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Influence of Dietary Supplementation of Eucalyptus Leaves Extract (*Eucalyptus globulus*) on Performance, Lipid Profiles, Digestive Enzymes, Microbial Content, Antioxidant Indices and Immune Responses of Growing Japanese Quail

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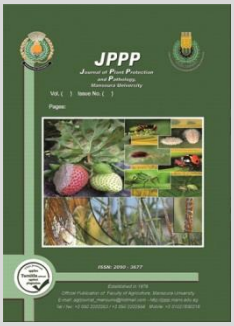

ABSTRACT

This investigation was initiated to evaluate the impact of an extract made from eucalyptus leaves (*Eucalyptus globulus*) (ELEx) on the quail's physiological state, growth performance, some blood parameters, and intestine bacterial numbers. Three hundred and sixty Japanese quail chicks, aged ten days, were split up into four groups, each with six cages and every cage containing fifteen chicks. While the initial treatment was treated with a basal diet (considered the control treatment), the following groups were administered the basal diet supplemented with ELEx at concentrations of 250, 500, and 750 mg ELEx/kg diet, correspondingly. Findings revealed significantly LBW and BWG, along with notable improvements ($p<0.001$) in feed conversion ratios with lowest feed intake for groups subjected to ELEx levels, notably the ones receiving 750 mg ELEx followed by 500 mg ELEx in comparison to the control group. Quails consuming a diet enriched with ELEx exhibited substantially heightened levels of digestive enzymes in contrast to the control group. The presence of intestinal *Lactobacillus sp.* was markedly augmented, while *Escherichia coli and Salamonella* populations were significantly reduced by the dietary incorporation of ELEx levels ($p<0.001$). Also, parameters such as ALT, AST, lipid profiles, antioxidant status, and immunity were significantly influenced by varying ELEx concentrations in compare to the basal ($p<0.001$). To summarize, the incorporation of ELEx at 750 mg followed by 500 mg ELEx/kg diet of quail exhibited the perfect growth performance, some microbial numbers, and blood biochemical constituents in growing quail.

***keywords*:** quail, *Eucalyptus globulus*, serum biochemistry, antioxidant indices, immunity, digestive enzymes, performance

INTRODUCTION

Poultry farmers are now looking for alternative growth promoters such as medicinal herbs because the European Union has banned the use of antibiotics in the poultry industry. Many countries have recently shown a tendency to restrict or eliminate chemical components because they are detrimental to both humans and animals, emphasizing the importance of utilizing natural promoters. Research has demonstrated that medicinal herbs can promote the growth of broiler chickens and provide a range of natural benefits, including stimulant, tonic, antimicrobial properties, and antiseptic characteristics (Burt, 2004). Thus, to improve growth, feed conversion efficiency, and lower feed costs in broiler diets, herbs and medical plants have been suggested as nutritional supplements or growth enhancers (Hashemi and Davoodi, 2012). Studies on the use of herbal plants in broiler chickens have shown increased weight gain, enhanced conversion of feed and lower rates of mortality (Hernandez et al., 2004 and Cross et al., 2007). Eucalyptus, belonging to the *Myrtaceae* family, is extensively cultivated worldwide (Sallam et al., 2007). Eucalyptus leaves contain essential oil up to 3.5% w/w, with eucalyptol (1, 8-cineole) being the primary constituent. Additionally, chemical components like α-phellandrene, ρ-cymene, γ-terpinene, ethanone, spathulenol have been identified in Eucalyptus (Akin et al., 2010). Aromatic oils are natural substances that are mostly made up of terpenes and their byproducts, as well as other non-terpene components (Edris, 2007). Salmonella and Staphylococcus aureus are two pathogens that eucalyptus globules oil suggests antimicrobial properties against (Sallam et al., 2007). Consequently, the objective of this research was to assess the impact of incorporating ELEx as a supplementary feed alternative on the growth performance, antioxidant concentrations, immune responses, some intestinal microbiota numbers, and digestive enzyme activity in growing quails.

MATERIALS AND METHODS

The investigation was carried out at the Poultry Research Center, situated within the Faculty of Agriculture at Fayoum University in Egypt. The study involving live animals adhered to the protocols sanctioned by the Institutional Animal Care and Use Committee at Fayoum University in Egypt (Code No. of the proposal: AEC 2409).

Experimental design and diets:

A total of 500 one day-old quail chicks were housed in electrically heated batteries and nourished with a basal diet comprising 24%crude protein with2900Kcal ME/kg of feed, from day-one to day-ten, following the guidelines of the National Research Council (NRC, 1994). Upon reaching the tenth day of age, 360 unsexed quail chicks were randomly allocated to four uniform groups, with each group consisting

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of 90 birds divided into six replicates of 15 birds each. The initial group was provided with the basal diet devoid of ELEx (control), whereas the subsequent groups were administered the basal diet supplemented with 250,500,and750mg ELEx/kg, respectively. The newly hatched chicks were tagged with small plastic bandsat ten days of age and raised in cages measuring 40×60×25cm3, with individual weight measurements of the quail chicks. The ambient temperature within the rearing facility during the first ten days of life was maintained at 34−35ºC, gradually decreasing by approximately 2ºC weekly until reaching 30-31ºC by the third week, following which the birds were reared under the standard environmental conditions of Fayoum University Poultry Farms. The birds were exposed to continuous lighting for 23-24 hours daily, with *ad libitum* access to feed and water throughout the duration of the experiment. Leaves from *Eucalyptus* species were harvested from Maoming, Guangzhou, China in December, air-dried naturally in the shade for seven days (moisture content18.45±3.06%. The crushed eucalyptus leaves were subjected to extraction using70% ethanol (v/v) through low-temperature continuous phase transformation at a pump speed of 35L/h for a total of four hours at 75°C. The resulting extraction solutions were vacuum-concentrated and spray-dried (parameters: In let air temperature:180°C; Out let air temperature:80°C; Atomizer speed:12000r/min; Pump flowrate:4L/h). The Eucalyptus leaves extract (ELEx) was manually incorporated into the feed with a small portion initially, gradually increasing while ensuring thorough mixing until achieving the desired uniformity. Following the completion of mixing, the feed blends were stored in sealed and labeled bags corresponding to each treatment to preserve the efficacy of the additives. The detailed composition of the basal diet can be found in Table1.

Table 1. Composition and calculated analysis of basal diet fed to growing Japanese quail.

Ingredients	%
Yellow corn	53.5
Soybean meal (44 %)	30.5
Corn gluten meal (60%)	9.50
Wheat Bran	1.50
Vegetable oil	0.50
DL-methionine	0.20
L-Lysine HCl	0.30
Salt (NaCl)	0.50
Vitamin and mineral premix*	0.50
Limestone	2.00
Di calcium phosphate	1.00
Total	100
Calculated Analysis**	
Metabolizable energy (kcal/kg)	2900
Crude protein %	24.11
Crude fiber %	3.60
Calcium %	1.24
Available phosphorus%	0.39
Lysine	1.35
Methionine	0.62
Methionine + Cysteine	0.89

***-Premix provided per kg of diet: vitamin A,12.000 IU; vitamin D3,2.400 IU; vitamin E,30mg; vitamin K3, 4mg; vitamin B1, 3mg; vitamin B2,7 mg; vitamin B6, 5mg; vitamin B12, 15µg; niacin, 25 mg, Fe, 80 mg; folic acid, 1mg; pantothenic acid, 10 mg; biotin, 45 mg; choline, 125,000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.**AccordingNRC,1994.**

Growth performance:

Live body weights of birds (LBW) were individually measured, and feed consumptions per pen were recorded on a weekly basis (FC). The uneaten feed was disposed of, and body weight gain (BWG) was determined as the difference between final and initial body weights. Additionally, the feed conversion ratio (FCR) was computed. Furthermore, the performance index (PI) was derived according to North (1981): PI=BWkg/FCR, and the growth rate was assessed based on Brody (1945):GR= (LBW38 - LBW10)/0.5(LBW10 + LBW38).

Blood biochemical, anti-oxidant and immunity:

At the termination of the trial period (38days), blood specimens will be obtained from the slaughtered quails, with two birds (1male and1female) randomly selected from each replicate. The avian subjects were initially weighed with precision, and then euthanized through the incision of the Jugular vein (following Islamic rituals). Subsequently, blood samples were individually collected in dry, sterile centrifuge tubes, resulting in a total of 48 samples. After centrifugation at755g for15 minutes, the serum was separated and stored at −20°CinEppendorf tubes until further analysis. The subsequent quantitative analysis encompassed determinations of total cholesterol(TC), triglycerides(TG), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT). All biochemical parameters were assessed using calorimetric methods with diagnostic kits manufactured by Spectrum Diagnostics Company in Egypt. Amylase and lipase activities were evaluated following the protocol by Friedman and Young (2005), while the trypsin enzyme was quantified using the Bovine Trypsin ELISA Kit MBS706461. Glutathione peroxidase (GPx, EC 1.11.1.9) levels were calorimetrically determined based on the procedure described by Paglia and Valentine (1967), and the analysis of thiobarbaturic acid-reactive substances (TBARS) followed the method outlined by Yagi (1998) with diagnostic kits from Cayman Chemical Company (USA). Chicken Immunoglobulins Isotypes IgG, IgM, and IgA were assayed using the Sandwich ELISA technique as detailed by Erhard et al. (1992), with absorbance readings takenat450nm on an ELISA plate reader.

Microbial analysis:

Subsequent to the slaughtering process, the intestinal contents were promptly collected in sterile glass receptacles, followed by thorough mixing of the digesta. The sesealed containers were then stored at 4°C in the laboratory until the commencement of microbial enumeration. Samples weighing 1g each were transferred into sterile test tubes, diluted1:10 in sterile 0.1% peptone solution, and homogenized for 3 minutes using a Stomacher homogenizer. Serial dilutions up to 10-7 were prepared for each sample in nine ml of 0.1% sterile peptone solution. The enumeration of *Salmonella spp, Escherichia coli (E.coli)*, and *Lactobacilli spp* was subsequently conducted. One millilitre of each serial dilution was plated on sterile Petri dishes containing the appropriate medium. *Lactobacillus spp.* colony counts were determined using MRS agar (Biokar Diagnostic, France) following anaerobic incubation at 37°C for72 hours. The enumeration of *Salmonella and E.coli* colonies was performed on brilliant green agar plates, followed by incubation at 37°C for 24 hours. The total colony count for *Lactobacilli, Salmonella, and E.coli* was then calculated based on the number of colonies and the reciprocal of the dilution factor. The microbial counts were expressed as colony forming units (cfu) per gram of the sample.

Statistical analysis

The results were examined through the application of statistical techniques, particularly analysis of variance, within the framework of Infostat as detailed by Di Rienzo (2017). The analytical model employed for this study can be represented as follows:

$$Yij=\mu+Ti+eij.$$

Wherein:

Yij: signifies the observation of traits, μ: represents the overall mean, Ti: denotes the treatment effect, and eij: accounts for random error. Subsequently, the comparison of all means was conducted utilizing the multiple range test established by Duncan in1955, at a significance level of 0.05.

RESULTS AND DISCUSSIONS

Results

Growth performance

The impact of dietary ELEx on the growth performance on growing Japanese quail was demonstrated in Table 2. Quails fed a diet treated by 750 mg ELEx showed notable enhancements in LBW, BWG, FCR, PI, and faster GR when compared to the control treatment. Moreover, the group that received 500 mg ELEx exhibited the lowest FI 10-38 value, although it was not statistically different from the 750 mg ELEx group.

Table2. Effect of dietary eucalyptus leaves extract on growth performance in growing Japanese quail.

Item Treat.	Control	ELEx 250 mg/kg	ELEx 500 mg/kg	ELEx 750 mg/kg	SE	P-value
Initial LBW (g)	57.73	57.72	57.77	57.71	0.53	0.9999
$LBW_{38}d$ (g)	222.08^{d}	232.71^{c}	238.29^{b}	244.40^{a}	1.37	0.0001
BWG_{10-38} (g)	164.10^{d}	174.22^{c}	180.58^{b}	185.40^{a}	1.28	0.0001
FI_{10-38} (g)	610.55^{a}	587.20^{b}	585.47^{b}	585.73^{b}	1.19	0.0001
FC_{10-38} (g/g)	3.76^{a}	3.38^{b}	3.25^{c}	3.17^{d}	0.03	0.0001
GR_{10-38}	1.17^{c}	1.20^{b}	1.22^{a}	1.22^{a}	0.01	0.0001
PI_{10-38}	6.01^{d}	6.93^{c}	7.37^{b}	7.76^{a}	0.09	0.0001

Abbreviations: LBW: Live Body Weight, BWG: Body Weight Gain, FI: Feed Intake, FC: feed conversion, SE: Standard Error, ELEx: Eucalyptus leaves extract, $^{a-d}$: Means within the same row with different superscript are significantly different(P≤0.05).

Serum biochemistry

Table 3 presented the impact of ELEx on serum biochemistry parameters. Quails fed a diet supplemented with 500 mg ELEx/kg exhibited significantly decreased levels of RBS, total cholesterol, LDL, and ALT with increased trypsin levels compared to the control group. On the other hand, the group fed a diet with 750 mg/kg displayed lower TG and AST levels, alongside higher levels of digestive enzymes (amylase and lipase). Additionally, quails on a diet with 250 mg ELEx/kg showed significantly higher HDL levels compared to the control group.

Table3. Effect of dietary eucalyptus leaves extract on lipid profile, liver functions and digestive enzymes in growing Japanese quail.

Items Treat.	Control	ELEx 250 mg/kg	ELEx 500 mg/kg	ELEx 750 mg/kg	SE	P-value
RBS	141.50^{a}	127.00^{b}	109.83^{c}	114.83^{c}	3.40	0.0001
lipid profiles						
Total Chol. mg/dL	183.67^{a}	170.83^{b}	157.33^{c}	160.33^{c}	2.87	0.0001
TG, mg/dL	143.33^{a}	96.00^{b}	94.67^{b}	94.50^{b}	3.09	0.0001
HDL, mg/dL	31.83^{b}	36.83^{a}	30.17^{b}	33.00^{b}	1.23	0.0032
LDL, mg/dL	128.83^{a}	115.83^{b}	110.33^{b}	111.50^{b}	2.56	0.0001
liver functions						
ALT, U/L	6.17^{a}	4.83^{b}	4.50^{b}	5.00^{b}	0.26	0.0002
AST, U/L	209.83^{a}	195.33^{b}	188.33^{b}	180.17^{c}	2.55	0.0001
Digestive Enzymes						
Amylase, U/L	511.33^{b}	673.50^{a}	677.00^{a}	683.50^{a}	11.55	0.0001
Lipase, U/L	71.82^{c}	89.28^{b}	91.38^{b}	94.33^{a}	0.97	0.0001
Trypsin, U/L	75.83^{b}	94.33^{a}	97.00^{a}	95.50^{a}	1.34	0.0001

Abbreviations: RBS: random blood sugar, Total Chol: Total Cholesterol, TG: triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine Aminotransaminase, AST: Aspartate Amino transaminase, MDA: Malondialdehyde, SE: Standard Error, ELEx: Eucalyptus leaves extract, $^{a-c}$: Means within the same row with different superscripts are significantly different(P≤0.05).

Antioxidant and immunological indices

The effects of ELEx treatments on antioxidant parameters and immune responses were analyzed in Table 4. Inclusion of ELEx in quail diets led to a significant improvement in IgG, IgA, and IgM levels, as well as glutathione peroxidase, while reducing thiobarbaturic acid reactive substances compared to the control diet. Particularly, the group treated with 750 mg ELEx/kg feed demonstrated the most significant enhancements in all antioxidant parameters and immune responses.

Table4. Effect of dietary eucalyptus leaves extract on antioxidant parameters and immune response in growing Japanese quail.

Items Treat.	Control	ELEx 250 mg/kg	ELEx 500 mg/kg	ELEx 750 mg/kg	SE	P-value
Antioxidant Parameters						
GSH-PX(nmol/min/ml)	1879.67^{b}	1984.83^{ab}	1982.83^{ab}	2083.50^{a}	35.81	0.0030
TBARS(nmol/ml)	1.33^{a}	1.09^{b}	0.99^{b}	0.97^{b}	0.07	0.0034
Immune Indices						
IgG (mg/dl)	965.76^{c}	1056.73^{b}	1040.78^{b}	1092.76^{a}	9.88	0.0001
IgA (mg/dl)	87.43^{b}	99.03^{a}	98.44^{a}	101.30^{a}	1.00	0.0008
IgM (mg/dl)	177.18^{c}	190.70^{b}	191.04^{b}	196.62^{a}	1.43	0.0001

Abbreviations: GSH-PX: Glutathione Peroxidase, TBARS: Thiobarbutric Acid-Reactive Substances, IgG: Immunglobin G, IgA: Immunglobin A, IgM: Immunglobin M, SE: Standard Error, ELEx: Eucalyptus leaves extract, $^{a-c}$: Means within the same row with different superscript are significantly different(P≤0.05).

Microbial content

Table 5 elucidated the impact of dietary ELEx on intestinal bacteria in growing Japanese quail. Quails consuming a diet with 750 or 500 mg ELEx/kg displayed significantly reduced populations of *Salmonella and E. coli.* Notably, the group receiving 750 mg ELEx/kg showed the highest *Lactobacilli* population without significant differences in other treatment groups.

Table 5. Effect of dietary eucalyptus leaves extract on intestinal bacteria and intestinal environment in growing Japanese quail.

Items Treat.	Control	ELEx 250 mg/kg	ELEx 500 mg/kg	ELEx 750 mg/kg	SE	P-value
E.coli log 10cfug	7.39[a]	6.65[b]	6.29[c]	6.22[c]	0.08	0.0001
Salamonella log 10 cfug	7.24[a]	6.10[b]	6.22[b]	6.13[b]	0.06	0.0001
Lactobacillus log 10 cfug	5.85[c]	6.04[b]	6.20[b]	6.56[a]	0.07	0.0001

Abbreviations: SE: Standard Error, ELEx: Eucalyptus leaves extract, [a-c]: Means within the same row with different superscript are significantly different (P≤0.05).

Discussion

In the present trial, quails that were provided with a diet containing 750 mg ELEx exhibited a significantly higher LBW, BWG, PI, and faster GR in comparison to the basal treatment. This observed improvement in performance could potentially be ascribed to the increase in antioxidant activities, enhancement of nutrient digestibility, and maintenance of favorable microbial populations. Previous pharmacological investigations have underscored the multifaceted physiological processes of ELEx in the quail's body, which can be linked to its variety of active functions, diverse antioxidant phytochemicals, and bioactive elements (Dhakad et al., 2018). Additionally, the favorable effectiveness of ELEx on performance of quail chicks might be associated with the capacity of EEO bioactive compounds to induce the secretion of digestive and pancreatic enzymes, facilitate enhancements in gut structure, and boost immune response (Hashemipour et al., 2013, Giannenas et al., 2018 and Chowdhury et al., 2018). Furthermore, the possible impacts of medical plants and their various oils in stimulating appetite and exhibiting antimicrobial properties could contribute to the observed enhancement in growth (Windisch et al., 2008). Also, the studies on broilers and laying hens conducted by Mashayekhi et al. (2018) and Chen et al. (2018) showed that treated with 0.5% and 0.8 g/kg eucalyptus leaves powder and polyphenols extracted from eucalyptus leaves led to improvements in BWG and productive traits. Additionally, broiler feed with 1.8-cineole recorded the best growth performance and antioxidant capacity (Shirani et al., 2019). Lippens et al. (2005) provided evidence that broilers receiving plant extracts exhibited significantly higher BWG compared to other treatment groups. In this respect, Sarica et al. (2005) illustrated that broilers treated with herbal plants have an enlargement in the relative length of the small intestine with increment in absorption surface. Where, longer digestive tracts are associated with better digestion and absorption of nutrients, because the elongated gastrointestinal tract increases the surface area available for absorption and improves nutrient transportation mechanisms. This finding suggests that the functionality of the villi is activated following the consumption of ELEx in the diet (Awad et al., 2009). Al Salman and Al-Gharawi (2019) demonstrated a significant increase in body mass and weight gain with all aqueous extracts of Eucalyptus leaves. The improved growth performance may be due to ELEx's capacity to boost the production of digestive enzymes, encourage the proliferation of beneficial bacteria, specifically *Lactobacillus species*, and inhibit the growth of detrimental bacteria in the gut, resulting in enhanced digestion and absorption (Elbestawy et al., 2016). Awaad et al. (2016) documented that eucalyptus oil possesses antimicrobial properties, resulting in a significant improvement in body weight. Ibrahim et al. (2018) noted a remarkable increase in body weight in broiler chicks upon the addition of eucalyptus leaf oil. Additionally, Zeng et al. (2015) corroborated that eucalyptus oil acts as an antioxidant, thereby enhancing the growth performance of broilers treated with such oil. Conversely, Rehman et al. (2013) reported that broilers fed diets supplemented by oil extracted from eucalyptus did not have any influence on BW.

Concerning to FI and FCR, birds receiving ELEx in their diets exhibited best FCR, which can be attributed to the active compounds present in eucalyptus, particularly phenols, which decreasing pathogenesis bacteria and supported beneficial microorganisms particularly *Lactobacillus* on intestine tract, stimulating the secretion of digestive enzymes and enhancing the digestion and absorption. Where, increase the utilization of feed intake with improving feed conversion ratio and immunity (Elbestawy et al. 2016, Makouei and Mehmannavaz, 2018 and Ibrahim et al. 2018). Plant extracts demonstrate antimicrobial properties against pathogenic microorganisms, promoting the growth of beneficial microbes and enhancing the secretion of digestive enzymes, thereby enhancing dietary digestibility (Zeng et al., 2015). Eucalyptus oils positively influence digestive enzymes, absorption, and weight gain. Furthermore, eucalyptus oil enhances feed conversion rates (Awaad et al., 2016 and Makouei and Mehmannavaz, 2018). Previous studies by Cabuk et al. (2006) demonstrated that broilers treated by eucalyptus oil exhibited the best FCR due to increased nutrient absorption surface and nutrient availability. Also, broiler and quail chicks fed diet with 0.5% eucalyptus leaf powder recorded the best FCR (Hassan et al. 2011 and Mashayekhi et al. 2018). Moreover, Motaal et al. (2008) and Waly et al. (2019) noted that laying hens and growing white rabbits that received diets supplemented by eucalyptus leaves powder and its extract significantly decreased feed intake and enhancing FCR. Recently, Kaur et al. (2023) revealed that the active ingredients that present in eucalyptus leaves extract such as 1-8cineol, pinene and pinocarveoltrans, which can improve feed intake and enhancing FCR. In contrast, broiler and quail chicks that received a diet with eucalyptus oil or eucalyptus leaves powder didn't have any

significant influence on FI and FCR (Mustafa, 2019, Petrolli et al., 2019 and Fathi et al., 2020). Moreover, no effects on FCR were observed when the diet of rabbits was supplemented with varying levels of eucalyptus (Fathi et al., 2019). Growing rabbits consumed more feed when eucalyptus leaves were added to their diet, according to Ahmed et al. (2005), because the volatile oil in the leaves improves palatability.

Concerning glucose levels, eucalyptus extract can gradually increase insulin secretion of the cloned pancreatic βcell line. Hence, the insulin release effect of eucalyptus extract may be the reason for the anti-hyperglycemic effect (Gray and Flatt, 1998, Ismail, 2007 and Arise et al., 2009). In this respect, Mashayekhi et al. (2018) found that broilers fed a diet with 0.5% eucalyptus have the lowest blood glucose level. Recently, Kaur, et al., (2022) reported that laying hens supplemented a diet with 0.45 and 0.60% eucalyptus leaf powder significantly have lower glucose levels.

Regarding lipid profiles, the many active ingredients present in eucalyptus, which act as natural antioxidants, may contribute to the overall hypocholesterolemic effect of eucalyptus supplementation. In this respect eucalyptus have many phenolic compounds like tannins, flavonoids, phenolic acids and terpenes are accountable for the antioxidant properties. These ingredients exhibit the ability to attach with LDL, obstruct free radicals, and subsequently diminish the absorption of cholesterol into the bloodstream (Brenes and Roura, 2010). Through an *invitro* investigation, Kladniew et al. (2014) noted that 1,8 cineole, the primary component found in eucalyptus, could hinder stages in cholesterol production by obstructing 3-hydroxy-3-methylglutarylCoA reductase, a significant enzyme in the cholesterol synthesis pathway. Furthermore, outcomes from *invivo* trials have suggested that the plausible mechanism behind the reduced cholesterol production due to phytogenic products may stem from the suppressive actions of their active elements on the reductase enzyme3hydroxy3methylglutarylCoA or the escalated breakdown of lipoproteins (Chowdhury et al., 2018). Additionally, a segment of the decline in blood cholesterol levels might be linked to the recognized functions of *Lactobacillus* following the improvement of intestinal microbial equilibrium (Ashayerizadeh et al., 2018 and Jazi et al., 2018). Our findings align with those of Kaur et al. (2022), who showed that layer hens treated with 0.45% eucalyptus leaf powder had significantly lower blood levels of triglycerides and cholesterol, which is in line with what Mashayekhi et al. (2018), Duskaev et al. (2020), and Namamian et al. (2020) found in broilers. Also, Waly et al. (2019) and Hassan et al. (2011) reported comparable outcomes when eucalyptus leaves were added to the diets of rabbits and quails. Recently, Alagbe (2023) ascertained that cholesterol levels were lower in treated groups in compare to untreated group, indicating the hypocholesterolemic potential of ELEx, which could improve food safety and mitigate the risk of cardiovascular diseases (Alagbe et al., 2022 and Alagbe et al., 2023a). Furthermore, Mohebodini et al. (2021) illustrated a significant decline in serum cholesterol levels in broilers received varying levels of EEO. Additionally, Chen et al. (2018) determined that hens fed with 0.8 g/kg from polyphenols extracted from eucalyptus leaves could lessen the cholesterol levels in yolks. As per Arise et al. (2009), blood triglycerides, cholesterol, and LDL in rats were all diminished by an aqueous eucalyptus extract.

Concerning the digestive enzymes, quails that were provided with a diet enriched with 500 mg ELEx /kg diet exhibited a notably higher trypsin level in comparison to untreated one. Also, the group that fed a diet completed by 750 mg/kg diet showed elevated levels of amylase and lipase. When there is a compromise in digestive system, it impacts the digestive tract functions (absorption and digestion), potentially leading to compromised health and performance (Soumeh et al., 2019). Phytogenic products are believed to operate by enhancing the secretion of digestive substances such as mucin, saliva, bile acid, and enzymes. From this point, the implication of medical plants rich in carvacrol, cinnamaldehyde, and capsaicin in the diet of broiler chickens was shown to enhance nutrient digestibility (Jamroz et al., 2003). Furthermore, Hashemipour et al. (2013) observed that incorporating phytogenic products containing a balanced blend of thymol and carvacrol at various concentrations into broiler diets led to a progressive increase in digestive enzymes activities in the pancreas and intestines. Similarly, Lee et al. (2003) illustrated that broiler chickens consuming diets supplemented with essential oils like thymol and carvacrol displayed heightened ileal digestibility coefficients for starch and protein due to increased amylase activity in comparison to untreated treatment. Also, Hashemipour et al., (2013), Chowdhury et al., (2018), Giannenas et al., (2018) and Mohebodini et al., (2019) demonstrated that adding phytogenic products such ELEx to broiler diets improves intestinal structure, antioxidant levels, gut microbiota equilibrium, intestinal integrity, and immune responses, where may increase digestive enzymes secretion and improve the digestion and absorption of nutrients. The digestibility coefficients exhibited a linear and quadratic growth in reaction to the gradual addition of eucalyptus oil in the diet (Mohebodini et al., 2021).

The incorporation of Eucalyptus leaf extract (ELEx) into the dietary regimen of quails resulted in a statistically significant elevation ($p<0.001$) in glutathione peroxidase concentrations, alongside a marked reduction ($p<0.001$) in thiobarbutric acid reactive substances when juxtaposed with the control dietary formulation. This enhancement in efficacy may be ascribed to the inherent ability of aromatic botanicals or their essential oils to donate hydrogen or electrons, in addition to facilitating the reallocation of the unpaired electron within the aromatic framework (Jin et al., 2020). Furthermore, it has been established that eucalyptus leaves (EL) encompass a plethora of phytochemical constituents, including gemin D, pedunculagin, tellimagrandinI, tellimagrandinII, pentagalloylglucose, oenotheinB, among others, which demonstrate notable antioxidant properties and prospective anti-aging implications (Chen et al., 2014 a, b). A study conducted by Luis et al. (2016) posited that eucalyptus essential oil (EEO) could serve as feasible alternatives to synthetic compounds, owing to their capacity to scavenge free radicals and impede lipid peroxidation. The researchers correlated the documented antioxidant attributes with the presence of 1.8 cineole. Additionally, Horvathova et al. (2014) observed that 1.8 cineole exhibited varying degrees of reducing power, radical scavenging potential, chelating efficacy, and DNA

protective functions. The pivotal antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) fulfill an essential role in cellular protection by mitigating the formation of free radicals and bolstering antioxidant defenses (Mohebodini et al., 2019). Mohebodini et al. (2021) elucidated that the administration of Eucalyptus essential oils (EEO) within the dietary regimen led to an augmentation in the serum antioxidant capacity of broiler chickens, as evidenced by an increase in SOD activity and a concomitant decrease in malondialdehyde (MDA) levels. This observation suggests that EEO may confer a superior capacity for free radical scavenging in broiler chickens. Fathi et al. (2020) disclosed that the activity of GSH-Px was significantly influenced ($P \leq 0.05$) by the incorporation of eucalyptus leaves (EL) into the dietary composition. A progressive increase in GSH-Px activity was recorded with ascending levels of EL. It is postulated that the presence of antioxidant constituents in EL augments GSH-Px activity, thereby mitigating oxidative stress in chickens through the inhibition of oxygen free radical generation. Moreover, the supplementation of 0.8g/kg polyphenols derived from EL in the diets of laying hens resulted in an improvement in serum antioxidant status via the enhancement of enzymatic activities ((GSH-Px), total superoxide dismutase(T-SOD), and total antioxidant capacity(TAC)), thereby averting oxidative damage and imparting protective effects on hepatic tissue (Chen et al., 2018). Recently, Alagbe (2023) suggested that eucalyptus camaldulensis oil exhibits antioxidant properties, particularly α pinene and eucalyptol, which provide a safeguard against the deleterious impacts of free radicals. The protective mechanisms of antioxidants manifest across multiple levels, obstructing the initiation of chain reactions by neutralizing free radicals, disrupting the sequential processes involved in the scavenging of free radicals generated during biochemical reactions, and eliminating peroxidases, thus impeding the further synthesis of reactive oxygen species (Olafadehan et al., 2020). Similar results were reported in a preceding investigation that documented a beneficial response following the supplementation of natural plant extracts in the dietary regimen of rabbits (Ayodele et al., 2016; Oluwafemi et al., 2021; Singh, 2022; and Shittu et al., 2022).

With respect to immune responses, the incorporation of ELEx into the diets of quails resulted in a statistically significant augmentation ($p<0.001$) of the concentrations of IgG, IgA, and IgM when juxtaposed with the control dietary regimen. ELEx is comprised of bioactive constituents, notably eucalyptol and α pinene, which are characterized by their immune-enhancing properties (Alagbe etal.,2023b). In addition, phytochemicals and bioactive compounds are associated with a plethora of pharmacological effects, including but not limited to hepatoprotective, antimicrobial, antioxidant, antiviral, anti-helminthic, and antifungal activities (Oluwafemi et al.,2021). Phenolic compounds function as antioxidants or immune modulators, providing protective mechanisms against the invasion of pathogenic microorganisms (Olafadehan et al.,2020). Our results are consistent with the findings of Alagbe (2023), who delineated modifications in the immune response of weaned rabbits subjected to a dietary regimen augmented with E. camaldulensis oil (ECO). Where, the levels of Immunoglobulin A, immunoglobulin G, and immunoglobulin M were significantly altered ($P<0.05$) due to the inclusion of ECO in the dietary formulations for the rabbits. Immunoglobulins (A, G, and M) demonstrated elevated levels ($P < 0.05$) at dosages of 200 mg, 400 mg, and 600 mg/kg relative to the control cohort. Oloruntola et al. (2016) documented an increase in immunoglobulin concentrations in rabbits that were fed a diet supplemented with *Alchornea cordifolia* leaves. Analogous results were recorded when phytogenic additives (*Gliricidia* leaf) were incorporated into the diets of weaned rabbits (Oloruntola et al., 2016 and 2018). Furthermore, the essential oil derived from eucalyptus globulus possesses the ability to enhance the innate cell-mediated immune response, thereby providing a scientific basis for the additional application of this botanical extract, alongside its established antiseptic and anti-inflammatory effects in rodent models (Serafino et al., 2008).

In the domain of microbial ecology, quails subjected to nutritional regimens incorporating 750 or 500 mg ELEx/kg of feed exhibited a marked reduction in populations of *Salmonella and E. coli*, concomitant with an increase in *Lactobacilli* populations when compared to the control cohort, as evidenced by rigorous statistical evaluation ($p<0.001$). The intrinsic biological characteristic of phytogenic agents resides in their ability to mitigate the presence of detrimental and pathogenic microorganisms within the gastrointestinal milieu, as elucidated by Brenes and Roura (2010). Investigations pertaining to avian species have demonstrated that phytogenic substances, including cinnamon essential oil and herbal extracts abundant in phenolic compounds, possess the capability to inhibit the colonization of intestinal pathogens such as *E. coli and Clostridium perfringens*, attributable to the antibacterial properties inherent in their phenolic constituents (Chowdhury et al., 2018 and Giannenas et al., 2018). The mechanisms through which phytogenic agents exert their antibacterial influence vary according to the source of the product, with a prevalent hypothesis suggesting a mode of action that includes the alteration of bacterial cell permeability (Windisch et al., 2008), disruption of the pH gradient within bacterial cells (Jin et al., 2020), and provision of a growth substrate for lactic acid-producing bacteria residing in the intestines, such as *Lactobacillus*, which utilize phenolic compounds derived from essential oils as carbon sources (Pacheco-Ordaz et al.,2018). Moreover, existing literature indicates that phytogenic products can augment the synthesis of intestinal mucus in broiler chickens, potentially obstructing the adhesion of pathogenic bacteria, including *coliforms and E. coli*, thereby contributing to the maintenance of a balanced gut microbial community in these animals (Windisch et al.,2008). The effectiveness of eucalyptus essential oils (EEO) in counteracting pathogens such as *E. coli and Salmonella* has been prominently demonstrated in controlled laboratory settings (Ghalem and Mohamed, 2008; Mekonnen et al., 2016 and Dhakad et al., 2018). These outcomes are ascribed to the presence of bioactive compounds such as 1.8-cineole, limonene, and α pinene (Dhakad et al., 2018). Mohebodini et al. (2021) noted that avian subjects receiving dietary supplements of EEO displayed elevated *Lactobacilli* concentrations in the caecal digesta alongside diminished *E. coli* populations. The intestinal microflora plays a crucial role in preserving the integrity of the intestinal mucosa (Jazi et al., 2020). Disruption of this integrity may lead to a progressive

increase in mucosal permeability, thereby facilitating the incursion of pathogens. Furthermore, there is accumulating evidence suggesting that phytogenic compounds, in specific contexts, promote the proliferation of beneficial gut microbiota. Murugesan et al. (2015) reported that the addition of a commercial phytogenic feed additive (comprising 30 essential oils) in broiler diets resulted in elevated *Lactobacilli* counts in the caecal digesta. Additional studies have also indicated that essential oils such as thymol, eugenol, carvacrol, and cinnamaldehyde enhance gut microflora by diminishing populations of harmful bacteria while simultaneously increasing the prevalence of beneficial microbial entities (Jamroz et al., 2003 and McReynolds et al., 2009).

CONCLUSION

Supplementing quails' feed with750 and500 mg of ELEx/kg can enhance their productive performance, physiological parameters, lipid profile, antioxidant capacity, immunological parameters, and intestinal flora. As a result, ELEx can be used to promote health and stimulate growth in growing Japanese quail.

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تأثير التغذية على علائق مضاف إليها مستخلص أوراق الكافور (*Eucalyptus globulus*) على الأداء ومقاييس الدهون والإنزيمات الهاضمة والمحتوى الميكروبي ومؤشرات مضادات الأكسدة والاستجابات المناعية لطيور السمان الياباني النامي

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الملخص

هدفت الدراسة الحالية إلى تقييم تأثير مستخلص أوراق الكافور (Eucalyptus globulus) (ELEx) على الحالة الفسيولوجية للسمان وأداء النمو وبعض معايير الدم وأعداد البكتيريا المعوية . في هذه الدراسة تم تقسيم ثلاثمائة وستين كتكوتا من السمان الياباني، يبلغ عمرهم عشرة أيام، إلى أربع مجموعات، تتكون كل منها من ستة مكررات بكل مكرر 15 كتكوت . حيث تم إعطاء المجموعة الأولى عليقة أساسية بدون أي إضافات (عليقة الكنترول)، بينما تم إعطاء المجموعات التالية عليقة الكنترول مضافا إليها مستخلص أوراق الكافور (ELEx) بتركيزات 250 و 500 و 750 مجم / ELEx لكل كجم عليقة، على التوالي . كشفت النتائج أن المجاميع التى تم معاملتها بمستخلص أوراق الكافور (ELEx) أظهرت ارتفاع ملحوظ في وزن الجسم الحى ومعدل الزيادة في وزن الجسم ($p<0.001$) مع تحسن معنوي في كفاءة التحويل الغذائي (FCR)، واقل كمية علف مأكول (FI)، ولا سيما تلك التي تلقت 750 مجم من ELEx متبوعة بتلك التى تلقت 500 مجم من ELEx مقارنة بمجموعة الكنترول. أظهرت مجاميع السمان التي تناولت علائق مكملة بـ ELEx مستويات مرتفعة بشكل ملحوظ من الأميليز والتريسين والليباز ($p<0.001$) مقارنة بمجموعة الكنترول. سجلت المجاميع المعاملة بمستخلص أوراق الكافور (ELEx) زيادة ملحوظة في أعداد بكتيريا حامض اللاكتيك *Lactobacillus sp.* ، بينما حدث إنخفاض ملحوظ في أعداد بكتيريا القولون والسالمونيلا *Escherichia coli* و *Salamonella*. علاوة على ذلك حدث تحسن ملحوظ في وظائف الكبد ومقاييس الدهون وحالة مضادات الأكسدة والإستجابة المناعية للمجاميع المعاملة بمستويات مختلفة من ELEx مقارنة بمجموعة الكنترول. باختصار، أدت إضافة مستخلص أوراق الكافور ELEx بجرعة 750 مجم متبوعًا بجرعة 500 مجم إلى علائق السمان إلى إظهار أداءً مثاليًا للنمو وعددًا مثاليًا من الميكروبات المعوية والمقاييس الكيميائية الحيوية في مصل الدم لدى السمان الياباني النامي.