate the effects of dietary supplementation of *Eucalyptus camaldulensis* essential oil (ECEO) on the growth performance, nutrient digestibility and caecal fermentation of weaned rabbits.

2. Materials and Methods

Ethical approval, experimental site and duration

The experimental procedures and animal management were in line with the methods outlined by the animal welfare committee at Sumitra Research Institute, India. The study was carried out at the Livestock unit of the institute located between 23^{o} 13' N and 72^{o} 41' E between January to April 2021 and the experiment lasted for 56 days.

Source and processing of Eucalyptus camaldulensis leaves

Fresh *Eucalyptus camaldulensis* leaves were collected at Sumitra Research Teaching and Research farms, Gujarat, India. Detailed authentication was carried out by a certified taxonomist and leaves were washed to remove soil and other debris. Samples was later spread on a flat plastic tray to allow water drain from the plant, cut into smaller to reduce their surface area before they were transferred to the laboratory and stored in a sealed labeled container.

Extraction of Eucalyptus camaldulensis oil

The extraction procedure employed is steam distillation technique. The laboratory process requires; a steam generator, round bottom flask, beaker, condenser, safety tube, seperatory funnel thermometer and a distillation flask attached with a delivery tube which is used to connect the steam generator. 80 grams of sliced *Eucalyptus camaldulensis* leaf was transferred into 500 mL round bottom flask thereafter 250 mL distilled water was added. The distillation flask, condenser and the receiver was set up and the mixture in the round bottom flask was heated at 70^{o}C for 15 minutes the steam produced passes through the condenser and when it is cool, oil is collected in a receiver (beaker). Distillate is passed through the seperatory funnel to get obtain pure *Eucalyptus camaldulensis* essential oil (ECEO).

Bioactive profiling of Eucalyptus camaldulensis essential oil by gas chromatography and mass spectrometry (GC-MS)

Bioactive profiling of *Eucalyptus camaldulensis* essential oil was carried out with Labtron GC-MS (Model – 05GY-871, India). It is a portable and high precision gas chromatography mass spectrometry and it has the following technical specifications; maximum flow rate (10 ml/min), in let temperature (450ºC), pressure range (0 – 100 psi), heating rate (up to 120ºC/min), room temperature (4ºC - 450ºC), pressure control mode (electronic pressure control), split mode (split / split less), split ratio (1000:1) and temperature programming (7 stages / 8 platforms) while the mass spectrometry (Model-MS-89): mass range (1.5 – 1000amu), ion – source temperature (100ºC - 350ºC), GC-MS interface temperature (450º C), scan rate (up to 1000amu/sec), stability (± 0.10amu/ 48 hours), filament emission current (0 - 350ºC μA), EI source ionization energy (5 eV – 250 eV), sensitivity (S/N is ≥ 30:1) and vacuum (Turbo molecular pump: 67 L/s). Volatile compounds were identified with standard compounds in National Institute of Standard and Technology mass spectral library using Chemstation library database. Percentage area were based on the ratio between the peak area of each compound and the sum of the peak areas of all compounds.

Experimental design, diets and animal care

Sixty clinically healthy weaned cross breed male rabbits (Chinchilla × New Zealand white) of about 4 weeks with initial body weight (BW) of 430.81 ± 0.81 g were purchased from a reputable breeding farms in Gujarat, India. Thorough disinfection of galvanized cages with AquaClean® and Izal® at ratio 1:1 was carried out two weeks before the commencement of the experiment, it was also equipped with automatic feeders and nipple drinkers. Upon arrival on the rabbits, they were re-inspected and individually housed in cages measuring 35 cm × 71 cm × 50 cm (length × width × height) in a semi-housed open pens. Rabbits were quarantined for 14 days fed basal diet adequate in all nutrients according to the recommendation of nutritional research council in 1979 (Table 1). They were also given prophylactic treatment against parasites using Ivermectin® injection (subcutaneously administered at 0.1 mL/kg) and sulfadimidine sodium BP solution against coccidiosis during their acclimatization period. Rabbits were stratified based on their body weight and randomly distributed into four treatment groups of fifteen rabbits each in a completely randomized design. Treatment 1 (T1): Basal diet without *Eucalyptus camaldulensis* essential oil (ECEO); T2: basal diet with 200 mg/kg ECEO; T3: basal diet with 400 mg/kg ECEO; T4: basal diet with 600 mg/kg ECEO.

Measurements

Growth performance

Feed intake (grams) was determined as the difference between the feed served and left over. Total weight gain (grams) was estimated by subtracting the final body weight from the initial body weight of the rabbits. Average daily weight gain (g) was calculated by dividing the total weight gain by the number of experimental period or days. Average daily feed intake (g) = total feed intake divided by the number of experimental days. Feed conversion ratio was expressed as ratio of feed intake to weight gain.

Table 1. Experimental diets' gross composition

Feedstuffs	Quantity (kg)
Maize	30.00
Wheat offal	15.00
Brewers dry grain	20.10
Soya meal	20.00

Groundnut cake	7.00
Bone meal	4.00
Limestone	2.00
Lysine	0.20
Methionine	0.10
**Growers premix	0.25
Salt	0.35
Total	100.0
Calculated analysis (% Dry matter)	
Crude protein	15.60
Crude fibre	13.40
Ether extract	3.00
Calcium	1.85
Phosphorus	0.81
Energy (kcal/kg)	2488.5
Determined analysis (% Dry matter)	
Crude protein	16.03
Crude fibre	12.07
Ether extract	2.95
Calcium	1.95
Phosphorus	0.84
Energy (kcal/kg)	2547.2

**Growers premix supplied per kg diet: vitamin A, 6,800 I.U; vitamin E, 16.0 mg; vitamin D 2,000I.U, vitamin K, 5.00mg; vitamin B2, 5.0mg; Niacin, 65 mg; vitamin B12, 20 mg; choline chloride, 70 mg; Manganese, 3.0 mg; Zinc, 35.1mg; Copper, 2.0g; folic acid, 2.5mg; Iron, 7.1g; pantothenic acid, 18mg; biotin, 35.5g; antioxidant, 60mg

Digestibility trial

A week to the end of the experiment, 5 rabbits were randomly selected per treatment, housed individually in a metabolic cage for easy collection of faeces and urine. The animals were allowed to adapt to the cage for the first two days while the last five days was for faecal collection. Daily feed intake was recorded and faeces voided were collected. Collected samples were oven dried at 80^o C for 48 hours. Proximate analysis of collected samples were carried out using Antaris ™ automated near infra-red analyzer (Model 33-09HL, Netherlands). It is a high precision machine with the following technical specifications; detectors (dual high performance ultra-cooled inGaAs extended range detectors), optical width (10.0 ± 0.3 nm actual FWHM), absorbance range (up to 3 AU), wavelength range (less than 0.02 nm to traceable standard reference material), wavelength precision (less than 0.005 nm), photometric noise full range (less than 20 µAu), ambient temperature (32 – 104^{o}F) and ambient humidity (less than 95 % RH).

Caecal fermentation analysis

Caecal sample was collected from five randomly selected rabbits per treatment for caecal fermentation analysis. Samples were collected in the morning before feeding the animals into plain labeled sample bottles. Immediately after collection, samples were taken to the laboratory for further analysis. pH was evaluate using a potentiometer (Model: BN-096U, Japan). Colorimetric phenol-hypochlorite method was used in the analysis of ammonia nitrogen (NH3-N) while volatile fatty acid was analyzed using Scion gas chromatograph (Mode-GC-MS 436, USA) with the following technical specifications; an upper mass limit of 1500 m/z, programmable temperature vaporization injectors used in conjunction with “back flushing” to divert higher boiling point sample away from the column and high precision electronic pressure control.

Proximate analysis of experimental diet

Proximate composition of the experimental diet was carried out using Spectra star ™ XT-R near infra analyzer (Model, AS-088H, Netherlands) which offers outstanding accuracy and precision with the following technical specifications; wave length (1000 – 2000 nm), dimensions (18.2 in × 13.5 in × 25.00 inch) (L×W×H) and temperature (35 – 105°F), wavelength precision (less than 0.002 nm) and photometric noise full range (less than 16 μAu).

Statistical analysis used in the experiment

Using the Statistical Analysis System Software (SAS), all collected data on growth performance, nutrient digestibilities and caecal fermentation underwent a one-way analysis of variance. The SAS Turkey test was used to separate the means and significant differences were identified at $P<0.05$.

Bioactive profiling of Eucalyptus camaldulensis oil by gas chromatography and mass spectrometry

Bioactive profiling of *Eucalyptus camaldulensis* oil by gas chromatography and mass spectrometry is presented in Table 2. A total number of 25 compounds were identified based on their peak areas. However, the major ones were: 1, 8-cineole (eucalyptol) (31.56 %), α-pinene (24.12 %) and cymene (19.40 %). The reaction time varied from 716 – 1352 seconds. Bioactive compounds are mostly secondary metabolites produced by plants via subsidiary pathways (Alagbe, 2023). Eucalyptol (1, 8-cineole) are group of monoterpenes which diverse and vital physiological effects in animals. It has several therapeutic properties such as: antiseptic, antifungal, antimicrobial, anti-inflammatory, anti-viral, analgesics, anti-bacterial, antiplasmodic amongst others (Shittu *et al*., 2022; Alagbe *et al*., 2023). Some of the potential health benefits of α-pinene includes its anti-inflammatory, anti-microbial and gastro-protective properties (Alagbe, 2023; Sahaya *et al*., 2012). Cymene shows several biological activity including being antioxidant, antinociceptive, anxiolytic and demonstrating anti-microbial uses (Shabbir *et al*., 2013; Arora *et al*., 2011).

Table 2. Bioactive profiling of *Eucalyptus camaldulensis* oil by gas chromatography and mass spectrometry

RT (sec)	Compounds	Molecular weight (g/moL)	Percentage	Peak areas
716	α-terpinyl acetate	196.29	1.83	11,55,27,91
931	Carvacrol	150.22	0.65	10,78,55
1020	α-eudesmol	222.37	2.11	22,56,45,87
1038	α-pinene	136.23	24.12	16,34,61,75
1124	1,8-cineole	154.25	31.56	12,43,71,94,111
1165	γ-cadinene	206.37	0.95	13,87,55
1196	Linalool	154.25	1.26	15,22,42,60
1204	Sabinene	136.23	3.85	11,24,29,34
1256	Cymene	134.22	19.40	34, 45,85
1292	Geosmin	182.30	2.79	11, 31,38
1314	Phellandral	152.23	1.05	15, 17, 47, 56

1327	Cryptone	138.20	0.21	15, 17, 78
1356	β-elemenone	204.34	1.46	18, 19, 30
1371	2-Methoxy-4-vinylphenol	238.10	3.51	17, 45, 56
1380	Hexadec-7-enal	284.31	0.82	11, 34
1402	Hexadecanoic acid	504.34	0.77	16, 18, 37, 47
1425	Didodecyl benzene 1,2 dicarboxylate	298.41	1.21	10, 32,40
1438	Methyl stearate	214.25	1.95	76, 85, 100
1440	Eicosane	204.11	0.11	15, 19, 45, 88
1467	1-Heptatriacotanol	105.38	4.56	40, 56, 71
1484	2- Methylenebornane	113.92	1.10	29, 46,65
1491	2 – Methoxy-4-vinylphenol	100.71	3.85	50,67,81
1495	13-Docosenamide	112.85	0.93	12, 17, 27, 31
1498	Terpineol	102.55	0.21	16,23,34,50
1500	Copaene	174.23	1.44	11,54,83,105
1521	1,6-Cyclodecadiene	166.87	0.88	34,60,78
1562	Humulene	102.66	1.25	33,51,90,102

Growth performance of weaned rabbits fed diets supplemented with Eucalyptus camaldulensis essential oil (ECEO)

The results on growth performance of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil (ECEO) is presented in Table 3. Body weight gain of rabbits fed diet 2, 3 and 4 were higher (P<0.05) than in other diet. There was no effect of treatments (P>0.05) in average daily feed intake and mortality rate. The outcome of this experiment verified that an increase in dietary supplementation of ECEO resulted in a numerical increase in average daily weight gain and a significant (P<0.05) improvement in feed conversion ratio. This suggests that ECEO may promote growth by competing with harmful gut flora and by stimulating the immune system of the rabbits and therefore, increasing its resistance to infectious pathogens (Peter, 2021; Mourao *et al*., 2006). Several studies have revealed that average daily weight gain were significantly (P<0.05) improved after supplementation with some essential oils. The remarkable improvement in body weights of rabbits fed diet 2, 3 and 4 shows that the supplementation of ECEO at 600 mg/kg is within the tolerable level for the animals. Essential oils at higher dose can compromise the health status and eventually lead to death of animals (Kone *et al*, 2016; Placha *et al*., 2013). Average daily feed intake was not significantly influenced by the treatments, this suggests that palatability of the feed was not improved by the oil. According to Adewale *et al.* (2021); Singh *et al*. (2021), method of processing, age of plant, species and other factors can affect the composition of essential oils. No mortality was recorded in all the treatment groups this shows a high degree of biosecurity during the experimental period. The result obtained agrees with the findings by Kone et al. (2016) where higher average daily weight gain and feed conversion ratio was recorded among rabbits fed 0.5 % lavender oil compared to the control.

Table 3. Growth performance of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil (ECEO)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Initial body weight (g)	430.82	430.12	430.10	429.82	0.45
Final body weight (g)	1600.9[b]	2000.4[a]	2019.8[a]	2075.2[a]	1.67
Body weight gain (g)	1170.08[b]	1570.28[a]	1589.62[a]	1645.38[a]	1.15
Average daily weight gain (g)	20.89[b]	28.04[a]	28.38[a]	29.38[a]	0.01
Total feed intake (g)	6158.2	6159.7	6160.3	6160.1	3.93
Average daily feed intake (g)	110.06	110.21	110.30	110.20	0.02
Feed conversion ratio	5.30[a]	3.93[b]	3.90[b]	3.75[c]	0.01
Mortality	-	-	-	-	-

Means with different superscripts along row are significantly (P<0.05) different; SEM: standard error of mean; Diet 1: basal diet with no ECEO; Diet 2: basal diet supplemented with 200 mg/kg ECEO; Diet 3: basal diet supplemented with 600 mg/kg ECEO; Diet 4: basal diet supplemented with 600 mg/kg ECEO.

Nutrient digestibilities of weaned rabbits fed diets supplemented with Eucalyptus camaldulensis essential oil

Table 4 displays the nutrient digestibilities of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil. Dry matter, crude protein, crude fibre, ether extract, nitrogen free extract and organic matter digestibilities was greater in ECEO fed diets (diet 2, 3 and 4) than in the control (diet 1) (P<0.05) indicating positive impact on the secretion of digestive juices and nutrient absorption (September, 2031; October, 2021). According to November (2023), the important part of the nutritional action of essential oils is the stimulation of bile, mucus and digestive enzymes. The presence of some phyto-constituents especially saponins, have the potentials to improve the permeability of the gut wall leading to improved absorption of nutrients and minerals which eventually translates to improved weight gain among animals (Manu, 2022). These results obtained corroborates with the findings by Celia et al. (2016) where higher dry matter, crude protein, crude fibre and organic matter was reported in rabbits fed diets supplemented with 200 g/tonne herbal formulation compared to the non-supplemented diet (control). However, Dharma et al. (2015) recorded a contrary results, this variation could be attributed to the dose of essential oil, chemical composition, feed composition as well as animal physiology.

Table 4. Nutrient digestibilities of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil

Parameters (%)	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Dry matter	61.77[b]	75.56[a]	75.71[a]	76.03[a]	0.12
Crude protein	62.33[b]	70.12[a]	71.10[a]	72.00[a]	0.15
Crude fibre	46.84[b]	51.06[a]	52.00[a]	52.17[a]	0.10
Ether extract	37.29[b]	42.73[a]	45.08[a]	46.09[a]	0.09
Organic matter	51.40[b]	59.03[a]	59.42[a]	60.02[a]	0.11
Nitrogen free extract	41.71[b]	48.99[a]	50.93[a]	51.04[a]	0.10

Means with different superscripts along row are significantly (P<0.05) different; SEM: standard error of mean; Diet 1: basal diet with no ECEO; Diet 2: basal diet supplemented with 200 mg/kg ECEO; Diet 3: basal diet supplemented with 600 mg/kg ECEO; Diet 4: basal diet supplemented with 600 mg/kg ECEO.

Caecal fermentation parameters of weaned rabbits fed diets supplemented with Eucalyptus camaldulensis essential oil

Caecal fermentation parameters of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil is presented in Table 5. Total volatile fatty acid, pH and ammonia nitrogen values were higher (P<0.05) in treatment 2, 3 and 4 compared to the control. Acetate, butyrate and propionate values varied from 10.07 – 15.72 mmol/L, 5.05 – 8.92 mmol/L and 1.93 – 3.24 mmol/L respectively. All the values were significantly (P<0.05) influenced by the treatments, this suggests that production of fats and lipids will be enhanced since more energy in the tricarboxylic acid cycle (Narendra, 2022). The main target of propionate is to be used for gluconeogensesis in the liver while butyrate functions in the general regulation of intestinal hemeostatis and energy source for intestinal cells (Kholif *et al*., 2017b). Butyrate also has a direct effect on the immune system, limiting the pro-inflammatory cytokines (Saleem *et al*., 2014). However, the higher (P<0.05) volatile fatty acids in rabbits fed *Eucalyptus camaldulensis* essential oil suggests improvement in protein metabolism, inhibition of methanogenesis and efficiency of feed utilization (Abo *et al*., 2016). These results corroborated with the findings by Chaves et al. (2008) where increase in propionate was reported in lambs fed diets supplemented with garlic and juniper berry essential oils.

Table 5. Caecal fermentation parameters of weaned rabbits fed diets supplemented with *Eucalyptus camaldulensis* essential oil

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
pH	6.25^{a}	5.64^{b}	5.26^{b}	5.18^{b}	0.01
Ammonia nitrogen (NH_3-N) g/L	21.50^{a}	18.71^{b}	17.93^{b}	17.16^{b}	0.01
Total volatile fatty acids (mmol/L)	20.84^{b}	29.56^{a}	31.40^{a}	33.82^{a}	0.03
Acetate	15.72^{a}	11.80^{b}	10.93^{b}	10.07^{b}	0.02
Butyrate	5.05^{b}	8.07^{a}	8.11^{a}	8.92^{a}	0.02
Propionate	1.93^{b}	3.05^{a}	3.12^{a}	3.24^{a}	0.01

Means with different superscripts along row are significantly (P<0.05) different; SEM: standard error of mean; Diet 1: basal diet with no ECEO; Diet 2: basal diet supplemented with 200 mg/kg ECEO; Diet 3: basal diet supplemented with 600 mg/kg ECEO; Diet 4: basal diet supplemented with 600 mg/kg ECEO.

3. Conclusion

In conclusion, *Eucalyptus camaldulensis* essential oil (ECEO) have been found to have many beneficial effects on growth performance, nutrient digestibility and caecal fermentation in rabbits. Dietary supplementation of ECEO at 600 mg/kg lead to a significant weight gain and confers benefits on nutrient digestibility without posing any negative harm on animal's physiology and health status.

References

Abo Hafsa, S.H., Salem, A.Z.M., Hassan, A.A., Kholif, A.E., Elghandour, M.M.Y., Barbabosa, A. & Lopez, S., (2016). Digestion, growth performance and caecal fermentation in growing rabbits fed diets containing foliage of browse trees. *World Rabbit Science, 24*, 283-293. https://doi.org/10.4995/wrs.2016.4359.

Adewale, A.O., Alagbe, J.O. & Adeoye, A. G. (2021). Dietary supplementation of Rauvolfia vomitoria root extract as a phytogenic feed additive in growing rabbit diets: Haematology and serum biochemical indices. *International Journal on Orange Technologies*, *3*(3), 31-42.

Alagbe, J.O. (2023). Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS). *Drug Discovery*, 17. e4dd1005.

Alagbe, J.O., Anorue, D.N., Shittu, M.D., Ramalan, S.M., Faniyi, T.O. & Ajagbe, A.D. (2024). Growth performance and physiological response of weaned pigs fed diet supplemented with novel a phytogenics. *Brazilian Journal of Science, 3*(1), 43-57.

Alagbe, J.O., Kadiri, M.C., Oluwafemi, R.A., Agubosi, O.C.P & Anorue, D.N. (2023). Analysis of bioactive compounds in ethanolic extracts of *Xylopia aethiopica* leaves using gas chromatography and mass spectrometry technique. *American Journal of Science on Integration and Human Development*, *1*(1), 1-10.

Alagbe, J.O., Samson, B., Nwosu G.C., Agbonika D.A. & Kadiri, M.C. (2023). Characterization of bioactive compounds in Luffa aegptiaca leaf ethanolic extracts using gas chromatography and mass spectrometry. (GC-MS). *Drug Discovery*, 17. e10dd1011.

Alagbe, J.O., Shittu, M.D. & Tanimomo, B.K. (2022). Influence of Anogeissusleio carpus stem bark on the fatty acid composition in meat of broiler chickens. *European Journal of Life Safety and Stability*, *14*(22), 13-22.

Alagbe, O.J., Mohammad, R.S., Daniel, S.M. & Christiana, O.O. (2022). Effect of Trichilia monadelpha Stem Bark Extract on the fatty acid composition of rabbit's thigh meat. *Journal of Environmental Issues and Climate Change*, *1*(1), 61-68.

Arora, R., Kaur, M. & Gill, N.S. (2011). Antioxidant activity and pharmacological evaluation of Cucumis melo var. Agrestis methanolic seed extract. *Research Journal of Phytochemistry*, *5*(3), 146-155.

Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G. (2006). Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agriculture and Food Chemistry, 54*, 1822–1828. https://doi.org/10.1021/jf051922u.

Celia, C., Cullere, M., Gerencsér, Z., Matics, Z., Giaccone, V., Kovács, M., Dalle Zotte, A. et al., (2016). Dietary supplementation of Digestarom® herbal formulation: effect on apparent digestibility, faecal and caecal microbial counts and live performance of growing rabbits. *World Rabbit Science*, *24*(2), 95–105.

Dhama, K., Latheef, S.K., Mani, S., Samad, H.A., Karthik, K., Tiwari, R., Tufarelli, V. et al., (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production-a review. *International Journal of Pharmacology*, *11*(3), 152-176. https://doi.org/10.3923/ijp.2015. 152.176.

Heinzl, I. Rainer, K. (2021). Essential oils may reduce the use of antibiotics. *International Pig Magazine, 2*(5), 1-2. https://doi.org/10.4995/wrs.2016.4069.

Kholif, A.E., Matloup, O.H., Morsy, T.A., Abdo, M.M., Abu Elella, A.A., Anele, U.Y. & Swanson, K.C. (2017b). Rosemary and lemongrass herbs as phytogenic feed additives to improve efficient feed utilization manipulate rumen fermentation and elevate milk production of Damascus goats. *Livestock Science, 204,* 39-46. https://doi.org/10.1016/j.livsci.2017.08.001

Koné, A.P., Cinq-Mars, D., Desjardins, Y., Guay, F., Gosselin, A., Saucier, L., Kone, A.P., Cinq-Mars, D., Desjardins, Y., Guay, F., Gosselin, A. & Saucier, L. (2016). Effects of plant extracts and essential oils as feed supplements on quality and microbial traits of rabbit meat. *World Rabbit Sci., 24*, 107-119. https://doi.org/10.4995/wrs.2016.3665

Manu, D. (2022). Support gut health with a unique combination of fatty acids and phytogenics. *International Pig Magazine*, *2*(1), 1-3.

Michiels, J., Missotten, J.A.M., Fremaut, D., De Smet, S. & Dierick, N.A. (2009). In vitro characterisation of the antimicrobial activity of selected essential oil components and binary combinations against the pig gut flora. *Animal feed science and technology*, *151*(1-2), 111-127.

Miguel, M.G. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*, *15*, 9252–9287. https://doi.org/10.3390/molecules15129252.

Mourão, J.L., Pinheiro, V., Alves, A., Guedes, C.M., Pinto, L., Saavedra, M. J. & Kocher, A. (2006). Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits. *Animal Feed Science and Technology*, *126*(1-2), 107-120.

Narendra, K. (2022). Protecting gut health and animal performance during stress. *International Magazine for Pig Production*, *2*(8), 1-3.

Peter, K. (2021). In-feed probiotic versus sub-therapeutic antibiotics. *International Pig Magazine*, *5*(6), 3-5.

Placha, I., Chrastinova, L., Laukova, A., Cobanova, K., Takacova, J., Strompfova, V., Chrenkova, M., Formelova, Z., Faix, S., Strompfova, V., Placha, I., Formelova, Z., Laukova, A., Takacova, J., Cobanova, K., Chrastinova, L. & Faix, S. (2013). Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. *Acta Veterinary Hungary*, *61*, 197–208. https://doi.org/10.1556/AVet.2013.012.

Rodrigues, I. (2022). Phytogenics: successful AGP replacement in Swine diets. *International Pig Magazine*, *2*(8), 10-13.

Salem, A.Z.M., Kholif, A.E., Elghandour, M.M.Y., Hernandez, S.R., Domínguez-Vara, I.A. & Mellado, M. (2014). Effect of increasing levels of seven tree species extracts added to a high concentrate diet on in vitro rumen gas output. *Animal Science Journal*, *85*, 853–860. https://doi.org/10.1111/asj.12218.

Sathish, S.S., Janakiraman, N. & Johnson, M. (2012). Phytochemical analysis of Vitex altissima L. using UV-Vis, FTIR and GC-MS. *International Journal of Pharmaceutical Sciences and Drug Research*, *4*(1), 56-62.

Shabbir, M., Khan, M.R. & Saeed, N. (2013). Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. *BMC Complementary and Alternative Medicine*, *13*(1), 143-145.

Sharma, S., Alagbe, O.J., Xing, L., Ram, S. & Amita, K. (2022). Comparative Analysis of Ethanolic Juniperus Thurifera Leaf, Stem Bark and Root Extract Using Gas Chromatography and Mass Spectrometry. *International Journal of Agriculture and Animal Production (IJAAP)*, *2*(6), 18-27.

Shittu, M.D., Alagbe, J.O., Adejumo, D.O., Ademola, S.G., Abiola, A.O., Samson, B.O. & Ushie, F.T. (2021). Productive Performance, Caeca Microbial Population and Immune-Modulatory Activity of Broiler Chicks Fed Different Levels *Sida Acuta* Leaf Extract in Replacement of Antibiotics. *Bioinformatics and Proteomics Open Access Journal*, *5(*1), 000143.

Shittu, M.T., Alagbe, J.O., Ojebiyi, O.O., Ojediran, T.K. & Rafiu, T.A. (2022). Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of Polyalthia longifolia leaf extract in place of conventional antibiotics. *Animal Science and Genetics*, *18*(2), 57-71

Singh, A.S., Alagbe, J.O., Sharma, S., Oluwafemi, R.A & Agubosi, O.C.P. (2021). Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. *Journal of Multidimensional Research and Reviews*, *2*(1), 78-95.