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To Cite:

ANASO EU. Immune Status, Reproductive Potential, Caecal Microbial and Fermentative Characteristics of Rabbits Supplemented Rolfe (*Daniellia Oliveri*) Leaf Extract Essential Oil Based Diet. *Discovery* 2025; 61: e19d3121
doi: <https://doi.org/10.54905/disssi.v61i338.e19d3121>

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Peer-Review History

Received: 12 March 2025
Reviewed & Revised: 25/March/2025 to 18/June/2025
Accepted: 23 June 2025
Published: 03 July 2025

Peer-Review Model

External peer-review was done through double-blind method.

Discovery

pISSN 2278–5469; eISSN 2278–5450



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Immune Status, Reproductive Potential, Caecal Microbial and Fermentative Characteristics of Rabbits Supplemented Rolfe (*Daniellia Oliveri*) Leaf Extract Essential Oil Based Diet

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ABSTRACT

This study investigated the effects of essential oil extracted from *Daniellia oliveri* leaf (DOLE) on immune function, oxidative stress markers, semen quality, and caecal microbial and fermentation characteristics in male rabbits. Animals were randomly assigned to three dietary treatments: a control group fed a basal diet, and two test groups receiving the basal diet supplemented with DOLE at two different inclusion levels. Blood, semen, and caecal samples were collected and analyzed at the end of the trial. Rabbits supplemented with DOLE showed improved immune responses, as indicated by higher levels of immunoglobulins and antioxidant enzymes than the control group. Levels of oxidative stress markers, such as malondialdehyde and cortisol, were significantly reduced in DOLE-supplemented groups. Semen characteristics—including ejaculatory volume, motility, and sperm concentration—were enhanced with DOLE supplementation, while sperm abnormalities and reaction time were lower compared to the control. Semen color and pH remained consistent across all groups. Microbial analysis revealed a reduction in harmful bacteria, such as *Escherichia coli*, coliforms, and *Streptococcus* species, in rabbits fed DOLE-supplemented diets, alongside an increased beneficial *Lactobacillus* species. Caecal pH remained stable across treatments. Ammonia nitrogen levels were reduced in rabbits receiving the higher dose of DOLE. Additionally, total volatile fatty acids increased with DOLE supplementation, while the concentrations of acetic acid was highest in the control group, the butyric, and propionic acids showed significant increase in animals supplemented DOLE. In conclusion, DOLE supplementation enhanced immune function, mitigated oxidative stress, improved reproductive traits, and positively influenced gut microbial ecology in rabbits. The higher inclusion level of DOLE demonstrated the most pronounced benefits, suggesting its potential as an effective natural dietary additive for improving overall health and productivity in small mammals.

Keywords: Rabbit bucks, tropical phytochemicals, oxidative stress indices, semen, ceecal microbes and fermentation.

1. INTRODUCTION

The livestock industry has recently had to deal with widespread incidence of ailments, particularly in Africa, due primarily to antibiotic resistance and inefficiency (Anaso and Alhassan, 2025). In order to create healthy food products (Anaso et al., 2021a and b) and livestock-production system with little impact on environmental pollution (Anaso et al., 2023c; Anaso et al., 2024c; Anaso et al., 2025), this issue has resulted in or caused the indefinite ban of the use of antibiotics in animal production in developed countries. This has prompted a search for non-chemical alternatives, such as phytochemical feed additives.

In addition, rabbit farming in Africa, especially in Nigeria, is challenged by multitudinous issues, which have led to a severe meat shortage to feed the nation's growing population (Anaso, 2023a; Anaso, 2023b; Anaso et al., 2024b). One extremely important environmental element influencing rabbit production in the tropics is high temperatures, or heat. Essential oils are a significant aromatic component of plant materials (source) that can be harvested, extracted, and isolated, according to Anaso (2023a; 2023b) (Anaso and Alagbe, 2025).

A new study initiative aims to improve the health of Nigeria's farmed rabbits by utilizing alternative feed additives to antibiotics, as a result of concerns over the widespread and careless use of antibiotics in the country's rabbit production (Anaso et al. 2025). Using medicinal plants, which are natural, less harmful, residue-free, and have been shown to contain several bioactive compounds that give them the capacity to carry out a variety of biological functions, is one such option (Wang et al., 2008; Park et al., 2015; Anaso 2023a).

Daniellia oliveri is one of the possible plants; it is abundant in vitamins, minerals, amino acids, and other secondary metabolites. *Daniellia oliveri* (Rolfe), a member of the Fabaceae family, is called West African copal tree. It is an evergreen tree that is widely available but not farmed, especially in Nigeria's savannah region (Olafadehan and Okunade, 2018; Olafadehan et al., 2020). The plant is also found in South America, Africa, the Amazon areas, and temperate and tropical regions of the world. It is a widely available, uncultivated, evergreen tree, especially in Nigeria's savannah zone (Olafadehan et al., 2020).

Additionally, the plant can be found in the Amazon region, South America, Africa, and temperate and tropical parts of the planet (Adubiario et al., 2016). Osuntokun et al. (2016) claim that *Daniellia oliveri* leaves produce a liquid oleoresin that is made up of a variety of phytochemicals, particularly oleoresins, in large but variable amounts. The Oleoresins is traditionally used as an anti-inflammatory agent, as well as in the treatment of skin and genito-urinary tract disorders. The leaves are also rich in bioactive chemicals such as alkaloids, flavonoids, tannins and saponins which confers it the ability to act as anti-inflammatory, antibacterial, anti-fungal and antioxidant agent (Daniels et al., 2013; Edeoga, 2006). Anaso et al. (2025) examined the impact of *Piliostigma thonningii* essential oil (PEO) on the nutrient and feed intake, serum biochemistry, and immune/oxidative stress responses of rabbits. They concluded that PEO supplementation decreased oxidative stress indices and improved feed utilization, as well as the immune and general health status of the experimental rabbits, even though there have been numerous studies on the beneficial effects of some plant extracts in animal nutrition (Hernandez et al., 2004; Cross et al., 2007).

Similarly, Olafadehan et al. (2020) examined the performance and haemato-biochemical parameters of broiler chicks given *Daniellia oliveri* leaf extract as an antibiotic alternative and concluded that feeding birds DOE up to 40 ml/litre enhanced overall performance and did not have any detrimental effects on the animal. Anaso et al. (2023c) investigated whether the inclusive administration of *Piliostigma thonningii* essential oil (PEO) in rabbits' diet was beneficial to their general reproductive traits and concluded that it posed no threat. Although there isn't much information available, feeding *Daniellia oliveri* to rabbits will also help close the gap between food safety and production.

2. METHODOLOGY

Collection of test material (*Daniellia oliveri* leaf) and preparation of the extract

Several mature leaves of *Daniellia oliveri* were collected from trees within the premises of the Federal University of Agriculture in Mubi, Nigeria. The mature leaves of *Daniellia oliveri* had light mid-veins which was dark green and slightly glossy. A certified taxonomist from the Forestry Research Institute of Nigeria's Department of Biological Science verified the plant. In order to eliminate soil and other

binding particles, the *Daniellia oliveri* leaf was properly rinsed with running tap water and then distilled water. It was then let dry until it reached a consistent weight and blended into a meal. To make the leaf extract, 250 grams of *Daniellia oliveri* powder were combined with 1000 milliliters of ethanol (80% BDH) and let to soak for 48 hours in an airtight container. The resulting extract was stored in a refrigerator at 4 °C for additional analysis after being filtered through regular filter paper using Whatman No. 1 filter paper.

Chemical analysis

The extract was subjected to standard chemical tests for the detecting of saponins, flavonoids, phenolics, alkaloids, steroids and glycosides using standard methods described by Harbone (1973); Odebiyi and Sofowora (1978). Tannins (Van-Burden and Robinson, 1981) and flavonoids (Boham and Kocipai-Abyazan, 1974) contents were also determined in the extracts. Proximate analysis of the leaf was carried out using the method of AOAC (2000). The mineral content of the leaf was determined using Atomic Absorption Spectrophotometer (AAS) model 392 A.

Experimental site

The experiment was done in Mubi town, which is located in Northern Senatorial District of Adamawa State, Nigeria, at the Federal University of Agriculture Mubi Teaching and Research Farm's Monogastric Unit, the Morugo Agricultural/Research site was the study's location. Situated at the base of the Mandara Mountains, which divide Nigeria from the Republic of Cameroon, Mubi is 1906 feet above sea level and falls between latitude 10.27 and longitude 13.28 (10.2801° N, 13.2774° E).

Experimental animals, management and treatment

Forty-five clinically certified healthy weaned male Dutch rabbits, which weighed an average of 0.27 kg and approximately five weeks of age, were utilized in the experiment. The rabbits were purchased from the National Animal Production Research Institute at Ahmadu Bello University in Zaria, Nigeria. The hutches and the surrounding area were sterilized and disinfected two weeks before the rabbits arrived using Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and Morigad antiseptic. The animals were quarantined for precisely two weeks and given prophylactic medication. The preventive measures included administering an anti-stress drink (Vitalyte®), injecting a parenterally administered intramuscular injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg BW, and injecting a subcutaneous dose of an anti-parasitic drug (Avomec®) at 0.5 mg/kg of the animal body weight (BW) to control endo and ecto parasites. Additionally, as advised by the manufacturer, rabbits were given a single subcutaneous treatment with coccidiostat (Sulphadimidine Sodium BP solution) at the start of the study at a dose of 1 mL/rabbit. The sporadic hutches were cleaned every day using a strong disinfectant. The rabbits were separated into three groups, each consisting of exactly fifteen rabbits per group. Once body weight (BW) was balanced, each group's rabbits were randomly assigned to one of three treatments, with their starting BWs being numerically identical. Table 1 shows the base control diet that was developed for rabbits based on the recommendations given by the NRC (1994).

For a duration of 12 weeks, water and feed were given freely, with feeding occurring twice a day at 8:00 and 16:00. In the initial treatment, a baseline control food was given to the rabbits. In the other treatments, five and ten millilitres of DOLE per kilogram of the control food were added.

Table 1: Ingredient composition (%) of the experimental diet

Ingredient	Quantity
Maize	30.00
Cowpea husk	20.00
Soybean meal	7.00
Corn bran	20.00
Groundnut cake	19.40
Bone meal	2.00
Salt	0.30
Limestone	1.00
Premix	0.30
Total	100.00

Blood collection, immune response and oxidative stress analyses

Blood samples were taken on the last day of the trial from all the rabbits in each treatment. The marginal ear veins were the site for blood collection from individual rabbits in the morning before access to feed and water. Samples were collected into several 5 ml vacuum tubes, placed in ice packs and were immediately analyzed.

Plasma was harvested for the analysis of biochemical indicators by centrifugation of whole blood at 3000 rpm over 15 min. in a laboratory centrifuge (NOP-350R, NOP medical instruments, Punjabi, India) at a temperature of 4°C. The plasma was analyzed within four hours of collection. Total protein and total cholesterol were determined, along with the activities of the following enzymes: alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate amino transaminase (AST) by calorimetric methods using Bio Maxima reagent sets (Lublin, Poland) in a Metrolab 5.0, Norway, Oslo. Albumin was determined using the Bromocresol Green (BCG) method, as explained by Anaso et al. (2025). Glucose was determined using the spectrophotometric method, as described by Allain et al. (1974). The concentration of globulin was obtained by deducting albumin values from the total protein. Also, high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine, triglyceride, uric acid and bilirubins were analysed using specific kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's recommendations, as described by Elghalid et al. (2020).

Immunoglobulins (IgA, IgG and IgM) were analysed using specific kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's recommendations, as described by Elghalid et al. (2020).

Triiodothyroxine (T3), total antioxidant capacity (TAC) and activity of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, cortisol and glutathione reductase were analyzed using specific kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's recommendations, as described by Elghalid et al. (2020).

Semen collection and Evaluation

The semen was collected by means of an artificial vagina filled with a warm liquid of about 45°C. The doe was fitted with this device and presented to bucks in their respective hutches (Boiti et al., 2005). Semen samples were evaluated immediately for volume by direct reading of the graduated Collection vial. The result was expressed in millilitres (mL). The sample were then kept in a water bath at 37°C, where necessary evaluations were made in sequence according to CBRA, (1998) animal manual. The appearance of the semen was determined by visualization of consistency of ejaculates and classified as: Creamy marble, creamy, thick milky, milky and watered. Smear of each semen sample was prepared, air dried, labelled and kept for further examination. The progressive motility was determined by placing 10 µL of semen into 1 mL of Tris dilution buffer (Hydroxymethylaminomethane (3.0 g), sodium citrate (2.0 g) and fructose (1.0 g)). A 10 µL-aliquot of the diluted semen sample was placed between preheated slide and coverslip (37°C) and evaluated under optical microscope (100 × magnification). The progressive motility was expressed as percentage. The concentration of the spermatozoa was determined using a haemocytometer crossed with microscopic grids containing 25 large squares, each containing sixteen smaller squares. The total number of smaller squares on the haemocytometer was 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares comprising 80 smaller squares (Rekwot et al., 1997). Before to counting, formaldehyde was used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The haemocytometer was mounted on the microscope stage and an absorbable tube and O-no pipette was used to pipette a drop of the solution into the haemocytometer chamber. The absorbable tube and the O-no pipette was blown before pipetting to avoid air bubbles in the O-no pette. The result obtained was recorded and used to calculate the sperm cell concentration for the sample as: sperm concentration per ml × volume of ejaculate. Sperm morphology was determined from 95 slide smears stained with 7.5% Giemsa (Doles Laboratory); the slides were immersed in the Giemsa solution for two hours, afterwards the slides were kept upright until they completely dried and viewed under the microscope to get the normal and abnormal sperm percentage. Normal spermatozoa and spermatozoa abnormalities were classified according to principles used for rabbits by Barth and Oko, (1989). The live to dead sperm ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosin stain immediately after collection. A drop of semen sample was placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample on the slide at an angle of 45 degree to make contact with the semen sample slide and carefully drawn apart to prepare a thin smear. This was allowed to dry and thereafter labelled. This was done for each sample. After that, the slides were viewed under the microscope to count

the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained. The procedure above was developed by Hancock, (1951).

Caecal fermentation and microbial profile

Caecal 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 quantified 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 °C. 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).

Statistical analyses

Data on intake, serum biochemical indices, serum minerals, immune status and oxidative stress indices, were subjected to analysis of variance in a thoroughly randomized design (approach) using SPSS (23.0). Duncan's multiple range test of the same software was used to test the differences between the means at $P \leq 0.05$ level of significance.

3. RESULTS AND DISCUSSION

Immune and oxidative stress parameters of rabbits supplemented *Daniellia oliveri* leaf extract

Immune and oxidative stress parameters of the experimental treatments are shown in Table 2. Immunoglobulin G and M, Superoxide dismutase, catalase, glutathione peroxidase, total antioxidant capacity and glutathione reductase were lower ($P < 0.05$) in T1 relative to T2 and T3, which had similar ($P > 0.05$) values, while Immunoglobulin A was significantly higher in T3, and lowest ($P < 0.05$) in T1. Melanodialdehyde and cortisol were lower ($P < 0.05$) in T2 and T3 than in T1. In order to prevent attachment to epithelial cells and neutralize toxic substances, immunoglobulins bind to pathogens (Woof and Kerr, 2005).

DOLE's antioxidant properties may be the cause of the treatment groups' (T2 and T3) greater levels of IgG, IgM, and IgA. Rabbits in T3, which received the greatest dose, had the highest immunoglobulin levels, which rose in groups given DOLE. Alagawany et al. (2021) reported that the experimental immunoglobulin levels (IgM, IgG, and IgA) were higher than those of the control group. The current study's results support their findings.

Elghalid et al. (2020) state that triiodothyronine plays a vital role in body thermoregulation (temperature, heart rate, and respiratory rate) as well as systemic physiology (growth, development, and metabolism). Similar findings were made by El-Gindy et al. (2020), who found that the essential oil treatments considerably increased the blood immunoglobulin M concentration when compared to the control. El-gogary et al. (2018) claim that MDA is a sign of cell membrane damage, and that lipid peroxidation determines how much of this marker is present. The antioxidant capacity of DOLE's phenolic component may be the reason for the treatment groups' reduced MDA. By scavenging superoxide anion and hydroxyl radicals from antioxidant chemicals, phenolic ketone derivatives, catechins, and volatile oils, certain phenolic components in feed additive combinations have demonstrated antioxidative action (Elkirdasy et al., 2015).

Elghalid et al. (2020) reported lower MDA due to the combination of phyto-genic additives with phenolic compounds that increased glutathione levels in cells, enhanced the activity of cellular antioxidant enzymes (catalase, superoxide dismutase, and glutathione

peroxidase), and scavenged reactive oxygen species to elicit an antioxidant effect. The results of reduced MDA and cortisol, while increased SOD, catalase, glutathione peroxidase, and reductase were comparable to the findings of this study (Borek, 2001). In a similar vein, Lin et al. (2003) found that consuming herbs reduced MDA levels. Therefore, as components of sesquiterpenes and monoterpene hydrocarbons, bioactive oleic acid, heptadecanoic acid, hexadecane, limonene, alpha-pinene, and beta-caryophyllene reduced the MDA, a marker of oxidative stress, and cortisol, which is released in response to stress and low blood sugar, through their antioxidant, antibiotic, and anti-inflammatory properties and characteristics (Hoehn and Marieb, 2009).

The treatment groups T2 and T3 had the highest SOD. SOD is essential for protecting cells from oxidative damage, but specific elements in the diet are necessary for this process to take place (Ashour et al., 2014). The findings are also in line with those of Lin et al. (2003), who discovered that the activity of general serum antioxidant enzymes was elevated by herb use.

DOLE may have contributed to the rise in glutathione in T2 and T3, suggesting that the production of glutathione disulfide was not inhibited. Alagawany et al. (2016) claim that glutathione prevents the peroxidation of the apolipoprotein B protein, prevents oxidative degradation of macromolecules, and protects cells from harm caused by free radicals.

The control group's blood cortisol level rose as a result of increased acetylcholine breakdown, which decreased nutritional intake and absorption and resulted in low blood sugar (Elghalid et al., 2020). In order to facilitate appropriate digestion and nutrient absorption, DOLE inhibits the breakdown of acetylcholine, which is responsible for the contraction of smooth muscles, including the involuntary peristaltic movement of the gastrointestinal muscles, through its bioactive hydrocarbon, which also serves as an acetylcholinesterase inhibitor (Anaso et al 2025).

Catalase and glutathione peroxidase and reductase are important for preventing, preserving, and essentially catalyzing the breakdown of hydrogen peroxide to water and oxygen as well as shielding cells from the scavenging effect of reactive oxygen species (ROS) (Chelikani et al., 2004; Deponte, 2013).

Table 2. Immune and oxidative stress responses of rabbits supplemented DOLE based diet

Parameter	T1	T2	T3	SEM
Immunoglobulin G (mg/dL)	85.77 ^b	124.58 ^a	130.22 ^a	4.31
Immunoglobulin A (mg/dL)	68.08 ^c	80.64 ^b	96.36 ^a	4.91
Immunoglobulin M (mg/dL)	25.07 ^b	29.99 ^{ab}	32.58 ^a	2.16
Triiodothyroxine (mg/mL)	1.65 ^b	2.84 ^a	3.62 ^a	0.38
Melanodialdehyde (nmol/mL)	94.17 ^a	72.36 ^b	69.36 ^b	2.68
Superoxide dismutase (nmol/mL)	45.35 ^b	62.41 ^a	66.71 ^a	1.83
Catalase (nmol/mL)	36.19 ^c	72.92 ^b	75.78 ^a	1.05
Glutathione peroxidase (nmol/mL)	8.65 ^b	11.77 ^a	12.07 ^a	0.40
Cortisol (nmol/mL)	19.58 ^a	13.37 ^b	13.07 ^b	0.85
Total antioxidant capacity (umol/L)	2.32 ^b	4.22 ^a	4.62 ^a	0.39
Glutathione reductase (ng/mL)	2.53 ^b	4.41 ^a	4.51 ^a	0.37

Means with the different superscripts in the same row are significantly ($P < 0.05$) different

T1, 0 ml DOLE; T2, 5 ml DOLE; T3, 10 ml DOLE/kg diet

Semen characteristics of rabbits supplemented *Daniellia oliveri* leaf extract

Table 3 shows the semen parameters of rabbits fed DOLE supplemented diet. The semen colour was similar (creamy) across treatments (T1, T2 and T3). Semen pH was 6.00 for each of the treatments and was not affected by treatments. Ejaculatory volume varied from 0.60 to 1.14 ml, sperm motility from 58.37 to 75.29% and semen concentration varied from 226.92 to 314.21 ($\times 10^6$), presenting lower ($P < 0.05$) values in T1 compared to T2 and T3 which were similar ($P > 0.05$). Reaction time from 10.66 to 24.62 secs and sperm abnormalities 9.55 to 23.31% presented higher ($P < 0.05$) values in T1 compared to T2 and T3 which were similar ($P > 0.05$).

The semen of the experimental rabbits was similar in color to that of domestic animals, as reported by Anaso et al. (2024d), Anaso et al. (2023c), and Ososanya et al. (2013). The look of high-quality semen is creamy white. Transparent semen indicates low concentration, whereas blood spots and an odd color suggest contamination or poor quality. Therefore, high-quality semen is indicated by the

identical semen colors of all rabbits, regardless of treatment. This further suggests that the quality of the semen would not be affected if DOLE supplements were added in amounts up to 10 ml/kg diet.

When it comes to sperm motility and viability in terms of their capacity to fertilize an egg, semen pH is a critical element (Zhou et al., 2015). In keeping with the findings of Zhou et al. (2015), who discovered no discernible difference in the pH of the semen from animals administered thyme essential oil (TEO) in contrast to the control group, the pH difference in the semen was not statistically significant. Rabbits have slightly acidic pH values, ranging from 6.00 to 6.33. Zhou et al. (2015) state that unusual pH readings that are higher than normal could be a sign of an underlying infection. Therefore, it could affect its feasibility. When growing rabbits were fed potato peel extract, a potent antioxidant, El-Gindy et al. (2020) saw improved semen features, such as increased volume, motility, concentration, and fewer abnormalities. The study by Anaso et al., (2023c) showed that the treatment groups of growing rabbits that received dietary thyme essential oil and phyto-genic supplement had significantly improved semen qualities. Season, collectors' factor, pooled volume, collecting frequency, or management can all lead to situations of reduced ejaculate volume (Anaso et al., 2023c). They could also be brought on by inadequate diet. Thus, a modest variation in semen volume could be due to the oxidative and antibacterial properties of the bioactive compounds in several phyto-genic feed additives (Anaso et al., 2024a).

According to Osinowo (2016), sperm motility is linked to sperm viability, a metric that indicates a high or low sperm count. Motile cells are often always viable, while the viability of non-motile cells determines whether they are alive or dead. Osinowo (2016) states that sperm motility of 65% or more is considered satisfactory. In highly concentrated ejaculates, strong, progressive motility—often seen as swirling, wave-like motions—is a crucial sign of the sperm's capacity to survive.

According to Anaso et al. (2024a), there is a clear correlation between elevated feed intake and increased semen concentration, which can also be brought on by changes in temperature and ejaculation frequency. According to Anaso et al. (2023c) and Anaso et al. (2024d), the increased sperm concentration in T2 and T3 and improved nutrient delivery for the spermatogenic process may be due to the potent antioxidant properties of beta-pinene and caryophyllene found in PEO supplements. Similar to the current findings, El-Ratel et al. (2021) found that developing rabbits given extra virgin olive oil (EVOO), betaine (BET), and ginger (GIN) as natural antioxidants showed improvements in progressive motility, vitality, sperm cell concentration, sperm outputs, and fertility.

Increased libido scores in rabbits given DOLE supplements demonstrate the impact of the essential oil's bioactive constituents, particularly its antioxidative qualities. Reaction time determines the libido score. Lower reaction time is inversely correlated with higher libido. Increasing rabbits' sexual performance is currently the focus of more research and attention than other livestock species, despite the Libido test becoming a standard procedure in breeding soundness testing for domestic animals. In general, male fertility is significantly influenced by libido, or sex drive. Gonadal and extragonadal sperm stores, semen quality, body weight, growth pace, and masculinity have little bearing on it (Anaso, 2024e).

Table 3. Semen characteristics of rabbits supplemented DOLE based diet

Parameter	T1	T2	T3	SEM
Semen colour	Cream	Cream	Cream	
pH	6.00	6.00	6.00	0.01
Semen volume (ml)	0.60 ^b	0.85 ^{ab}	1.14 ^a	0.20
Motility (%)	58.37 ^b	70.67 ^b	75.29 ^a	1.18
Semen concentration (x10 ⁶)	226.92 ^b	297.07 ^a	314.21 ^a	6.29
Abnormalities (%)	23.31 ^a	12.17 ^b	9.55 ^b	1.28
Live dead ratio	1.84 ^c	3.00 ^b	3.84 ^a	0.11
Libido/reaction time (secs)	24.62 ^a	12.32 ^b	10.66 ^b	0.67

^{abc} Means with the different superscripts in the same row are significantly ($P < 0.05$) different

T1, 0 ml DOLE; T2, 5 ml DOLE; T3, 10 ml DOLE/kg diet

Cecal microbial profile of rabbits supplemented DOLE

Table 4 shows the cecal microbial profile of rabbits fed DOLE supplemented diet. Counts of cecal *Escherichia coli*, *Coliform* species and *Streptococcus* species varied from 4.03 – 8.39, 4.23 – 7.65 and 1.54 – 4.02 Log₁₀CFU/g respectively with T1 having higher counts ($p < 0.05$)

than T2 and T3 which had similar ($p > 0.05$) counts. Contrarily, *Lactobacillus* species (11.19 to 18.32 Log₁₀CFU/g) counts increased as the PEO supplementation level increased ($p < 0.05$).

Compared to the control group, the numbers of *Streptococcus* species, *Escherichia coli*, and coliform bacteria were considerably reduced by treatments (T2 and T3). Elghalid et al. (2020) and Placha et al. (2013) found that rabbits fed a diet supplemented with 0.5 g/kg DM of thyme essential oil had less coliforms in their caecum. Rabbits given a meal supplemented with a blend of essential oils, herbs, spices, and extracts known as a natural herbal formulation shown a constant and significant decrease in bacterial divergency in their caecum, according to Elghalid et al. (2020) and Celia et al. (2016).

A discernible decline in pathogenic bacteria favors species that are good for the body, changing the microbial ecology of the gastrointestinal tracts, especially the gut (Michiels et al., 2009). As a result, intestinal villus regeneration is enhanced by epithelial cells, increasing the intestinal absorption capacity (Mourao et al., 2006). Phenolic chemicals found in essential oils, herbs, and extracts effectively suppress the adhesion, colonization, and proliferation of *Escherichia coli* and many other pathogens in the digestive tracts of domestic broilers (Jang et al., 2004; Simitzis, 2017). Elghalid et al. (2020) showed that a number of recognized Eos and aromatic plants possess phytogetic extracts with broad antimicrobial action (antibacterial, antifungal, and antiviral properties). Pathogenic bacteria are frequently discovered in rabbits' intestinal tracts and are responsible for a number of digestive diseases that have been linked to increased rates of morbidity and death in young and growing rabbits (Elghalid et al., 2020).

Anaso et al. (2025), who added *Piliostigma thoninngii* essential oil to rabbits' diet, revealed that the highest dosage of *P. thoninngii* EO T3 (four milliliters per kilogram of diet) increased the quantity of *Lactobacillus* spp. bacteria, which is advantageous, in a manner similar to that of Elghalid et al. (2020). It has been demonstrated that eos, natural herbs, and general phytogetic extracts can decrease dangerous bacteria while promoting *Lactobacillus* spp. and other helpful microbes (Elghalid et al., 2020; Simitzis 2017). *Lactobacillus* species are a common and well-known component of the rabbit intestinal microbiota that promotes normal gut activity and function (Elghalid et al., 2020; Celia et al., 2016). *Lactobacilli* bacteria interact with the gut immune complex system and provide short-term protection against both simple and complex medical illnesses by continuously producing low-molecular weight peptides that trigger immunological activation (Muir et al., 2000). Furthermore, study by Rinttila and Apajalahti (2013) was supported by Elghalid et al. (2020), who explained that altering the receptors these bacteria use increases the number of *Lactobacilli* bacteria and improves their resistance to colonization by harmful germs. Antibacterial properties of DOLE were found to be potent against the harmful microorganisms in the current investigation.

Table 4: Cecal microbial profile of rabbits supplemented DOLE based diet

Parameter (Log ₁₀ CFU/g)	T1	T2	T3	SEM
<i>Escherichia coli</i>	8.39 ^a	4.63 ^b	4.03 ^b	0.36
Coliform	7.65 ^a	4.77 ^b	4.23 ^b	6.04
<i>Lactobacillus</i> species	11.19 ^c	15.85 ^b	18.32 ^a	0.31
<i>Streptococcus</i> species	4.02 ^a	1.92 ^b	1.54 ^b	0.22

^{abc} Means with the different superscripts in the same row are significantly ($P < 0.05$) different

T1, 0 ml DOLE; T2, 5 ml DOLE; T3, 10 ml DOLE/kg diet

Cecal fermentation characteristics of rabbits supplemented DOLE

Table 5 shows the cecal fermentation characteristics of rabbits fed DOLE supplemented diet. The cecal pH ranged from 5.99 to 6.01 and no showed no significance ($p > 0.05$) among treatment groups. The ammonia nitrogen (17.33 – 18.10 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 (19.88 – 24.60 meq/100 ml) increased along treatment groups with lowest ($p < 0.05$) value in the control and highest in T3 (10ml DOLE). Acetic acid (13.03 – 14.80 meq/100 ml) was similar ($p > 0.05$) in rabbits supplemented with DOLE which had lower concentrations ($p < 0.05$) than the control. Butyric and propionic acid varied between 2.70 to 4.73 meq/100 ml and 0.89 to 2.97 meq/100 ml respectively. Higher and similar ($p > 0.05$) values were observed in groups supplemented with DOLE, while lower ($p < 0.05$) values were seen in the control. Total volatile fatty acids (VFA), acetic acid, butyric acid and propionic acid) Ammonia-N and pH values are general measures of caecum microbial activity.

In contrast to control animals, the DOLE-supplemented groups show increased levels of VFA and ammonia-N in the caecum (Abd El-Azeem et al., 2019). These modifications to the VFA and ammonia-N fermentation patterns point to significant shifts in the microbes in the caecum. The observed increase in T2 and T3 levels may indicate enhanced metabolic bacterial activity, decreased absorptive capacity of the rabbits' gut wall, or little to no bacterial overgrowth (Krieg et al., 2009).

Gidenne (2006) discovered a positive relationship between increased VFA and propionic acid and the antioxidant activity displayed by various essential oils, uronic acids, and phytochemicals. The additional increase in rabbits' energy requirements is caused by VFA (Bassiony et al., 2015; Anaso et al., 2025). Butyric acid is necessary for the enhancement of enterocytes, especially in the lower digestive tract (Butzner et al., 1994). This might result in an energy deficit for the colonocytes, which would lower their capacity to absorb (Krieg et al., 2009).

Furthermore, it is believed that higher intestinal proteolytic activity is indicated by I-butyric acid (Cardona et al., 2005), and a change in its level indicates a shift in the way bacterial proteins are metabolized. The results of Krieg et al. (2009) and Bassiony et al. (2015), who used a variety of herbal feed additives on growing rabbits, are consistent with the current findings.

Table 5: Cecal fermentation characteristics of rabbits supplemented DOLE based diet

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 in the same row are significantly ($P < 0.05$) different T1, 0 ml DOLE; T2, 5 ml DOLE; T3, 10 ml DOLE/kg diet

4. CONCLUSION

In the light of the present findings, DOLE supplementation improved immune and oxidative stress parameters, semen characteristics and enhanced rabbits' caecal fermentation characteristics in the context of the current findings. Supplementing rabbits with *P. thoningii* 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.). DOLE supplementation at 10 ml/kg diet is superior to 5 ml/kg diet due to better results obtained for some parameters.

Acknowledgement

No acknowledgements.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Ethical approval & declaration

In this article, the animal regulations are followed as per the ethical committee guidelines of Department of Animal Science, Federal University of Agriculture, Mubi, Adamawa, Nigeria; the authors observed the Immune Status, Reproductive Potential, Caecal Microbial and Fermentative Characteristics of Rabbits Supplemented Rolfe (*Daniellia Oliveri*) Leaf Extract Essential Oil Based Diet. The Animal ethical guidelines are followed in the study for species observation, identification & experimentation. Also, the ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES

1. Abd El-Azeem AE, Al-Sagheer AA, Daader AH, Bassiony SM. Effect of dietary supplementation with betaine thyme oil and their mixtures on productive performance of growing rabbits. *Zagazig J. Agric. Res.* 2019; 46: 815–28.
2. Adubiaro H, Olaofe O, Ogunbusola E, Adeyeye E. Nutritional and antinutritional attributes of underutilized tree crops: *Adansonia digitata* *Albizia lebbek* and *Daniellia oliveri* seeds. *Ekiti State Univ. J. Sci.* 2016; 2: 24–33.
3. Alagawany M, Ashour EA, Feda FM. Effect of dietary supplementation of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) on growth performance carcass traits blood profile and oxidative status in growing rabbits. *Ann. Anim. Sci.* 2016; 16: 489–505.
4. Alagawany M, El-Saadony MT, Elnesr SS, Farahat M, Attia G, Madkour M, Reda FM. Use of lemongrass essential oil as a feed additive in quails nutrition: Its effect on growth carcass blood biochemistry antioxidant and immunological indices digestive enzymes and intestinal microbiota. *Poult. Sci.* 2021; 100: 101172.
5. Anaso EU, Al-Hassan SM. Identification and antimicrobial susceptibility of *Pseudomonas aeruginosa* strain PAO1 isolated from African catfish skin scrapings. *Sci. Lett.* 2025; 13: 1324350sl.
6. Anaso EU, Olafadehan OA, Chibuogwu IC, Alagbe JO. Seminal morphology and organ morphometrics of rabbit bucks fed *Piliostigma thonningii* essential oil supplemented diet. *Sci. Lett.* 2024b; 12: 70–75.
7. Anaso EU, Olafadehan OA, Chibuogwu IC. Nutritive effects and physiologic responses of rabbits supplemented with camel's foot (*Piliostigma thonningii*) essential oil-based diet. *Vet. Arhiv.* 2025; 95(3): 307–318
8. Anaso EU, Olafadehan OA, Chibuogwu IC. Semen characteristics of rabbits fed camel's foot (*Piliostigma thonningii*) essential oil supplemented diet. *Discov.* 2023c; 59: e36d1037.
9. Anaso EU, Olafadehan OA, Emeka FS. Carcass and meat quality of rabbits supplemented with camel's foot (*Piliostigma thonningii*) essential oil-based diet. *Arch. Zootech.* 2024c; 27: 36–51.
10. Anaso EU, Olafadehan OA, Oluwafemi RA. Biodegradation of agro-industrial by-products as a panacea to feed scarcity in Nigeria amidst farmer insecurity and global pandemic. *Proc. 46th Annu. Conf. Niger. Soc. Anim. Prod.* 2021a; 46: 616–20.
11. Anaso EU, Olafadehan OA, Shoyombo AJ, Emeka FS. Body weight scrotal parameters and semen characteristics of Kano Brown bucks fed *Pleurotus ostreatus* solid-state fermented sugarcane scrapings. *Turk. J. Food Agric. Sci.* 2024d.
12. Anaso EU, Olafadehan OA, Shoyombo AJ. Semen characteristics of Kano Brown bucks fed white rot fungi (*Pleurotus ostreatus*) biodegraded sugarcane scrapings-based diets. *Proc. 46th Annu. Conf. Niger. Soc. Anim. Prod.* 2021b; 46: 625–28.
13. Anaso EU. Bioactive compounds of *Piliostigma thonningii* essential oil detected by gas chromatography-mass spectrometry. *EJABS* 2023b; 3: 68–72.
14. Anaso EU. Macro and micro anatomy of the male genitalia of the Nigerian laughing dove (*Spilopelia senegalensis*). *East. J. Agric. Biol. Sci.* 2024a; 4: 31–44.
15. Anaso EU. Phytochemicals and essential oil supplementation in rabbit and monogastric production a panacea to improved animal production and antibiotic resistance challenge in Nigeria. *EJABS* 2023a; 3: 45–51.
16. Anaso UE, Alagbe JO. Body thermoregulatory adaptation and blood serum mineral metabolic profile of rabbits supplemented camel's foot (Schum) seed essential oil based diet. *J. Chem. Sci.* 2025; 2: 1–6.
17. Anaso UE, Olafadehan OA, Chibuogwu IC, Shoyombo AJ, Mailafia S, Anaso JN, Fidelis ES. Haematological profile and fertility potential of rabbits supplemented with camel's foot (*Piliostigma thonningii*) essential oil-based diet. *Turk. J. Agric. Food Sci. Technol.* 2024e; 12: 2470–77.
18. AOAC. Official method of analysis. 15th ed. Washington D.C.: Assoc. Off. Anal. Chem.; 2000. p. 70–88.
19. Bacteriological Analytical Manual (BAM). 8th ed Rev. A. 1998.
20. Barth AD, Oko RJ. Abnormal morphology of bovine spermatozoa. Ames IA: Iowa State Univ. Press; 1989.

21. Bassiony SSM, Elhindawy MM, Attia AE, Ismail IE. Effect of some bioactive components of essential oils on growing rabbits performance. *Zagazig J. Agric. Res.* 2015;42: 1171-1182
22. Boham BA, Kocipai AC. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *Vaccinium calcycinium*. *Pac. Sci. J.* 1974; 48: 458–63.
23. Borek C. Antioxidant health effects of aged garlic extract. *J. Nutr.* 2001; 131: 1010–15.
24. Butzner JD, Meddings JB, Dalal V. Inhibition of short-chain acids in the caecum of the conscious rabbit. *Br. J. Nutr.* 1994; 36: 61–70.
25. Cardona ME, Collinder E, Stern S, Tjellstrom B, Norin E, Midtvedt T. Correlation between faecal iso-butyric and iso-valeric acids in different species. *Microb. Ecol. Health Dis.* 2005; 17: 177–82.
26. CBRA. Manual de exame e avaliação de sêmen animal. 2nd ed. Belo Horizonte: Col. Bras. Reprod. Anim.; 1998.
27. Celia C, Cullere M, Gerencsér ZS, Matics ZS, Giaccone V, Kovács M, Bónai A, Szendrő Zs, Dalle-Zotte A. Dietary supplementation of Digestarom® herbal formulation: effect on apparent digestibility faecal and caecal microbial counts and live performance of growing rabbits. *World Rabbit Sci.* 2016; 24: 95–105.
28. Chelikani P, Fita I, Loewen PC. Diversities of structures and properties among catalases. *Cell. Mol. Life Sci.* 2004; 6: 192–208.
29. Cross DE, McDevitt RM, Hillman K, Acamovic T. The effect of herbs and their associated essential oil on performance dietary digestibility and gut microflora in chickens from 7–28 days of age. *Br. Poult. Sci.* 2007; 48: 496–504.
30. Daniels AO, Famurewa O, Malomo O, Alamu EA. Screening of *Daniellia oliveri* against three bacteria and one fungus. *Res. J. Pharm. Biol. Chem. Sci.* 2013; 4: 940–45.
31. Deponte M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim. Biophys. Acta* 2013; 1830: 3217–66.
32. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 2006;47: 685–88.
33. Elghalid OA, Kholif AE, El-Ashry GM, Matloup OH, Olafadehan OA, El-Raffaa AM, Abd-El-Hady AM. Oral supplementation of the diet of growing rabbits with a newly developed mixture of herbal plants and spices enriched with special extracts and essential oils affects their productive performance and immune status. *Livest. Sci.* 2020;238: 104082.
34. El-Gindy YM, Abu Hafsa SH, Dosoky WM. Effects of potato peel extract on semen quality sex hormones and immunity of rabbit bucks under intensive breeding system. *J. Am. Oil Chem. Soc.* 2020. 52(11):e13869.
35. El-Gogary MR, El-Said EA, Mansour AM. Physiological and immunological effects of rosemary essential oil in growing rabbit diets. *J. Agric. Sci.* 2018;10: 485.
36. Elkirdasy A, Shousha S, Alrohaimi AH, Arshad MF. Hematological and immunobiochemical study of green tea and ginger extracts in experimentally induced diabetic rabbits. *Acta Pol. Pharm.* 2015;72: 497–506.
37. El-Ratel IT, Attia KAH, El-Raghi AA, Fouda SF. Relief of the negative effects of heat stress on semen quality reproductive efficiency and oxidative capacity of rabbit bucks using different natural antioxidants. *Anim. Biosci.* 2021;34: 844–54.
38. Gidenne T. The caecal ecosystem in the growing rabbit: impact of nutritional and feeding factors. *Proc. 3rd Rabbit Congr. Amer.* 2006; Maringa Brazil. HAL-02751977.
39. Hancock JI. A staining technique for the study of temperature shock in semen. *Nature* 1951; 197: 323–23.
40. Harbone IB. A guide to modern techniques to plant analysis. 2nd ed. New York: Chapman and Hall; 1973.
41. Hernandez F, Madrid J, Garcia V, Orengo J, Meglea MD. Influence of two plant extracts on broiler performance digestibility and digestive organ size. *J. Poult. Sci.* 2004; 83: 169–74.
42. Hoehn K, Marieb EN. Human anatomy and physiology. 8th ed San Francisco: Benjamin Cummings 2009. <https://doi.org/10.1016/j.psj.2021.101172>
43. Jang IS, Ko YH, Yang HY. Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. *Asian-Australas. J. Anim. Sci.* 2004;17: 394–400.
44. Krieg R, Vahjen W, Awad W, Sysel M, Kroeger S, Zoehner E, Hulan H, Arndt G, Zentek J. Performance digestive disorders and the intestinal microbiota in weaning rabbits are affected by a herbal feed additive. *World Rabbit Sci.* 2009;17: 87–95.
45. Lin CC, Wu SJ, Chang CH, Ng LT. Antioxidant activity of *Cinnamomum cassia*. *Phytother. Res.* 2003;17: 726–30.
46. Michiels J Missotten JAM Fremaut DS. Smet De, Dierick NA. In vitro characterisation of the antimicrobial activity of selected essential oil components and binary combinations against the pig gut flora. *Anim. Feed Sci. Technol.* 2009;151: 111–27.
47. Mourao JL, Pinheiro V, Alves A, Guedes CM, Pinto L, Saavedra MJ, Spring P, Kocher A. Effect of mannan oligosaccharides on the performance intestinal morphology and cecal fermentation of fattening rabbits. *Anim. Feed Sci. Technol.* 2006;126: 107–20.

48. Muir WI, Bryden WL, Husband AJ. Immunity vaccination and the avian intestinal tract. *Dev. Comp. Immunol.* 2000;24: 325–42.
49. NRC. Nutrient requirements of poultry. 9th ed Washington D.C.: Natl. Acad. Press 1994.
50. Odebiyi A, Sofowora AE. Phytochemical screening of Nigerian medicinal plants. Part III. *Lloydia* 1978;41: 234–36.
51. Olafadehan OA, Oluwafemi RA, Alagbe JO. Performance haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Drug Discov.* 2020;14(33): 135–45.
52. Osinowo OA. Introduction to animal reproduction. 2nd ed Sophie Academics Services Ltd 2016.
53. Ososanya TO, Adewumi MK, Oyeyemi MO, Adeosun AO. Semen characteristics of pubertal West Africa dwarf rams fed pineapple waste silage as replacement for dried cassava peel. *Afr. J. Agric. Res.* 2013;9(4): 501–06.
54. Osuntokun OT, Fasusi OA, Ogunmodede AF, Thonda OA. Phytochemical composition and antimicrobial activity of *Daniellia oliveri* extracts on selected clinical organisms. *Int. J. Biochem. Res. Rev.* 2016;14(1): 1–13.
55. Park HJ, Lim EJ, Zhao RJ, Oh SR, Jung JW, Ahn EM, Lee ES, Koo JS, Kim HY, Chang S, Shim HS, Kim KJ, Gwak YS, Yang CH. Effect of the fragrance inhalation of essential oil from *Asarum heterotropoides* on depression-like behaviors in mice. *Journ Austr-Trad Med Society* 2015; 21(2):129-130.
56. Placha I, Chrastinova L, Laukova A, Cobanova K, Takacova J, Stropfova V, Chrenkova M, Formelova Z, Faix S. Effect of thyme oil on small intestine integrity and antioxidant status phagocytic activity and gastrointestinal microbiota in rabbits. *Acta Vet. Hung.* 2013;61: 197–208.
57. Rekwot PI, Oyedipe EO, Dawuda PM, Sekoni VO. Age and hourly related changes of serum testosterone and spermogram of pre-pubertal bulls fed two levels of nutrition. *Vet. J.* 1997;153: 341–47.
58. Rinttila T, Apajalahti J. Intestinal microbiota and metabolites—Implications for broiler chicken health and performance. *J. Appl. Poult. Res.* 2013;22: 647–58.
59. Said MMA, Shehata SA, Khalil BA. Effect of water thyme extract on growing rabbits performance. *Zagazig J. Agric. Res.* 2016;43(6A): 2023–35.
60. Simitzis PE. Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. *Medicines* 2017;4: 1–21.
61. Wang ML, Suo X, Gu JH, Zhang WW, Fang Q, Wang X. Influence of grape seed proanthocyanidin extract in broiler chickens: Effect on chicken coccidiosis and antioxidant status. *Poult. Sci.* 2008;87: 2273–80.
62. Warner ACI. Production of volatile fatty acids in the rumen: Methods of measurements. *Nutr. Abstr. Rev.* 1964;34: 339.
63. Woof MJ, Kerr MA. Functions of immunoglobulin A in immunity. *J. Pathol.* 2005;208: 270–82.
64. Zhou L, Zhao Y, Wang S, Han S, Liu J. Lead in the soil–mulberry (*Morus alba* L.)–silkworm (*Bombyx mori*) food chain: Translocation and detoxification. *Chemosphere* 2015; 128:171–77.