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Nutritive effects and physiological responses of rabbits supplemented with camel's foot (*Piliostigma thonningii*) essential oil based diet

Emmanuel U. Anaso[1*], Olurotimi A. Olafadehan[1] and Ijeoma C. Chibuogwu[2]

[1]Department of Animal Science, University of Abuja, Abuja, Nigeria
[2]Department of Veterinary Theriogenology, University of Abuja, Abuja, Nigeria

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ABSTRACT

This study investigated the effect of *Piliostigma thonningii* essential oil (PEO) on the nutrient and feed intake, serum biochemistry and immune/oxidative stress responses of rabbits. The young male rabbits were randomly divided into three treatment groups, with 15 rabbits per group, and balanced for their body weight such that the rabbits in each group had a similar average initial body weight (BW) of 262.89±22.36 g in a completely randomized design. Treatment 1 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. Intake of nutrients, final BW, immunoglobulin A and M increased ($P<0.05$) with an increasing level of PEO supplementation. Serum total protein, albumin, globulin, glucose, high density lipoprotein, immunoglobulin G, triiodothyroxine, total anti-oxidant capacity, superoxide dismutase, catalase, glutathione perioxidase and glutathione reductase were higher ($P<0.05$) in T2 and T3 than in T1. Serum cholesterol, triglyceride, aspartate transaminase, alanine transaminase, alkaline phosphatase, low density lipoprotein, malondialdehyde and cortisol were higher ($P<0.05$) in T1 than in T2 and T3. Serum creatinine, uric acid, total and direct bilirubin were not affected ($P>0.05$) by treatments. It was concluded that PEO supplementation reduced oxidative stress indices and improved feed utilization, and the immune and general health status of the experimental rabbits. However, 4 ml PEO/kg diet was the optimum supplementation level, as it was more effective in enhancing the nutritional, physiological and general wellbeing of the rabbits.

Key words: bucks; phytogenic; feed intake; blood; immune and oxidative stress; serum biochemistry

Introduction

In the world today, research has shown a steady rise in animal protein demand due mainly to the swift increase of the human population and increased income, along with changing material alternatives or preferences (FAO, 2009). In Nigeria, the acute scarcity of meat due to the farmer-herder crises in Nigeria, the global pandemic, and poor economic policies have compelled livestock farmers to improve feed resource utilization, health status and meat production from their animals (ANASO et al., 2021). This shortage can be overcome by

* Corresponding author:
Emmanuel Ugochukwu Anaso, Department of Animal Science, University of Abuja, Abuja, Nigeria, e.mail:dranasoeub@gmail.com

the farming of highly prolific monogastrates with short production cycles, such as rabbits (ANASO et al., 2024).

Recently, the livestock industry, especially in Africa, has been dealing with the widespread emergence of illnesses due mainly to antibiotic resistance and inefficacy**.** This problem has led to or caused the indefinite ban of the use of antibiotics in animal production in developed countries, necessitating a search for non-chemical alternatives, such as phytogenic feed additives, in order to develop healthy food products, while also evolving a livestock-production system with minimal influence on environmental pollution (ANASO et al., 2023).

In addition, rabbit farming in Africa, especially in Nigeria, is challenged by multitudinous problems, which have resulted in a gross insufficiency of meat to meet the increasing population challenge in the country (ANASO, 2023a). High temperatures (heat) are a critically transient climatic factor affecting rabbit production in the tropics. ANASO (2023a; 2023b) described essential oils as an important aromatic substance of plant materials (source) which could be harvested, extracted and isolated.

Camel's foot (*Piliostigma thonningii* Schum.) Milne – Rech (*Caesalpiniaceae*) is a small tree often found in the savannah, which is of crooked growth with dark brown to black fissured bark. *Piliostigma thonningii* has been adequately utilized for a long time in the treatment of dermatosis and malaria by Nigerian traditionalists, and has been shown to possess typical flavoring, anti-oxidant, insecticidal and antimicrobial properties (ANASO et al., 2024).

There is no information on the nutritive value and physiological responses of rabbits supplemented with PEO. The goals of this investigation were, therefore, to: 1) evaluate the nutrient and feed intake of rabbits fed a PEO supplemented diet, 2) determine the serum biochemical responses, 3) determine the immune and oxidative stress indices of a PEO supplemented diet.

Materials and methods

Collection of P. thonningii seeds and extraction of the essential oil. Piliostigma thonningii seeds were sourced from within the premises of the University of Abuja, Nigeria, located within the southern Guinea savannah agro ecological zone. A certified taxonomist from the Forestry Research Institute of Nigeria (FRIN) recognized and authenticated the seeds.

The steam distillation method of extraction was used for essential oil (EO) extraction. The EO was extracted using Clevenger apparatus, as explained by ANASO (2023b). The *P. thonningii* seeds were shade-dried, finely crushed, and kept at room temperature until extracted. Approximately 100 g of dried ground material was suspended in 700 mL of distilled water using the distillation process at 100℃ for about 3 hours by placing the ground seed sample in the steel apparatus, and allowing the softening of the sample and production of the essential oil in vaporized form by heating it up after connecting the condenser to a water inlet and outlet. The vaporized essential oil droplets produced mixed with the steam (the carrier) and converged in the cooling system. The EO was then collected via a collection tube, and the percentage of the oil content calculated using the formula below:

% Oil content (v/w) = volume extracted (ml)/ original sample weight (g) x 100

Experimental Location (site). The experiment was carried out at the Monogastric Unit of the University of Abuja Teaching and Research Farm. The project site lies between latitudes $08^0$51' and $09^0$37'N and longitudes $007^0$20' and $007^0$51' E. Annual rainfall ranges from 1145-1631 mm. The temperature in the dry season is between 36-42℃ and 25.8-30.2℃ during the rainy season. Relative humidity is about 60% during the rainy season and 30% during the dry season (ITIOWE et al., 2019).

Experimental animals, management and treatment. Forty-five, clinically healthy, weaned male Dutch rabbits of about five weeks of age, with an average initial BW of 262.89±22.36 g, were used for the experiment. The rabbits were purchased from a highly recognized research institute (NAPRI, ABU Zaria Nigeria). Two weeks precisely before the arrival of the rabbits, the hutches and their immediate surroundings were cleaned (swept and washed) thoroughly and disinfected with antisep-

tic (Morigad, producer, city, country) and Hypo® (sodium hypochlorite, caustic soda and de-mineralized water). The animals were placed in the hutches under clinical confinement for two weeks and administered prophylactic treatment. The treatment included the administration of anti-stress mixture (Vitalyte®, Interhatch, Chesterfield, United Kingdom) in the drinking water, a subcutaneous injection with an anti-parasitic drug (Avomec®, Yuanzheng Pharmaceutical Co LTD, Shijiazhuang City, Hebei Province, China) at 0.5 mg/kg body weight (BW), for the prevention and efficient control of endo- and ecto- parasites, and injection with oxytetracycline HCl (a broad-spectrum antibiotic) at 1.0 mL/10 kg BW via the parenteral intramuscular route. The rabbits were also administered coccidiostat (Sulphadimidine Sodium BP solution, Bioveta, a.s., Komenskeho 212/12 683 23 Ivanovice na Hane, Czech Republic) subcutaneously once at the onset (take-off) of the experiment at 1 mL/rabbit, adhering to the manufacturer's recommendation.

Individual rabbits were held in individual open-sided metabolic hutches, which separate fec- es from urine. Cleaning of the hutches was done daily using a potent disinfectant. All the rabbits were weighed individually using a weighing scale to determine their initial BW. The rabbits were placed into three groups (T1, T2 and T3) with fif- teen rabbits per group. They were balanced for their BW, such that rabbits in each individual group had a comparable average initial BW. This was all done in a fully randomized manner (design).

The basal control diet was formulated according to NRC (1984) requirements for growing rabbits. Ad libitum water and food were provided for a period of 12 weeks, while feeding was done twice a day at 08:00 hrs and 16:00 hrs. The rabbits in the first treatment group were fed a basal control diet. In the other treatments, the basal control diet was supplemented with 2 and 4 mL of PEO/kg of the diet.

Treatment one (T1): basal control diet (no PEO)

Treatment two (T2): T1 + 2.0 ml PEO/kg diet

Treatment three (T3): T1 + 4.0 ml PEO/kg diet

To ensure consumption of the full dose of PEO by the rabbits in treatments T2 and T3, the appropriate respective dose of the oil was mixed with a small known quantity of the basal control diet before being served. The basal control diets were consumed by the rabbits almost immediately; thereafter, they were served the basal control diet *ad libitum*.

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

Table 2. Chemical composition of experimental diet

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

Feed intake. The weight of the leftover feed was subtracted from the weight of the feed that was provided the day before to calculate feed intake (FI). The amount of nutrients an animal consumed was calculated by multiplying its feed intake (in DM) by the nutrient (in DM) derived from the diets' chemical compositions.

Blood collection and 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 taken for analysis.

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 analysed 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 PETER et al. (1982). 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).

Immune response. 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).

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

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.

Results

Dry matter and nutrient intake of rabbits fed a basal diet supplemented with Piliostigma thonningii essential oil. Table 3 shows the DM and nutrient intakes of rabbits fed a basal diet supplemented with PEO.

Table 3. Dry matter and nutrient intake of rabbits fed *Piliostigma thonningii* essential oil supplemented diet

Intake (g/day)	T1	T2	T3	SEM
Dry matter	28.32^{c}	34.66^{b}	40.80^{a}	1.08
Crude protein	4.68^{c}	5.73^{b}	6.74^{a}	0.18
Ether extract	0.64^{c}	0.78^{b}	0.92^{a}	0.02
Crude fiber	3.77^{c}	4.61^{b}	5.42^{a}	0.14
Neutral detergent fiber	9.07^{c}	11.11^{b}	13.08^{a}	0.35
Acid detergent fiber	5.30^{c}	6.48^{b}	7.63^{a}	0.20
Ash	2.67^{c}	3.27^{b}	3.85^{a}	0.10
Organic matter	25.64^{c}	31.39^{b}	36.95^{a}	0.98
Nitrogen-free extract	16.56^{c}	20.26^{b}	23.85^{a}	0.63

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

T1, 0 ml of *P. thonningii* essential oil/kg diet; T2, 2 ml of *P. thonningii* essential oil/kg diet; T3, 4 ml of *P. thonningii* essential oil/kg diet

Serum biochemical parameters of rabbits fed Piliostigma thonningii essential oil supplemented diet. Table 4 shows the serum biochemical parameters of rabbits fed PEO. Serum total protein, serum albumin, globulin, glucose, and high-density lipoprotein followed a similar trend as T1 having

Table 4. Serum biochemical parameters of rabbits fed *Piliostigma thonningii* essential oil supplemented diet

Parameter	T1	T2	T3	SEM	RV
Total protein (g/dL)	4.12[b]	7.33[a]	7.61[a]	0.54	4.0-8.3
Albumin (g/dL)	2.40[b]	4.50[a]	4.77[a]	0.24	2.4-6.4
Globulin (g/dL)	1.72[b]	2.84[a]	2.84[a]	0.37	1.5-2.8
Albumin globulin ratio (g/dL)	0.96	1.13	1.22	0.06	0.7-1.89
Glucose (mg/dL)	104.05[b]	135.76[a]	148.58[a]	6.08	75-155
Cholesterol (mg/dL)	52.50[a]	34.19[b]	24.18[b]	2.10	10-80
Uric acid (mg/dL)	3.43	3.47	3.64	0.23	2.0-4.0
Creatinine (mg/dL)	2.14	2.25	2.30	0.24	0.5-2.5
Triglyceride (mg/dL)	120.70[a]	74.47[b]	64.74[b]	14.76	15-160
High density lipoprotein (mg/dL)	19.07[b]	25.93[a]	26.11[a]	0.16	NA
Low density lipoprotein (mg/dL)	71.91[a]	47.95[b]	42.37[b]	3.48	NA
Total bilirubin (umol/L)	4.88	4.43	4.73	0.30	0-12
Direct bilirubin (umol/L)	0.73	0.78	0.66	0.08	0-0.7
Aspartate amino transaminase (u/L)	60.16[a]	38.66[b]	35.16[b]	3.60	14-113
Alanine transaminase (u/L)	61.28[a]	48.05[b]	46.17[b]	2.26	48-80
Alkaline phosphatase (u/L)	11.27[a]	6.70[b]	6.44[b]	0.46	4-16

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

T1, 0 ml of P. *thonningii* essential oil; T2, 2 ml of P. *thonningii* essential oil; T3, 4 ml of P. *thonningii* essential oil/kg diet

RV: reference values as stated by BEERS (2006).

Table 5. Immune and oxidative stress responses of rabbits fed *Piliostigma thonningii* essential oil supplemented diet

Parameter	T1	T2	T3	SEM
Immunoglobulin G (mg/dL)	83.18[b]	113.81[a]	117.00[a]	12.17
Immunoglobulin A (mg/dL)	65.80[c]	79.09[b]	91.76[a]	2.11
Immunoglobulin M (mg/dL)	20.06[c]	27.77[b]	31.84[a]	0.90
Triiodothyroxine (mg/mL)	1.01[b]	2.85[a]	3.08[a]	0.26
Melanodialdehyde (nmol/mL)	93.53[a]	75.15[b]	71.05[b]	3.92
Superoxide dismutase (nmol/mL)	44.03[b]	61.21[a]	65.11[a]	2.96
Catalase (nmol/mL)	34.66[b]	61.61[a]	65.96[a]	3.94
Glutathione peroxidase (nmol/mL)	7.45[b]	10.61[a]	10.73[a]	0.29
Cortisol (nmol/mL)	16.72[a]	14.31[b]	13.60[b]	0.32
Total antioxidant capacity (umol/L)	1.91[b]	3.58[a]	3.63[a]	0.24
Glutathione reductase (ng/mL)	2.60[b]	3.86[a]	4.40[a]	0.41

Means with the different superscripts in the same 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

a lower value (P<0.05) than T2 and T3, which had similar (P>0.05) values. Serum cholesterol was lower in T2 and T3 (P<0.05) than in T1. Low density lipoprotein, triglyceride, AST, ALT and ALP were higher (P<0.05) in T1 than in T2 and T3, which were insignificantly affected (P>0.05).

Immune and oxidative stress responses of rabbits fed Piliostigma thonningii essential oil supplemented diet. Immune and oxidative stress parameters of the experimental treatments are shown in Table 5. Immunoglobulin G, Superoxide dismutase, catalase, gluthionine peroxidise, 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 Immunoglobin A and M were significantly higher in T3, and low- est (P<0.05) in T1. Melanodialdehyde and cortisol were lower (P<0.05) in T2 and T3 than in T1.

Discussion

Dry matter and nutrient intake of rabbits fed the Piliostigma thonningii essential oil supple- mented die. Dry matter intake (DMI) generally de- pends on a variety of factors, including the chemi- cal composition, characteristics and palatability of feeds (OLAFADEHAN and ADEBAYO, 2016). OSOSANYA (2010) described feed intake as a crucial factor in feed utilization by livestock, and a critical determinant of energy and protein availability, as well an important performance index in livestock. Feeds low in digestibility are generally perceived to cause constraint on DMI due to delayed passage through the digestive tract (ALLEN, 1996). Similarly, OLAFADEHAN and OKOYE (2017) described DMI as a crucial factor in the utilization of feed, and also a critical determinant of energy availability and performance in domestic livestock.

P. thonningii EO, which is a monoterpene hydrocarbon, contains beta-mycerne and limonene which have flavoring properties. It appears that these flavor characteristics were more pronounced at the higher dose of PEO, which led to a greater feed intake in T3. These feed intake results agree with the work of EL-NOMEARY et al. (2020) where garlic oil, cinnamon and Jupiter EO significantly increased DMI in comparison to the control group. Similar results relating to DMI could possibly be due to the similarity in method of delivery or supply of herbs and dietary essential oils to the animals, the taste and odor emanating from the active substances contained in the plants, and the dose of the EO which all encourage intake by the animals. In other words, some herbs are appetizing (EL-NOMEARY et al., 2020). From the current study, before approaching the feed, the animals were strongly influenced by the aroma that emanated.

Higher crude protein intake, ether extract intake, crude fiber intake, ADF intake, NDF intake, OM intake and NFE intake in T3 indicates the bioactive ability of the essential oil to improve the palatability of the feed by influencing the taste of the diet. The increased nutrient intake of the treatment groups, particularly T3, is obviously due to the increased DM intake. OMER et al. (2012) reported that adding medicinal plants (fennel seeds or oregano leaves) to rabbit diets significantly improved DM and CP. OLAFADEHAN et al. (2014) asserted that nutrient intake is a function of DM intake and the composition of the nutrient in a diet. This was obvious in the current study, as the nutrient intake followed the same pattern as DM intake.

Serum biochemical parameters of rabbits fed the Piliostigma thonningii essential oil supplemented diet. Serum biochemistry is a generalized procedure of assessing the health status of animals, and it reveals pathophysiological states which lead to identification of pathogenesis of disease (OLAFADEHAN et al., 2014).

Serum proteins (albumin and globulin) are important in osmotic regulations, immunity, and transport of several substances in the body (JAIN, 1986). The results obtained were within the standard reference range for clinically (apparently) healthy rabbits, as stipulated by MITRUKA and RAWNSLEY (1981) and KHAN (2006).

According to OLAFADEHAN et al. (2023), serum albumin and globulin function by preventing blood from spilling out of blood vessels, and helps fight infection. They also help transport hormones, vitamins, and other cellular substances to the peripherals of the body. Normal protein values within

the stipulated range therefore function to maintain cellular processes and maintain animal health. Low albumin levels may be a sign of liver disease, such as liver cirrhosis, kidney disease, malnutrition, and thyroid infection or disease, while high albumin levels may be a sign of severe dehydration and diarrhea. Low globulin levels may be a sign of liver or kidney disease, while high globulin levels may be due to certain types of blood cancers and hemolytic anemia. (OLAFADEHAN et al., 2023).

The higher albumin and globulin in T2 and T3 (animals supplemented with *P. thonningii* EO) is in line with the observation by OLORUNTOLA et al. (2018) who observed higher serum proteins in rabbits administered varying doses of EO relative to the control, which could be ascribed to the antioxidative property of phytogenics. Similarly, our findings were consistent with earlier studies by ALZAWQARI et al. (2016) and ALAGAWANY et al. (2021) who observed increased serum total protein and globulin with the addition of lemongrass EO, and lemongrass leaves, respectively, to a basal diet. The higher serum albumin and glob- ulin of T2 and T3 implies improved immune re- sponse as a significant response to the protective and potential antioxidative effects of the supplemented essential oil.

Serum triglyceride was at the highest level in the control. High triglyceride levels in the blood above stipulated normal range may imply cardiovascular problems, such as atherosclerosis, which narrows the blood vessels by the clogging and calcification of the arteries, which increases the threat or chances of major heart diseases. Though triglyceride levels were significant among the treatments in the current study and they were within the stipulated range (KHAN, 2006), implying that PEO had no toxic effect on the research animals.

The glucose values of the experimental rabbits, which were within the stipulated ranges, indicate the lack of a challenge to the carbohydrate metabolism in animals supplemented with PEO. The higher glucose levels in T2 and T3 imply improved dietary energy utilization, which was indicative of the fact that the animals were not suffering from glycaemia and glycosuria (WEISSMAN and KARRER, 2009). This result is supported by the higher OM digestibility because, according to ANASO et al. (2023), OM is a good index of energy availability.

The decreased cholesterol and low-density lipoprotein of the treatment groups was similar to what ELGHALID et al. (2020) observed, that is, a decrease in cholesterol, triglycerides, and LDL cholesterol, indicating a favorable modulatory influence on cholesterol metabolism and turnover, tissue protection from lipid peroxidation, and considerable lipid lowering action in rabbits following these treatments. These outcomes support the cardiovascular preventive effect of phytogenic additive supplementation. The additive's active components and phenolics have the ability to impact lipid metabolism in animal tissues by enhancing the activity of antioxidant enzymes, and inhibiting the generation of reactive oxygen species and off-flavors produced from the peroxidation of polyunsaturated fatty acids (MIGUEL, 2010). Additionally, reducing the concentrations of cholesterol and LDL cholesterol may be linked to the biosynthesis of cholesterol in cells (FUHRMAN et al., 2000) and a decrease in intestinal absorption of cholesterol, which raises the excretion of neutral lipids in the feces (PUROHIT and VYAS, 2006). According to LEE et al. (2004), phytogenics, which include flavonoids and polyphenolics, have the capacity to impede the action of the liver's 3-hydroxy-3-methylglutaryl coenzyme A reductase, an essential enzyme that regulates the synthesis of cholesterol. ELSHATER et al. (2009) found that a decrease in cellular cholesterol biosynthesis is linked to an increase in LDL receptor activation, which improves LDL elimination from the circulation and lowers serum or plasma cholesterol levels. Also, a major sesquiterpene and monoterpene hydrocarbon (beta-caryophyllene and oleic acid) binds to the CB2 receptors generally located on the immune cells, thereby decreasing cholesterol, and preventing osteoporosis (MANDAL, 2019). Generally, the normal serum cholesterol levels within stipulated ranges for apparently healthy rabbits suggest that meat from the experimental animals should be safe and wholesome for human consumption, and thus not likely to elevate cholesterol levels in consumers. However, the reduced values for T2 and T3 indicate that the meat from

these rabbits may be safer or healthier for human consumption.

P. thonningii EO treatments decreased the concentration of liver enzymes in the experimental animals. Similarly, ABDEL-WARETH and METWALLY (2020) who added thyme essential oil (TEO) to male rabbits, reported a significant decrease in ALT and AST activity in comparison to the control group. Contrary to the current findings, EL-RATEL et al. (2021) reported that the activity of AST and ALT in rabbits was significantly improved by oral administration of phytogenic (5 or 10 mg allicin) compared with the control. The AST and ALT levels were normal and within the reference ranges, indicating that there was no health risk to the experimental animals' hearts and livers, as these enzymes are typically seen in high concentrations in the blood when the heart or liver is injured. ALP readings within the reference ranges suggest that no diseases (abnormalities) existed in the experimental animals. ALP is an enzyme that is released into the bloodstream in a variety of conditions, including obstructive jaundice and sever- al bone ailments, and it also regulates hydrolysis. Also, blood pH and illnesses can also alter ALP levels (SMITH, 2009). The low levels of AST, ALT and ALP in the T2 and T3 treatment groups are an indication of better excretory and liver function in the rabbits supplemented with PEO in their diet. Again, the normal values for all treatments indicate no hepatic damage.

Immune and oxidative stress responses of rabbits fed a Piliostigma thonningii essential oil supplemented diet. Immunoglobulins act by binding to pathogens, thereby inhibiting adhesion to epithelial cells and neutralizing harmful toxins (WOOF and KERR, 2005).

The higher IgG, IgM and IgA in the treatment groups (T2 and T3) could be ascribed to the antioxidant property of the *P. thonningii* EO. The immunoglobulin levels increased in groups administered *P.thonningii* EO and were highest in rabbits in T3, which were given the highest dose. The results of the current research concur with ALAGAWANY et al. (2021), who stated that the experimental immunoglobulin levels (IgM, IgG, and IgA) were higher than those of the control group.

According to ELGHALID et al. (2020) triiodothyronine performs a crucial activity in systemic physiology (such as growth, development and metabolism) and body thermoregulation (such as temperature, heart rate and respiratory rate). El-GINDY et al. (2020) similarly reported serum immunoglobulin M concentration to be significantly higher with the essential oil treatments in comparison to the control.

According to El-GOGARY et al. (2018), MDA is a marker of damage to cell membranes, and the amount of this marker is dependent on lipid peroxidation. The lower MDA of the treatment groups could be attributed to the antioxidant ability of the phenolic ingredient of PEO. Some phenolic components in feed additive mixtures have been shown to have antioxidative activity by scavenging superoxide anion and hydroxyl radicals from antioxidant compounds, phenolic ketone derivatives, catechins, and volatile oils (ELKIRDASY et al., 2015).

The results of reduced MDA and cortisol, while increased SOD, catalase, gluthanione peroxidase and reductase, were similar to the work of ELGHALID et al. (2020), who reported decreased MDA due to the combination of phytogenic 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 (BOREK, 2001). Similarly, LIN et al. (2003) reported that herb intake caused a decrease in MDA levels. Therefore, bioactive oleic acid, heptadecanoic acid and hexadecane, limonene, alpha-pinene and beta-caryophyllene, as constituents of sesquiterpenes and monoterpene hydrocarbons, through their antioxidant, antibiotic, anti-inflammatory properties and characteristics, decreased the MDA, a marker of oxidative stress and cortisol, which is released in response to stress and low blood sugar (HOEHN and MARIEB, 2009).

SOD was highest in the treatment groups T2 and T3. SOD is crucial in defending cells against oxidative damage, but for this process to occur, the diet must have certain nutrients (ASHOUR et al., 2014). The results are likewise consistent with those of LIN et al. (2003), who found that consuming herbs

increased the activity of general serum antioxidant enzymes.

The increase in glutathione in T2 and T3 might have been due to PEO, implying that there was no inhibition of the glutathione disulfide synthesis. According to ALAGAWANY et al. (2016), glutathione shields cells from free radical damage, stops macromolecules from oxidatively deteriorating, and stops the peroxidation of the apolipoprotein B protein.

Increased breakdown of acetylcholine, leading to reduced intake and absorption of nutrients causing low blood sugar, led to the subsequent rise in blood cortisol level in the control group (ELGHALID et al., 2020). PEO, via its bioactive hydrocarbon, which functions and acts as an acetylcholinesterase inhibitor, prevents the breakdown of acetylcholine accountable for the contraction of smooth muscles, such as the involuntary peristaltic movement of the gastrointestinal muscles, aiding in proper digestion and absorption of nutrients.

According to CHELIKANI et al. (2004) and DEPONTE (2013), catalase and glutathione peroxidase and reductase play a major role in protecting, preventing, maintaining and basically catalyzing the decomposition of hydrogen peroxide to water and oxygen, and protecting cells from the scavenging effect of ROS.

Conclusions

P. *thonningii* essential oil supplementation enhanced feed and nutrient intake, as well as the serum blood profile, oxidative status and immune response of the rabbits. PEO supplementation at 4 ml/kg diet was superior to the 2 ml/kg diet due to the better results obtained for some parameters.

Declaration of competing interest

No potential conflicting interest was reported by the authors.

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SAŽETAK

U radu je istraživan učinak eteričnog ulja mahunarke *Piliostigma thonningii* (PEO) na unos hranjivih tvari, biokemijske pokazatelje u serumu i odgovor na imunosni/oskidacijski stres u kunića. Prema randomiziranom modelu, mladi mužjaci kunića nasumično su podijeljeni u tri pokusne skupine, s 15 jedinki po skupini. Tjelesna masa kunića u skupinama bila je približno jednaka i iznosila 262,89 ± 22,36 g. Kunićima u pokusnoj skupini 1 (kontrolna skupina) ponuđena je osnovna prehrana. U pokusnoj skupini 2 u osnovnu je prehranu dodano 2 mL PEO/kg hrane, a u pokusnoj skupini 3 hrani je dodano 4 mL PEO/kg hrane. Unos hranjivih tvari, tjelesna masa na završetku istraživanja, te imunoglobulini A i M povećani su ($P<0,05$), s povećanim unosom PEO-a. Vrijednosti ukupnih serumskih proteina, albumina, globulina, glukoze, HDL-a, imunoglobulina G, trijodotiroksina, ukupnog antioksidacijskog kapaciteta, superoksid-dismutaze, katalaze, glutation-peroksidaze i glutation-reduktaze povećane su ($P<0,05$) u pokusnim skupinama 2 i 3 u odnosu na kontrolnu skupinu. Vrijednosti serumskog kolesterola, triglicerida, aspartat-transaminaze, alanin-transaminaze, alkalne fosfataze, LDL-a, malondialdehida i kortizola bile su veće ($P<0,05$) u pokusnoj skupini 1 nego u pokusnim skupinama 2 i 3. Vrijednosti serumskog kreatinina, mokraćne kiseline te ukupnog i direktnog bilirubina nisu bile znakovito različite između istraženih skupina kunića ($P>0,05$). Zaključeno je da dodatak prehrani PEO smanjuje oksidacijski stres i poboljšava iskorištavanje hranjivih sastojaka, imunost te opće zdravstveno stanje pokusnih kunića. Pritom je dodatak od 4 mL PEO/kg u prehrani bio optimalan s obzirom na učinkovitost u poboljšanju hranjivih sastojaka, fiziološkog odgovora i opću dobrobit kunića.

Ključne riječi: kunić; fitogeni; unos hrane; krv; imunosni i oksidacijski stres; biokemijski pokazatelji