Biotechnology in Animal Husbandry 41(1), 37-55, 2025
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.086'636.92
https://doi.org/10.2298/BAH2501037A

NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE, CEACAL MICROBIAL PROFILE AND FERMENTATION CHARACTERISTICS OF RABBIT BUCKS FED *PILIOSTIGMA THONNINGII* ESSENTIAL OIL SUPPLEMENTED DIET

Emmanuel Ugochukwu Anaso[1], Olurotimi Ayobami Olafadehan[2], Ijeoma Chika Chibuogwu[3], Ayoola J. Shoyombo[4]

[1]Department of Animal Science, Faculty of Agriculture, Federal University of Agriculture Mubi Adamawa, Nigeria
[2]Department of of Animal Science, Faculty of Agriculture, University of Abuja, Nigeria
[3]Department of Theriogenology, Faculty of Veterinary Medicine, University of Abuja, Nigeria
[4]Department of Animal Science, College of Agricultural Science, Landmark University, Omu-aran, Kwara, Nigeria
Corresponding author: Emmanuel Ugochukwu Anaso, dranasoeub@gmail.com
Original scientific paper

Abstract: The purpose of this current experimental research (study) was to examine the effects of *Piliostigma thonningii* essential oil on the nutrient digestibility, growth characteristics, caecal microbes and fermentation characteristics of rabbits. The rabbits were randomly assigned to individual groups of 3, comprising precisely 15 rabbits per group, and were balanced for body weight so that each group's average initial body weight (BW) was similar—262.89 ± 22.36 g—in a fully randomised design. The first treatment (T1) was a basal control diet. In treatments 2 and 3, the basal control diet was supplemented with 2 ml PEO/kg diet and 4 ml PEO/kg diet respectively. Results for final body weight, total weight, average daily gains, feed conversion ratio, protein efficiency ratio, caecal *Lactobabacillus* spp, total volatile fatty acid ($p < 0.05$) improved with increasing level of PEO supplementation. Except for ether extract digestibility and acid detergent fibre digestibility, apparent digestibility of dry matter and all nutrients, caecal butyric and propionic acid were higher ($p < 0.05$) in T2 and T3 than in T1. Acid detergent fibre digestibility was higher ($P < 0.05$) in T3 than T1 with T2 being intermediate ($p > 0.05$) between T1 and T3, while the ether extract digestibility was highest in T3 ($p < 0.05$) and similar for T1 and T2 ($p > 0.05$). Caecal *E. coli*, Coliform bacteria and *Streptococcus* spp. counts and acetic acid were significantly higher ($p < 0.05$) in T1 than in T2 and T3. Caecal pH was not affected ($p > 0.05$) by treatments. Ammonia nitrogen was higher ($p < 0.05$) in T1 relative to T3 while T2 was intermediate between T1 and T3 ($p > 0.05$). It is concluded that the third group administered 4 ml PEO/kg diet is the optimum supplementation level, as it was more efficient in improving the performance of the

rabbit bucks and that *P. thonningii* essential oil supplementation improved feed utilisation, nutrient digestibility, growth performance of the experimental rabbits and reduced caecal pathogenic bacterial counts thereby improving health status of the experimental research bucks.

Key words: feed effects, feed utilization, gut bacteria and metabolism, phytogenic feed substance, pseudo ruminants.

Introduction

Livestock production in the tropics have faced several challenges in terms of poor performance and feed utilization coupled with the incessant misuse of synthetic antibiotics to boost production. These all have led to production of unhealthy and unwholesome meat for human consumption. The use of phytogenic feed additives has therefore been encouraged and currently been adopted to bring an end to this undesirable outcome in the finished products (Anaso and Alagbe, 2025). The aromatic qualities of plant materials where essential oils can be extracted and isolated are how Anaso (2023a) categorized essential oils. The primary source of essential oils, also known as volatile oils, is plant material. Specifically, fragrant oily liquids are extracted through distillation from plant components such flowers, buds, leaves, twigs, bark, and organic fruits and roots (Anaso, 2023a). Anaso (2023a) further described essential oils as steam-volatile or rather organic-solvent extracts that have been used traditionally for centuries in many parts of the world (Anaso et al., 2024a; Anaso et al., 2024b; Anaso et al., 2024c). They are mostly renowned for having a pleasant flavor and scent, as well as preservation characteristics. A wide variety of chemicals, including terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters, are typically present in large quantities in essential oils (EOs) (Anaso et al., 2024b; Anaso, 2023b; Negi, 2012). These compounds can protect against bacterial, fungal, and insect attacks. EO studies have found numerous benefits in terms of feed utilisation, animal health, and rabbit live performance (Anaso, 2023a; Celia et al., 2016). Camel's Foot (*Piliostigma thonningii* Schum.), also known as Monkey Bread, is a small, crooked tree commonly found in savannahs. Belonging to the *Caesalpiniaceae* family and classified by Milne-Redhead, it has dark brown to black fissured bark (Anaso et al., 2024b). It has been discovered that Piliostigma thonningii possesses distinctive taste, antioxidant, insecticidal, and antibacterial properties. Nigerian traditionalists have long used it to treat dermatosis and malaria (Anaso et al 2024b; Anaso, 2023b). Although, a fair number phytogenics and EOs have been studied in livestock diets and had positive outcomes. Regarding the impact on rabbit growth traits, digestibility, and ceacal parametric values, little is known about camel's foot (Piliostigma thonningii Schum) EO as a continuation of research Anaso et al.

(2024b); Anaso et al. (2024c); Anaso (2023a); Anaso (2023b); Anaso et al., (2023).

Many essential oils and their plant extracts as reported by Simitzis (2017); Abd El-Azeem et al. (2019); Elghalid et al. (2020); El-nomeary et al. (2020) are highly acclaimed for their significant antioxidative effects, which are primarily determined by the specific phenolic compounds in the oil and other phytochemical fractions.

The goals of this investigation were, therefore, to:
1) Evaluate the nutrient digestibility of experimental rabbits
2) Access the growth parametric characteristics and
3) Determine the caecal microbial and fermentation characteristics of rabbits fed PEO supplemented diet.

Materials and Methods

Experimental site

The Monogastric Unit of the University of Abuja Teaching and Research Farm served as the site where the experiment was conducted. As a continuation to experiment carried out by Anaso et al. (2023). The project location is between longitudes 007020' and 007051' E and latitudes 08051' and 09037'N. The range of annual rainfall is 1,145–1,631 mm. In the rainy season, the temperature ranges from 25.8 to 30.2°C, whereas in the dry season, it is between 36 and 42°C. According to Anaso et al. (2023) and Itiowe et al. (2019), relative humidity is roughly 60% during the rainy season and 30% during the dry season.

Collection of P. thonningii seeds and extraction of the essential oil

The Piliostigma thonningii seeds were obtained from the southern Guinea savannah agro ecological zone using the same methods as Anaso et al. (2023). The seed was identified and confirmed at the Department of Biological Science by a qualified taxonomist from the Forestry Research Institute of Nigeria (FRIN) similar to researches conducted (Anaso et al., 2024b; Anaso et al., 2024c; Anaso and Alagbe, 2025).

The essential oil was extracted using the steam distillation process. Using the Clevenger apparatus, the essential oil was extracted in accordance with the methodology that Anaso et al. (2023) adopted from Mohamed et al. (2006). Before being extracted, the P. thonningii seeds were finely crushed, shade-dried, and kept at room temperature. About 100 g of dried ground sample was suspended in 700 ml of distilled water using a distillation process at 100°C for about three hours, as described by Anaso (2023b), where the condenser was connected to a water input and exit, and the ground seed sample was placed in a steel device to heat up and soften the essential oil in vaporized form (Anaso et al., 2024b; Anaso et al., 2024c).

The resultant vapourized essential oil droplets congregated in a cooling system after mixing with steam, the carrier. Using the same process as Anaso et al. (2024b); Anaso et al. (2024d); Anaso et al. (2023), the essential oil was then extracted using a collection tube, and the following formula was used to determine the percentage of oil content.

% Oil content (v/w) = volume of the oil extracted (ml)/weight of the sample taken (g) x 100

Ethical Acceptance

After the research proposal was presented on November 17, 2022, the Animal Ethics and Conduct Board of the Department of Animal Science University of Abuja, Nigeria, gave ethical approval with approval registration number 19/501/ANSJ/002. The approval, which was given prior to the thesis's advancement, was closely followed and examined to ensure that it complied with international guidelines for rabbit research (Kiani et al., 2022; Anaso and Olafadehan, 2025).

Management and treatment of experimental animals

The trial was a follow-up to an experiment by Anaso et al. (2023) in which 45 male Dutch rabbits, at 5 weeks of age, weighing approximately 262.89 ± 22.36 g and in good clinical health, were weaned. The rabbits were purchased from the National Animal Production Research Institute (NAPRI, ABU, Zaria) in Nigeria. The hutches and the area around them were completely cleaned, including sweeping and washing, two weeks before to the rabbits' arrival. After that, Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and Morigad antiseptic were used to disinfect the area. The animals were kept apart and given preventative care for a period of two weeks. As part of the treatment, drinking water was infused with electrolyte supplement (Vitalyte®). To control endo and ecto parasites, an intramuscular parenteral injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg BW and a subcutaneous injection of an anti-parasitic medication (Avomec®) at 0.5 mg/kg of the animal body weight (BW) were used. In addition, at the start of the study, rabbits received a single 1mL subcutaneous injection of coccidiostat (Sulphadimidine Sodium BP solution), administered in accordance with the manufacturer's recommendations.

The rabbits were kept in separate metabolic hutches with open sides so that urine and faecal waste could be kept apart. As described in previous research by Anaso et al 2024b, a strong disinfectant was used every day to clean the hutches. A weighing scale was used to weigh each rabbit individually in order to determine their starting body weight. Three groups of fifteen rabbits each were formed. They were balanced for BW so that rabbits in each group had a comparable average

beginning BW and then assigned to one of three treatments in a completely randomised design.

A control diet was formulated in compliance with the National Research Council's (NRC's) 1994 guidelines for growing rabbits. For a duration of 12 weeks, water and food were given freely, with feeding occurring twice a day at approximately 8:00 a.m. and precisely 4 p.m. Rabbits in T1 (first group) were fed a basal/control (general) diet. In the other treatments, the control (general) diet was supplemented precisely two and four mL of Piliostigma thonningii essential oil per kilogram of the general diet.

Table 1. Composition of ingredients (%) in the experimental diet

Ingredient	Quantity
Corn	30.00
Bean husk	20.00
Soy flour	7.00
Maize bran	20.00
Peanut cake	19.40
Bone-based compost	2.00
Sodium chloride	0.30
Calcium carbonate	1.00
Vitamin: Mineral Premix	0.30
Total Quantity	100.00

Growth performance

Precise weight of individual experimental animal was determined weekly using a commercial weighing scale. Total weight gain (TWG) was obtained by subtracting the starting (initial) body weight (INBW) from the final body weight (FBW), while the average daily weight gain (ADWG) was obtained by division of the TWG by the complete number of experimental days, feed conversion ratio as the division of the average daily feed intake (ADFI) by the average daily gain, protein efficiency ratio (PER) as the division of the ADG by the crude protein intake all as explained and stated by Olafadehan et al. (2014) using the mathematical equations: TWG = FBW – INBW; ADG = TWG/Experimental days; FCR = AFI/ADG; PER = ADG/CPI.

Digestibility trial

At the 15th week of the experiment at 5 months of age of experimental rabbits, for the final 7 days of the trial, daily feacal samples were obtained in the early hours of the day prior to feeding to determine total tract digestibility. Every day, faecal sample was obtained from each treatment's animals using the technique

outlined by Aregheore (2004). The daily faecal production for each animal under the particular treatment was calculated, and twenty percent of the total amount collected was reserved for the analysis of dry matter. Samples of the excrement were oven dried for 48 hours at 70°C in a force-air oven, according to Olafadehan et al. (2014). The dried faecal samples of individual rabbit were then bulked over the 7 days collection period and sub-samples was taken and milled with a simple laboratory mill. The milled sub-samples were then stored in airtight bottles pending analysis.

Caecal fermentation and microbial profile

Ceacal content was collected from euthanized rabbits from subsequent incised caecum using a cotton swab stick. The total number of caecum bacteria content (*Escherichia coli*, coliform, *lactobacilli* and *streptococcus* populations) was quantifed according to document reported by American Public Health Association (Elghalid et al., 2020; Anaso and Al-hassan, 2025). Isolation of bacteria from the caecal epithelium was effectively achieved using the method as explained by the Food and Drug Administration (BAM, 1998). A reciprocal value of the dilution exponent is used to characterize the dilution factor. The units that combine to form colonies are called colony forming units, or CFU/g (Barnes and Impey, 1970). This value is stated in this way (Elghalid et al., 2020). Using the CFU technique, an incubation period of two to seven days was observed at around 30 ℃. The caecal microbiological contents of exactly 8 rabbits per treatment were gathered. On the proper selective and non-selected agar plates, the numerical quantities of anaerobic bacteria, lactose enterobacteria, and coliform bacteria were enumerated (Elghalid et al., 2020). After that, the colony count was represented in log 10 CFU in relation to 1 g of material (Abd El-Azeem et al., 2019; Anaso and Al-hassan, 2025). The caecum and ileum digesta were extracted using a gentle finger stripping technique in order to measure the pH, total volatile fatty acids (TVFAs), and ammonia-N levels (Abd El-Azeem et al., 2019). According to Said et al. (2016), the pH readings were ascertained right away. The digesta residue was separated into two halves and stored in a deep freezer at -20 °C until needed for additional analysis. The consort pH meter model P 107 with combined electrode was used to measure the pH values. The distillation method was used to estimate the ammonia-N concentration in accordance with Association of Official Analytical Chemists (AOAC, 2000). Warner (1964) noted that steam distillation was used to evaluate the quantity of the TVFA (Said et al., 2016).

Analytical methods

Feed and faecal samples of each treatment was ground to pass a 1 mm sieve in a Wiley mill and stored for further analysis. Proximate analyses of crude protein (CP), ash, ether extract (EE) and crude fibre (CF) of the experimental samples were carried in accordance with the procedures of AOAC (2000). Neutral

detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the methods of Van Soest et al. (1991).

Statistical analyses

Data on nutrient digestibility, BW gain and nutrient utilization, caecal fermentation and microbial profile were subjected to variance analysis in a thoroughly randomized design with SPSS (23.0). The Duncan multiple range test was performed using the same software at a significance level of $p < 0.05$ to determine the difference between means as described and adopted from Anaso et al. (2024b); Olafadehan et al. (2023). Below is the statistical model that was employed.

$Yij = \mu + ti + eij$

Where:
Yij is the overall response to the particular parameter in question."μ," Each observation's unique overall mean, when it comes to measurable parameters, tij is the fixed effect of dietary interventions (treatments) (i = 3). For every exact estimate, eij is the random error term.

Results

Experimental diet composition

Table 2 shows the experimental diet's chemical composition.

Table 2. **Chemical composition of experimental diet**

Chemical components	% composition
Dry matter (% fresh material)	88.06
Crude protein	16.54
Ether extract	2.26
Crude fibre	13.30
Ash	9.44
Neutral detergent fibre	32.06
Acid detergent fibre	18.72
Organic matter	90.56
Nitrogen free extract	58.46

Nutrient digestibility of rabbits bucks

Table 3 shows the nutrient digestibility of rabbits supplemented with PEO in the diet. The dry matter digestibility (DMD) (60.10 – 70.98%), crude protein digestibility (CPD) (68.72 – 76.50%), crude fiber digestibility (CFD) (34.69 – 46.03%), Neutral detergent fibre digestibility (NDFD) (38.09 – 54.26%) and organic matter digestibility (OMD) (58.35-66.39%) followed the same trend with similar and higher ($p < 0.05$) digestibility in rabbits supplemented with PEO (T2 and T3) compared to the control group. Ether extract digestibility (EED) (53.92 – 73.26%) was higher ($p < 0.05$) in T3 than T1 and T2, which had similar ($p > 0.05$) digestibility. Acid detergent fibre digestibility (ADFD) (29.22 – 45.37%) was highest in T3 and lowest in T1 ($p < 0.05$) but was similar ($p > 0.05$) between T1 and T2, and T2 and T3.

Table 3. **Nutrient digestibility of rabbits supplemented PEO**

Parameter (%)	T1	T2	T3	SEM
Feed intake	26.03^{b}	27.36^{ab}	29.62^{a}	1.17
Dry matter digestibility	60.10^{b}	66.44^{a}	70.98^{a}	2.54
Crude protein digestibility	68.72^{b}	75.28^{a}	76.50^{a}	2.27
Ether extract digestibility	53.92^{b}	60.87^{b}	73.26^{a}	3.99
Crude fibre digestibility	34.69^{b}	41.85^{a}	46.03^{a}	2.83
Neutral detergent fibre	38.08^{b}	48.27^{a}	54.26^{a}	3.49
Acid detergent fibre	29.22^{b}	36.57^{ab}	45.37^{a}	4.23
Organic matter digestibility	58.35^{b}	64.08^{a}	66.39^{a}	2.31
Nitrogen free extract	43.19^{b}	51.79^{a}	57.60^{a}	2.92

^{abc} Means with the different superscripts along the row are significantly ($p < 0.05$) different
T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

Feed utilisation and growth performance of rabbits supplemented PEO

Table 4 shows the feed utilisation and growth parameters of animals administered PEO supplemented in diet. There were not any significant ($p > 0.05$) differences in the INBW among the three experimental groups. FBW (1096.00 – 2536.67 g), TWG (828.00 – 2275.33 g), ADG (9.90 – 27.08 g/d) and protein efficiency (2.10 – 3.99) followed the similar trend and increased with increasing level of PEO supplementation ($p < 0.05$). FCR (1.80 – 3.61) decreased with increasing level of PEO supplementation ($p < 0.05$).

Table 4. **Feed utilisation and growth performance of rabbits supplemented PEO**

Parameter	T1	T2	T3	SEM
Initial body weight (g)	268.00	259.33	261.33	56.06
Final body weight (g)	1096.69^{c}	1664^{b}	2536.67^{a}	175.93
Total weight gain (g)	828.00^{c}	1404.67^{b}	2275.33^{a}	146.44
Average daily gain (g/d)	7.89^{c}	13.38^{b}	21.67^{a}	1.40
Total feed intake (g)	2926.63^{b}	2984.95^{ab}	3162.40^{a}	92.71
Average daily feed intake	32.16^{c}	39.36^{b}	46.34^{a}	1.23
Feed conversion ratio	4.52^{a}	3.01^{b}	2.23^{c}	0.34
Protein efficiency ratio	2.10^{c}	2.90^{b}	3.99^{a}	0.25

^{abc} Means with the different superscripts along the row are significantly ($p < 0.05$) different
T1, 0 ml of *P. thonningii* essential oil; T2, 2 ml administration of *P. thonningii* essential oil; T3, 4 ml of *P. thonningii* essential oil/kg diet.

Ceacal microbial profile of rabbits supplemented PEO

Table 5 shows the ceacal microbial profile of rabbits fed PEO supplemented diet. Counts of ceacal *Escherichia coli*, coliform bacteria and *Streptococcus* species varied from 4.53 – 7.13, 3.72 – 6.42 and 1.59 – 3.01 Log_{10}CFU/g respectively with T1 having higher counts ($p < 0.05$) than T2 and T3 which had similar ($p > 0.05$) counts. Contrarily, *Lactobacillus* species (12.11 to 17.40 Log_{10}CFU/g) counts increased as the PEO supplementation level increased ($p < 0.05$).

Table 5. **Ceacal microbial profile of rabbits supplemented PEO**

Parameter (Log_{10}CFU/g)	T1	T2	T3	SEM
Escherichia coli	7.13^{a}	4.76^{b}	4.53^{b}	0.57
Coliform	6.42^{a}	3.72^{b}	4.03^{b}	0.38
Lactobacillus species	12.11^{c}	14.14^{b}	17.40^{a}	0.92
Streptococcus species	3.01^{a}	1.99^{b}	1.59^{b}	0.40

^{abc} Means with the different superscripts along the row are significantly ($p < 0.05$) different
T1, 0 ml *P. thonningii* essential oil; T2, 2 ml of *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

Ceacal fermentation characteristics of rabbits supplemented PEO

Table 5 shows the ceacal fermentation characteristics of rabbits fed PEO supplemented diet. The ceacal pH ranged from 5.88 to 6.06 and no showed no significance ($p > 0.05$) among treatment groups. The ammonia nitrogen (16.82 – 17.61 mg/100 ml) was highest in T1 and lowest in T3 ($p < 0.05$) but was similar ($p > 0.05$) between T1 and T2, and T2 and T3.

Total volatile fatty acid (19.79 – 23.20 meq/100 ml) increased along treatment groups with lowest ($p < 0.05$) value in the control and highest in T3 (4 ml PEO). Acetic acid (13.03 – 14.80 meq/100 ml) was similar ($p > 0.05$) in rabbits supplemented with PEO which had lower concentrations ($p < 0.05$) than the control.

Butyric and propionic acid varied between 2.65 to 4.11 meq/100 ml and 0.92 to 3.25 meq/100 ml respectively. Higher and similar ($p > 0.05$) values were observed in groups supplemented with PEO, while lower ($p < 0.05$) values were seen in the control.

Table 6. **Caecal fermentation characteristics of rabbits supplemented PEO**

Parameter	T1	T2	T3	SEM
pH	6.39	5.73	5.55	0.40
Ammonia nitrogen (mg/100 ml)	17.61^{a}	17.20^{ab}	16.82^{b}	0.24
Total volatile fatty acid (meq/100 ml)	19.79^{c}	21.01^{b}	23.20^{a}	0.32
Acetic acid (meq/100 ml)	14.80^{a}	13.81^{b}	13.03^{b}	0.33
Butyric acid (meq/100 ml)	2.65^{b}	3.88^{a}	4.11^{a}	0.19
Propionic acid (meq/100 ml)	0.92^{b}	2.13^{a}	3.25^{a}	0.47

^{abc} Means with the different superscripts along the row are significantly ($p < 0.05$) different
T1, 0 ml *P. thonningii* essential oil; T2, 2 ml of *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

Discussion

Chemical composition of experimental diet

According to Anaso et al. (2024b) and Anaso et al. (2024c), the diets' normally high DM is crucial since it indicates that there is less moisture and more nutrients. DM is a specific feed component that contains the nutrients, according to Al-Hassan and Anaso (2024) and Mowlem (1992). The experimental diet had a moisture level slightly above 10%, as shown by the DM of 88.06% fresh matter. This low moisture level is sufficient to stop mold formation, increasing the diet's

ideal shelf life (Anaso et al., 2024b). The NRC (1984) report on raising rabbits said that the CP was between 16 and 17.5%. The current study's CP of 16.54% is comparable to the 16% crude protein requirement for rabbits raised in small and medium-sized businesses (Lebas, 2013; Anaso et al., 2025).

Nutrient digestibility of rabbits fed Piliostigma thonningii essential oil supplemented diet

Digestibility for DM, CP and OM was significantly highest for experimental animals supplemented with the highest (4 ml) PEO. The improved digestibility in this study was similar to the work of El Nomeary et al. (2020), who added garlic essential oil to the maturing rabbits' basic diet and reported that it affected the CPD and CFD due to increased amounts of protein available at the cellular level for deposition in the body tissues. The result deduced this study was also researched to be similar to the findings of Patra et al. (2001); Shehata et al. (2003) and Hernandez et al. (2004) who attributed improved digestibility in their various experiments to garlic essential oil supplementation at different levels. This current result which showed an improved digestion of several nutrients, especially CP, may be due to an evenly enhanced increment in healthy and good microbes which increase the rate of stomach passage and physiologically improve food digestion by stimulating the release of bodily gastrointestinal enzymes (feed intake) (Anaso et al., 2025).

Feed utilisation and growth performance of rabbits fed Piliostigma thonningii essential oil supplemented diet

Devandra and Sere (1993) reported and implicated the plane of nutrition to clearly affect body weight gain (BWG) in domestic animals. The insignificant difference in the INBW rabbits indicates the effectiveness of randomisation of the animals which further justified the adoption of complete randomised design.

FBW, TWG and ADG were highest in treatment supplemented with 4 ml PEO. FBW was in line with the work of Omer et al. (2012) who administered Fennel (*Foeniculum vulgare*) and Oregano (*Origanum vulgare L*) EO as supplementary dietary additives improved BWG. El-Radwan and Abdel-Khalek (2007) supplemented herb mixture revealed that TWG was improved.

Feed conversion was highest in control but most efficient in treatment supplemented with 4 ml PEO. El Radwan and Abdel-Khalek (2007) and El-Nomeary et al. (2020) reported essential oils and some herbal feed additives to improve ADG and FCR for rabbits. According to El Nomeary et al. (2020), the taste and odor of the active ingredients prevent animals from consuming them, which presents a number of difficulties for the provision of herbs and essential oils to animals. Generally, a lower FCR indicates the feed to be of high potential

proving that the PEO supplemented treatment ensured efficient feed consumption, which implies better output to less input (feed). Similar to result in the current findings, Ahmed et al. (2018) reported FCR to be improved by thyme essential oil supplementation under hot environmental conditions which attributes the positive bioactive compounds on digestive efficiency leading improved FCR.

It was tentatively finalized (concluded) that the improvement reported in growth parameters could definitely be directly caused by the synergistic properties of PEO. The beta-mycerne and limonene constituent via the bioactive hexadecane, 1-methyl naphthalene and oleic acid hydrocarbons was responsible to have enhanced the body weight due to improved feed intake in animals administered the PEO. The increased weight (FBW, TWG and average daily weight gain) of T3 (with highest PEO concentration) suggests that the bioactive hydrocarbons of the PEO were effective and beneficial in enhancing the growth performance of the rabbits.

The higher FBW, TWG, ADG, FCR and PER in T3 can be adduced to the higher FI and digestibility suggesting better feed palatability and protein availability which was of benefit to the research rabbits due to PEO supplementation.

Caecal microbial profile of rabbits fed Piliostigma thonningii essential oil supplemented diet

Treatments (T2 and T3) significantly decreased the numbers of coliform bacteria, *Escherichia coli,* and *Streptococcus* species in comparison to the control group. Less coliforms were discovered in the caecum of rabbits fed a diet supplemented with 0.5 g/kg DM of thyme essential oil, according to Elghalid et al. (2020) and Placha et al. (2013). According to Elghalid et al. (2020) and Celia et al. (2016), rabbits fed a diet supplemented with a combination of essential oils, herbs, spices, and extracts known as a natural herbal formulation showed a consistent and significant decrease in bacterial divergency in their caecum. The microbial ecology of the gastrointestinal tracts, particularly the intestine, is altered by a clearly noticeable decrease in pathogenic bacteria, favoring species that are beneficial to the body (Michiels et al., 2009). This leads to an improvement in the ability of epithelial cells to regenerate intestinal villus, thereby amplifying the intestine's absorptive capacity (Mourao et al., 2006). The adhesion, colonization, and proliferation of Escherichia coli and numerous other pathogens in the digestive tracts of domestic broilers are successfully inhibited by phenolic compounds present in essential oils, herbs, and extracts (Jang et al., 2007; Simitzis, 2017). Elghalid et al. (2020) demonstrated that phytogenic extracts containing wide antimicrobial action (antibacterial, antifungal, and antiviral qualities) are present in several known EOs and aromatic plants. Numerous digestive disorders that have been connected to higher rates of morbidity and mortality in young and growing

rabbits are caused by pathogenic bacteria, which are commonly found in the intestinal tracts of rabbits (Elghalid et al., 2020).

Similar to the work of Elghalid et al. (2020), it was clearly demonstrated from the current experiment that the maximum dose of P. thoninngii EO (T3) increased the numbers of Lactobacillus spp. bacteria, which are favorable. EOs, natural herbs, and general phytogenic extracts have been shown to reduce harmful bacteria while fostering beneficial microbes such *Lactobacillus* spp. (Elghalid et al., 2020; Simitzis 2017). One prevalent and well-known resident of the rabbit intestinal microbiota that supports healthy gut activity and function is *Lactobacillus* spp. (Elghalid et al., 2020; Celia et al., 2016). By continuously generating low-molecular weight peptides that cause immunological activation, lactobacilli bacteria interact with the gut immune complex system and offer temporary protection against both simple and complex medical disorders (Muir et al., 2000). Additionally, Elghalid et al. (2020) agreed with research conducted by Rinttila and Apajalahti (2013), explaining that changing the receptors that these bacteria employ, brings about an increase in Lactobacilli bacteria thereby enhancing colonization resistance against pathogenic microorganisms.

Antibacterial properties of sesquiterpene and monoterpene hydrocarbons through bioactive hexadecanoic acid, limonene and alpha-pinene of *Piliostigma thonningii* essential oil was found to be potent against the harmful microorganisms in the current investigation.

Caecal fermentation characteristics of rabbits fed Piliostigma thonningii essential oil supplemented diet

Total volatile fatty acids (VFA), acetic acid, butyric acid, propionic acid, ammonia-N and pH values are general measures of caecum microbial activity.

According to Abd El-Azeem et al. (2019), the groups supplemented with PEO exhibit a higher level of VFA and ammonia-N in the caecum in comparison to control animals. These alterations in the fermentation pattern of VFA and ammonia-N suggest major changes in the caecum microorganisms. The observed rise in T2 and T3 levels could suggest the presence of minimal or no bacterial overgrowth, elevated metabolic bacterial activity, or reduced absorptive ability of the rabbits' gut wall (Krieg et al., 2009).

Gidenne (2006) found a favorable correlation between elevated VFA and propionic acid and antioxidant activity exhibited by phytogenics, uronic acids, and different essential oils. VFA is responsible for an extra rise in rabbits' energy needs (Bassiony et al., 2015). For enterocytes to be enhanced, particularly in the lower digestive tract, butyric acid is essential (Butzner et al., 1994). This could cause the colonocytes to experience an energy deficit and reduce their ability to absorb (Krieg et al., 2009). Moreover, I-butyric acid is thought to be a sign of elevated intestine proteloytic activity (Cardona et al., 2005), and a variation in its level

signifies a change in the metabolism of bacterial proteins. The current findings are in line with those of Krieg et al. (2009) and Bassiony et al. (2015), who employed a combination of herbal feed additives on growing rabbits.

Conclusion

In the light of the present findings, PEO supplementation improved nutrient digestibility, feed utilisation efficiency, growth performance and enhanced rabbits' caecal fermentation characteristics in the context of the current findings. Supplementing rabbits with *P. thonningii* essential oil enhanced their caecal microbial profile by decreasing the number of harmful bacteria (E. coli, Coliform, and *Streptococcus* spp.) and increasing the population of beneficial bacteria (*Lactobacillus* spp.). PEO supplementation at 4 ml/kg diet is superior to 2 ml/kg diet due to better results obtained for some parameters.

Svarljivost hranljivih materija, performanse rasta, mikrobni profil u cekumu i karakteristike fermentacije zečeva hranjenih obrokom sa dodatkom eteričnog ulja *Piliostigma thonningii*

Emmanuel Ugochukwu Anaso, Olurotimi Ayobami, Ijeoma Chika Chibuogwu, Ayoola J. Shoyombo

Rezime

Cilj ovog eksperimentalnog istraživanja bio je da se ispitaju efekti esencijalnog ulja *Piliostigma thonningii* na svarljivost hranljivih materija, karakteristike rasta, mikrobiom cekuma i karakteristike fermentacije kod zečeva. Zečevi su nasumično raspoređeni u pojedinačne grupe od po 3 jedinke, koje su obuhvatale tačno 15 zečeva po grupi, i bili su uravnoteženi po telesnoj masi tako da je prosečna početna telesna težina (TT) svake grupe bila slična - 262,89 ± 22,36 g - u potpuno randomizovanom dizajnu. Prvi tretman (T1) bio je bazalna kontrolna dijeta. U tretmanima 2 i 3, bazalna kontrolna dijeta je dopunjena sa 2 ml PEO/kg obroka i 4 ml PEO/kg obroka, respektivno. Rezultati za konačnu telesnu masu, ukupnu masu, prosečne dnevne priraste, konverziju hrane, efikasnosti proteina, *Lactobabacillus* spp. u cekumu, ukupne isparljive masne kiseline ($p< 0,05$) su poboljšani sa povećanjem nivoa suplementacije PEO. Osim svarljivosti etarskog ekstrakta i ADF svarljivosti vlakana, prividne svarljivosti suve materije i svih hranljivih materija, cekalna buterna i propionska kiselina bile su veće ($p < 0,05$) kod T2 i T3 nego kod T1. ADF svarljivost vlakana bila je veća ($P < 0,05$) kod T3 nego kod T1, pri čemu

je T2 bio na sredini ($p > 0,05$) između T1 i T3, dok je svarljivost etarskog ekstrakta bila najveća kod T3 ($p < 0,05$) i slična za T1 i T2 ($p > 0,05$). Broj *E. coli*, koliformnih bakterija i *Streptococcus* spp. u cekumu i sirćetna kiselina bili su značajno veći ($p < 0,05$) kod T1 nego kod T2 i T3. Tretmani nisu uticali na pH vrednost cekuma ($p > 0,05$). Amonijačni azot je bio veći ($p < 0,05$) kod T1 u odnosu na T3, dok je T2 bio na sredini između T1 i T3 ($p > 0,05$). Zaključeno je da je treća grupa, kojoj je dato 4 ml PEO/kg obroka, optimalan nivo suplementacije, jer je bila efikasnija u poboljšanju performansi zečeva i da je suplementacija eteričnim uljem *P. thonningii* poboljšala korišćenje hrane, svarljivost hranljivih materija, performanse rasta eksperimentalnih zečeva i smanjila broj patogenih bakterija u cekumu, čime se poboljšava zdravstveno stanje eksperimentalnih zečeva.

Ključne reči: efekti hrane, korišćenje hrane, crevne bakterije i metabolizam, fitogena hranljiva supstanca, pseudopreživari.

Conflict of interest
The authors declare that they have no conflict of interest.

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Received 17 January 2025; accepted for publication 11 April 2025