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INFLUENCE OF DIETARY SUPPLEMENTATION OF OLIVE LEAVES EXTRACT (*OLEA EUROPAEA*) ON PERFORMANCE, LIPID PROFILES, DIGESTIVE ENZYMES, MICROBIAL CONTENT, ANTIOXIDANT INDICES AND IMMUNE RESPONSES OF GROWING JAPANESE QUAIL

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ABSTRACT: The aim of the present study was to investigate the effects of incorporating an extract from olive leaves (*Olea europaea*), known as OLEx, as a natural additive in the diets of growing Japanese quail. The study examined various aspects of the quails' performance, including their productivity, levels of digestive enzymes, lipid profiles, liver functions, antioxidant levels, immune response, and gut ecology. A total of 360 Japanese quail chicks were divided into four treatment groups, each consisting of six replicates of 15 birds. The first group was given a standard diet without OLEx (control group), while the second through fourth groups were given the basal diet along with different concentrations of OLEx, being 150, 300, and 600 ppm, respectively. The findings revealed that quails fed diets supplemented with OLEx at concentrations of 300 and 600 ppm exhibited significantly higher body weight, body weight gain, and best feed conversion ratio. They also displayed a faster growth rate and had the best performance index compared to the control group. Furthermore, quails treated with OLEx at 300 and 600 ppm consumed less feed compared to the control quails. The lipid profiles (excluding HDL), plasma malondialdehyde levels, liver enzyme levels (ALT and AST), thiobarbaturic acid-reactive substances, and the population numbers of *Salmonella and Escherichia coli* were significantly lower in quails fed diets containing OLEx at levels of 300 and 600 ppm. Additionally, these quails exhibited higher antioxidant levels (GSH-PX and SOD), immune indices (IgG, IgA, IgM), and population numbers of *Lactobacilli* compared to the control group. In conclusion, the incorporation of OLEx at concentrations of 300 and 600 ppm resulted in improved productivity, antioxidant capacity, blood biochemical and immunological indices, as well as intestinal microbiota in growing Japanese quails.

keywords: antioxidant, immune, olive leaves (Olea europaea), quail, serum biochemistry

1 | INTRODUC TION

Nowadays, quail farms is one of the important agriculture activities in the World for providing the chance of meeting animal protein needs for humans. Therefore, using natural feed additives, extracts, phytogenic and essential oils in poultry nutrition is diffuse tradition for to lower feed costs, improve livestock products' quality, productivity, and health, and improve a variety of physiological characteristics ((Laudadio and Tufarelli, 2011, Surai, 2014). In addition, these natural feed additives and their extracts have many active component use as growth promoters and antimicrobial agents that have multiple health and nutritional benefits for poultry (Zeng, *et al.,* 2015). In this regard, natural antioxidants from olive trees such as oleuropein, dimethyl oleuropein, ligostroside, oleoside, phenolic substances including (caffeic acid, tyrosol, elenolic acid, catechol, rutin, tocopherols and hydroxytyrosol) and flavonoids like (apigenin, kaempferol, and luteolin) have significant medicinal potential (Ghanbari, *et al*., 2012).

According to (Gikas, *et al*., 2007), they showed that oleuropein is the important active component found in olive leaves, which has positive and health-promoting benefits coming from its antioxidant characteristic. Olive tree pruning results in a quantity of leafs (around 25 kg/ tree) (Paiva-Martins, *et al.*, 2009). Cellulosic compounds found in olive leafs can be harmful to the ecosystem. Therefore, using these leaves as livestock feed has considerable fiscal and environmental benefits (Abo Omar, *et al.*, 2012). The main polyphenol component in olive leaves is oleuropein and some of its derivatives (such as hydroxytyrosol) (Lee, *et al.*, 2009). Obied, *et al*., (2005) reported that *olea europaea L.* leaf extracts have an anti-microbial, anti-inflammatory, antithrombotic, anti-atherogenic, and antioxidant activities. In broiler chicks, (Jabri, *et al*., 2017) reported that OLE have a good works against harmful intestinal bacteria. El-Damrawy, *et al*., (2013)) conducted that the greatest immunity and serum chemical parameters recorded when olive leaves powder was added at 2% to Mandarah chicks diets, improving body weight and feed conversion. Additionally, broiler chickens' performance was improved by adding OLE to drinking water at a rate of 10 mL/L (Jabri, *et al*., 2017). In quails, Bahsi et al. (2016) found that quail diets treated by 400 ppm of oleuropein can enhance productive performance and breast muscle lipids quality. Ahmed, *et al.* (2017) reported that laying hens performance was improved with oleuropein supplements at 50, 100, and 150 ppm. Olive leaves' active ingredients might be a viable option for modulating intestinal population of microbes and enhancing growth performance. Oleuropein and olive leaf extract can therefore be used as growth stimulants and alternative additives in poultry nutrition, however, further researches is required to determine their effectiveness, mechanism of action, and antimicrobial properties. There are a few number of studies on the effects of olive leaf extract and oleuropein as a feed additive on productive performance, digestive enzymes, antioxidant parameters, antimicrobial activity and immunity of quail (Bahsi, *et al*., 2016). Therefore, this study targeted to investigate the influence of incorporating three dosages of OLEx on growth, serum biochemistry and antioxidant substances, immunity, bacteria species content of the intestinal and digestion enzymes in Japanese quails during growing period.

2 | MATERIALS AND METHODS

The experimental work of the current study was carried out at Poultry Research Center, Faculty of Agriculture, Fayoum University, Egypt.

2.1 | Preparation of olive leaves extract:

Olive leaves used in this study were collected in winter (February) from the farms of Faculty of Agriculture, Fayoum University, Egypt. The leaves collected were cleaned from impurities matter and shade-dried with natural ventilation and after that mashed into a fine powder. The dried leaves were milled using a blender with a special size to ensure the leaves powder homogeneity. After that, 10 g of olive leaves powder were extracted for 2 hrs with 200 ml of 70% (v/v) aqueous ethanol at 38 ºC by a thermo-shaker which is fixed to 180 rpm. Then,

the samples were centrifuged at 5000 rpm for 15 minutes and the separated parts of the samples were carried to a rotary evaporator to remove ethanol under reduced pressure at 38 ºC, 120 rpm. The remaining aqueous solutions were lyophilized at -50 ºC, 0.028 mbar, so the water was evaporated using a Freeze dryer (-40C) apparatus to obtain the extract powder, the crude extracts which was kept in refrigerator in glass bottles until using Aytul (2010).

2.2 | Experimental design, birds and diets:

Birds lodging and management methods during trial were in agreement with protocols of the Institutional Animal Care Committee of the Fayoum University (Code No. of the proposal: AEC 2215). A total number of 500 1-day- old Japanese quail chicks were reared in electrically heated batteries and were fed on a basal diet containing 24% crude protein with 2900 Kcal ME/kg of diet, from one up to 10 days of age, according to National Research Council (NRC, 1994). At day 10^{th} of age, 360 unsexed quail chicks (average weight of 60.46 ± 0.59 g) were randomly disturbed to four equal treatments, each treatment containing 90 birds in six replicates of 15 birds each. The first treatment fed a basal diet without additives (control group), the second, third, and fourth groups were received the control diet plus 150, 300 and 600 ppm olive leaves extract (OLEx), respectively. On day ten, chicks were individually wing-banded by using small size plastic bands, and were reared in cages with dimensions 60 × 40 × 25 cm^3. A continuous lighting program was applied throughout the experimental period. Water and feed were offered to birds *ad-libitum* all over the experimental period. Table 1 displays the feed ingredients and chemical structure of the experimental diets. Olive leaves extract was mixed to diets manually with a small amount of the feed, then the quantity was increased with good mixing until reaching the demanded homogeneity, after that the mixing was completed, then diets were mixing was stored in sealed and labelled bags according to each treatment for the purpose of maintaining the effectiveness of the additives.

2.3 | Growth performance:

Chicks of quail were individually weighed and feed intake (FI, g) per cage was recorded through the test period (10–38 days of age), the uneaten feed discarded, the body weight gain (BWG, g) was calculated by the following: $BWG_{10–38} = BW_{38} – BW_{10}$ and feed conversion ratio (FCR), was measured as follows: FI (g)/ BWG (g). Growth rate (GR) and performance index (PI), were calculated according to (North, 1981).

2.4| Blood biochemistry, immunity, and antioxidant parameters:

At the end of the experiment (38 days), blood samples were collected from the slaughtered quails using two birds (1 male and 1 female) randomly from each replicate. The birds were initially weighed to the nearest g, and slaughtered by cutting the Jugular vein (Islamic method), and in dry clean centrifugal tubes individual 48 blood, samples were gathered and by centrifugation for 15 minutes at 755 g value, the serum was isolated then stored at −20℃ in a tube of Eppendorf till analysis. Total cholesterol (total Chol), triglycerides (TG), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were estimated by James (2001). According to Paglia and Valentine (1967), total antioxidant capacity, glutathione peroxidase (GPx) and thiobarbaturic acid-reactive substances (TBARS) were measured and SOD activity in whole blood hemolysates was determined spectrophotometrically with an automatic biochemical analyzer RX Daytona (Randox Laboratories), using commercially available Ransod kit (Randox Laboratories), which is based on the original method of McCord and Fridovich(1969). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by Friedman and Young (2005). Also, Amylase and Lipase enzymes were assayed by Friedman and Young (2005) and according to Bovine Trypsin ELISA Kit MBS706461 trypsin enzyme was determined. Conforming to Erhard et al. (1992), the tool used to test birds immunoglobulins (IgG, IgA and IgM) in Sandwich ELISA was the absorbance measured on a 450 nm ELISA plate

reader. Concentration of MDA in plasma and liver was measured using HPLC, according to the methodology of Wong et al. (1987) modified by Fukunaga et al. (1995), and the quantification of MDA was performed using external standard (TEP, 5-point calibration curve). The procedure for the MDA determination in the liver was already described in Trebušak et al. (2014).2.5| Liver hepatosomatic index, digesta pH **and viscosity:** Liver and intestinal tract were removed, and weighed after-that stored at – 80℃ and homogenized until analysis. The contents of small intestine and caecum were squeezed out by finger pressure, collected in the Eppendorf tubes, and stored at −20℃ until microbial count was performed. Viscosity of small intestine contents was determined on the same day and placed directly in a centrifuge tube (10 ml). The centrifuge tubes with fresh digesta were immediately placed on ice in an isolated box until viscosity measurements within 1 h following slaughtered and Hepatosomatic Index was calculated according to Nur-Azri, *et al.,* (2018) using the following equation

Hepatosomatic Index (HSI) = $weight\ of$the $liver\ (g)$ / final body $weight\ (g)$ x 100

The pH of digesta from each bird was measured using a digital pH-meter (pH 315i, WTW Wissenschaftlich- TechnischeWerkstätten, Weilheim, Germany). The viscosity of intestinal digesta was determined according to Tsiouris, *et al.* (2013) using a Brookfield DV-II + PRO Digital Viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA). Two readings were taken from each sample and were represented in units of centipoise (CP).

2.6 | Microbial analysis: Intestinal content was immediately collected in sterile glass containers, digesta was evacuated and mixed. Samples (1 g of the mixed fresh mass) were taken into sterile test tubes, diluted 1:10 in sterile 0.1% peptone solution, and homogenised for 3 min in a Stomacher homogeniser. Ten-fold serial dilutions of up to 10−7 of each sample were prepared in 9 ml of 0.1% sterile peptone solution. Viable counts of *Salmonella ssp, Escherichia coli (E. coli)* and *Lactobacilli ssp.* were performed. One millilitre of the serial dilution was incubated into sterile Petri dishes and sealed with an appropriate medium. *Lactobacillus spp.* colony count was determined using MRS agar (Biokar Diagnostic) after incubation in an anaerobic chamber at 37°C for 72 h. *Salmonella and E. coli* colonies were counted on a brilliant green agar plate and incubated at 37°C for 24 h. After cultivation in Petri dishes, the total colony count for *Lactobacilli, Salmonella.* and *E. coli* was then calculated as the number of colonies by reciprocal of the dilution. The microbial counts were estimated as colony-forming units per gram (cfu/g) of the sample.

2.8 | Statistical analysis:

The results obtained were statistically analyzed using the statistics tools (analysis of variance) of Infostat (Di Rienzo, 2017), the following model was applied:

$Yij = \mu + Ti + eij$

Where: Yij: observation of traits, μ: overall mean, Ti: treatment effect, eij: random error. All means were compared using multiple range test (Duncan, 1955), at significance level of 0.05.

3 | RESULTS

3.1 | Growth performance:

The results depicted in Table 2 elucidated the impact of the inclusion of olive leaves extract (OLEx) in the diet on the performance of growing Japanese quail. The utilization of OLEx exhibited a significant influence ($p \leq 0.001$) on the growth performance observed within the period of 10 - 38 days of age (referred to as the growing period). Specifically, the chicks that were provided with diets supplemented with OLEx at concentrations of 600 and 300 ppm, respectively, displayed superior performance in terms of $LBW_{38}d$, BWG_{10-38}, FCR_{10-38}, and GR_{10-38}, when compared to the control group. The values for $LBW_{38}d$, BWG_{10-38}, FCR_{10-38}, and GR_{10-38} for the group receiving 600 ppm of OLEx were 243.49g, 182.90g, 3.23, and 1.20, respectively, while the corresponding values for the group receiving 300 ppm of OLEx were 242.77g, 182.13g, 3.24, and 1.20, respectively. Both the groups receiving OLEx at

concentrations of 600 and 300 ppm demonstrated superior performance in comparison to the control group. The optimal PI_{10-38} was observed for both cohorts nourished with OLEx 600 and 300 ppm, while the control group exhibited the lowest value of 7.58, 7.53, and 6.48, respectively. The birds that consumed diets containing OLEx 300 and 600 ppm demonstrated significantly ($p \leq 0.001$) lower feed intake values of 588.70 and 588.97g, respectively. Conversely, the OLEx 150 ppm group displayed the highest feed intake value of 598.17g, which did not differ significantly from the control group's value of 598.07g. Finally, the birds fed on control diets displayed significantly ($p \leq 0.001$) lowest $LBW_{38}d$, BWG_{10-38}, FCR_{10-38}, and GR_{10-38} values of 228.91g, 168.82g, 3.56, and 1.17, respectively.

3.2 | Blood biochemistry:

The findings presented in Table 3 indicated that the comprehensive lipid profile, encompassing parameters such as total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), were significantly influenced by the consumption of OLEx diets. Furthermore, excluding liver malondialdehyde (Liver MDA), the hepatic functions, specifically alanine transaminase (ALT), aspartate transaminase (AST), and (HIS) excluding liver malondialdehyde (Liver MDA), as well as the activity of digestive enzymes including amylase, lipase, and trypsin, were also significantly impacted by the OLEx diets. It was observed that chicks fed a diet supplemented with OLEx 600 ppm exhibited notably reduced levels of total cholesterol and VLDL, measuring 178.83 and 22.33, respectively, in comparison to the control group. Additionally, quails that received a diet treated with OLEx 300 ppm displayed significantly lower levels of TG and LDL, while exhibiting the highest HDL level, measuring 101.50, 121.50, and 39.67, respectively, when compared to the control group. All diets enriched with OLEx 300 and 600 ppm diet exhibited a significant ($p \leq 0.001$) reduction in liver enzymes (ALT and AST) and an increase in HIS, without eliciting any noteworthy impact on liver MDA levels when compared to the control group. Regarding the level of digestive enzymes (Amylase, Lipase, and Trypsin), the group supplemented with OLEx 300 ppm displayed a significantly higher ($p \leq 0.001$) level of Amylase at 842.67, surpassing the control group at 492.17. Also, quails consuming a diet supplemented with OLEx 600 ppm exhibited the highest Lipase and Trypsin levels (101.42 and 96.17, respectively) in comparison to the control group (67.05 and 69.33, respectively). In essence, the inclusion of graded levels of OLEx in the diets resulted in an improvement in digestive enzymes.

3.3 | Antioxidant capacity and immunity:

Both antioxidant parameters (GPx, TBAR, SOD, and Plasma MDA) and immunological indices (IgG, IgA, and IgM) were significantly influenced ($p \leq 0.001$) by all OLEx treatments, as depicted in Table 4. The incorporation of OLEx in diets resulted in an elevation of immune responses (IgG, IgA, and IgM), glutathione peroxidase, and SOD, while simultaneously reducing the presence of thiobarbaturic acid-reactive substances and plasma MDA in comparison to the control diet. Furthermore, the most notable effect ($p \leq 0.001$) was observed in the group treated with OLEx 600 ppm, where GPx, TBARS, SOD, plasma MDA, IgG, IgA, and IgM reached values of 526.67, 1.03, 1231.67, 0.51, 1085.25, 101.91, and 196.85, respectively, as opposed to the control group's values of 454.00, 1.57, 1091.67, 0.84, 927.75, 91.90, and 178.15. Overall, an increase in dietary OLEx levels led to improvements in the immunological indices (IgM, IgA, and IgG) as well as GPx values.

3.4 | Intestinal bacteria:

The data presented in Table 5 demonstrates the impact of dietary OLEx on the composition of intestinal bacteria in growing Japanese quail. In the current study, the addition of OLEx to the quails' diet resulted in a significant improvement in gut ecology ($p \leq 0.001$). This improvement was characterized by an increase in the population number of beneficial intestinal bacteria, specifically *Lactobacillus*. Furthermore, the addition of OLEx led to a

decrease in the population number of harmful intestinal bacteria, such as *Salmonella and E. coli*, when compared to the control group. Among the quails that were fed diets supplemented with OLEx at a concentration of 300 ppm, those with the lowest population numbers of *E. coli and Salmonella* were recorded, being 5.66 and 5.28, respectively. Additionally, this group exhibited the highest population number of *Lactobacilli* at 5.94. Moreover, the group that received OLEx at a concentration of 600 ppm displayed the highest viscosity and intestinal pH levels, being 2.79 and 6.80, respectively. In general, feeding the OLEx-treated diet resulted in a significant increase in the population number of beneficial *Lactobacilli*, as well as an increase in viscosity and intestinal pH. Furthermore, this diet led to a significant decrease in the population number of intestinal *E. coli and Salmonella*.

4 | DISCUSSION

In the present experiment quail chicks that received diets augmented with OLEx at 600 and 300 ppm, respectively presented the better $LBW_{38}d$, $BWG_{10\text{-}38}$, $FCR_{10\text{-}38}$, PI and faster $GR_{10\text{-}38}$ while, recorded significantly the lowest feed intake value compared to untreated group (control). These findings demonstrate that the addition of OLEx has led to an enhancement in growth performance. This improvement can potentially be attributed to the presence of various active constituents, such as oleuropein and its derivatives, hydroxytyrosol, caffeic acid, vanillic acid, vanillin, and rutin (Altiok, *et al.*, 2008). Furthermore, the extract obtained from olive leaves possesses a wide array of pharmacological activities, including antioxidant, anti-inflammatory and others (Carluccio, *et al.*, 2003). Additionally, (Toghyani, *et al.*, 2011) suggested that the observed enhancement in productive performance following the supplementation of OLEx may be linked to alterations in caecal microflora and blood metabolites (Zeng, *et al.*, 2015). Our data agree with (Erener, *et al.*, 2020) who reported that broilers fed a diet with $OLEx_{150}$, OLE_{300} and $OLEx_{600}$ had the higher daily BWG than the basal diet (control). In addition, Bahsi, *et al.*, (2016) discovered that the performance of quail was enhanced when they were provided with a diet supplemented with oleuropein. This finding was also observed by El-Damrawy, *et al.* (2013) and Jabri, *et al.* (2017) in broilers, as well as by Cayan and Erener (2015) and Ahmed, *et al.* (2017) in laying hens. Similarly, Oke, *et al.* (2017) demonstrated that broilers receiving 15 mL of OLEx exhibited significantly better final live body weight and body weight gain compared to those receiving 10 mL, 5 mL, and control. Mahmoud, *et al.* (2013) noted that average final body weight and body weight gain significantly increased in treatments supplemented with dried guava leaves or olive oil compared to the un-supplemented control. Similar results were reported by Rattanaphol and Rattanaphol (2009), who indicated that broiler chickens fed diets containing 0.5 and 2% olive oil had substantially improved final live body weight and average daily gain compared to the control group. The positive impact of olive oil on broilers' performance may be attributed to its ability to enhance digestion and nutrient absorption, including important fat-soluble vitamins. Additionally, olive oil has been shown to exhibit an antibacterial, anti-inflammatory, and antimicrobial effects that contribute to the improvement of chicks' digestive health (Pandey and Shweta, 2011 and Ryu, *et al*., 2012). In this respect, OLEx is a viable option to feed additives used as growth promoters because it has antimicrobial characteristics (Ocak, *et al*., 2008 and Amad, *et al*., 2011). Due to their greater livability with best FCR, the birds fed on olive leaves showed better European production index values (Varmaghany, *et al.*, 2013). Contrary to these publications, Xie, *et al*., (2022) noticed that the ADG of broiler was not substantially altered until the supplementing amount of OLE in the basal diet group reached more than 0.3%. Oleuropein was also found to have no discernible growth-promoting effects when added to the diets of quail at increasing levels (Sarica and Toptas, 2014). Furthermore, (Tarek, *et al.*, 2013) found that the performance of broiler chickens fed various levels of OLEx did not vary substantially.

Concerning to FI and FCR, quails fed diets containing OLEx 300 and 600 ppm recorded significantly the lowest feed intake value with the best FCR. In this regard, the improvement in FCR may be due to the good flavor with palatability of feed, augment digestive enzymes activity and nutrient absorption when fed with OLEx, also be connected to modifications in intestinal bacteria (Toghyani, *et al.*, 2011 and Zeng, *et al*., 2015), and in studied blood metabolites (Jemai, *et al*., 2008) when feeding OLEx. The results in the present study agree with, (Erener, *et al.*, 2020) who noticed that chicks fed diets supplemented with OLEx 300 and 600 ppm enhance the FCR with the highest FI compared with the OLEx 75, 150 and control groups. In this respect, Erener, *et al.* (2009) observed that adding olive leaf extract to chick diets increased BW and improved FCR. Similarly, Oke, *et al*., (2017) showed that the daily feed intake and total feed intake of the chicks received 15mL and 10mL OLEx in water were similar to but higher ($P < 0.05$) than those received 5 mL and control. Moreover, there was a significant improvement of FCR with increasing level of OLE. In the same trend, Ahmed et al. (2017) illustrated that birds fed diets treated by oleuropein at 50, 100 and 150 mg/ kg recorded significantly the best FCR compared to the control. While, there were no discernible variations in FI between the groups given varying doses of oleuropein compared to control group. Furthermore, El-Damrawy, *et al.* (2013) demonstrated that chick treated by olive leave powder substantially enhanced BW and FC. Additionally, Bahsi, *et al.*, (2016) reported that added oleuropein to Japanese quails diets by 400 ppm improves FCR. Although broilers given the diet supplemented with 600 mg kg-1 oleuropein had a higher FI, the better FCR and enhanced growth could be ascribed to OLE's antimicrobial effects, as reported by Bedford (2000). Contrarily, Cayan and Erener (2015) reported that layer chickens that treated with olive leaf powder had no impact on FI or FCR. According to Shafey, *et al*. (2013), chicks that fed a diet augmented with OLEx did not affect their FI and FCR during the trial.

The findings in the current trail revealed that total lipids profile, liver functions and digestive enzymes were significantly affected by OLEx diets. Chicks fed diet supplemented with OLEx 600 ppm showed significantly lower levels of total Chol and VLDL. While, quails received a diet-treated by OLEx 300 ppm showed significantly the lowest levels of TG and LDL with the highest HDL levels. Moreover, all diets supplemented with OLEx 300 and 600 ppm diet caused significantly an impacted liver enzymes (ALT and AST) and HIS without any significant effect on liver MDA levels. In this regard, oleuropein, hydroxytyrosol and oleuropein aglycone have an antioxidant qualities that able to spoiled free radicals by stopping the activity of reactive oxygen species and by catching radicals before they reach their cell goals (Srinivasan, *et al*, 2007). Additionally, scientific research has revealed that the majority of the phenolic compounds discovered in olive leaf extract possess activities that can reduce the concentrations of triglycerides in the serum and liver (Romani, *et al.,* 1999). These activities are attributed to their ability to alter the metabolism of cholesterol. The process by which this hypocholesterolemic action occurs involves the inhibition of dietary cholesterol absorption in the intestine, the reduction of hepatic production of cholesterol, the stimulation of biliary secretion of cholesterol, and the promotion of cholesterol excretion in the feces (Rezar, *et al.*, 2015). Similarly, Erener et al. (2009) showed that broilers fed a diet with OLEx have the lowest seurm cholesterol level. Additionally, Sarica and Topbas (2014) reported that quails fed diet augmented by 200 ppm oleuropein reduced serum total cholesterol and LDL levels, the reduction in the levels of serum and triglycerides due to main phenolic component in olive leaf (oleuropein), which have hypocholesterolemic properties. In this connection, olive leaves contain components called hydroxytyrosol and oleuropein that are known to block the enzyme 3-hydroxy-3-methyglutaryl coenzyme A, which is important for the synthesis of cholesterol, and to stop LDL from being oxidized (Cayan and Erener,

2015). Krzeminski, *et al*. (2003) showed that the reduction in serum cholesterol for chicks treated by OLEx might be due to reducing cholesterol absorption from intestinal, and lowering liver cholesterol synthesis. Also, Sung, *et al.* (2004) reported that isoflavone contents of OLEx have an inhibitory effect on catalytic domain of 3-hydroxy-3- methyl glutaryl (HMG) CoA reductase for cholesterol synthesis. Mahmoud, *et al.* (2013) found that birds received diets supplemented by olive oil recorded significantly ($P > 0.05$) reduction in lipids concentration except for LDL. In addition, chicks fed a diet with 3% olive oil have lower LDL triglycerides concentration with significant variation in HDL level (Bahrani, *et al.,* 2012). Furthermore, El-Damrawy, *et al*., (2013) found that birds fed with diet completed by olive leave powder have significantly reduction in serum cholesterol and triglycerides. Similarly, Parsaei (2014) reported that chickens fed with OLP caused a significant decrease in serum levels of triglyceride, cholesterol, LDL, VLDL, HDL and hepatic enzymes. Ahmed, *et al*. (2017) found that the birds given 150 ppm of oleuropein increased the total cholesterol levels. While, treatments receiving 100 and 150 ppm of oleuropein recorded significantly lower LDL and triglycerides levels. Whilst HDL levels did not significantly vary between the various experimental treatments. In the same trend, Younan,*et al.,* (2018) found that birds given OLEx have significantly ($P<0.05$) higher HDL levels with lower cholesterol, triglycerides and LDL. Furthermore, birds received diets treated by 200 or 400 ppm of OLEx showed lower serum cholesterol and triglycerides levels compared to basal diet (Agah, *et al*., 2019). In contrast, diets completed with olive leaf did not influence on lipid profile in laying hens (Zangeneh and Torki, 2011). In addition, Erener, *et al.* (2020) found that chicks fed a diet treated by OLEx at 75, 150, and 600 ppm have the higher triglycerides and HDL with lower LDL, without variation in cholesterol levels compared to the control group. Also, El-Bahra and Ahmed (2012) reported that addition of olive oil in broilers diets at level 2% didn't have any significant influence on cholesterol, triglycerides and VLDL levels. Recently, Xie, *et al*., (2022) found that chicks fed diet complemented by OLE not significantly effect on serum cholesterol, LDL, HDL and HDL/LDL.

Regarding to liver enzymes (ALT and AST), Parsaei, *et al* (2014) found that diets treated by OLP at 0.75 and 1 % had the best (lowest) concentrations of ALT and AST, respectively, compared to untreated diet (control). In this regard, olive leaf have many pharmacological properties on animals liver enzymes, and this coming from their antioxidant influence (Visioli, *et al.*, 2002). Some positive impacts of olive leaf ingredients include reducing oxidative stress, stopping amino transferase enzyme emigration and treating hepatic cells and hepatic poisoning (Tiot, *et al.*, 2001). In the same line, Agah *et al*. (2019) demonstrated that birds fed a diet with OLEx at 400 ppm reduced the production of ALT and ALP. Where, OLEx addition stopped the production of reactive oxygen species and terminated harmful impacts on hepatocytes. In this respect, the phenolic structure of OLEx serves to lessen liver damage caused by free radicals and this may be due to OLE's antioxidant traits.

Concerning the digestive enzymes level, the OLEx 300 ppm group significantly had the highest Amylase level. While, quails fed diet supplemented with OLEx 600 ppm had the highest Lipase and Trypsin levels. In this respect, the pancreatic secretion of trypsin, lipases, and proteases into chicks gut were enhanced with adding a mixture of phenol thymol components and carvacrol oil (Hashemipour, *et al*., 2013). Other studies by Zhang et al.(2013); Yu et al. (2015) and Zhang et al.(2015) reported that birds feeding on fermented Ginkgo leaves have the highest protease and lipase enzymes levels with enhanced absorption function within gut, and this could be due to improving villus height and villus height-to-crypt depth ratio. In addition, Wenk (2002) demonstrated that polyphenolic components stimulate the function of intestinal enzymes by reducing the number of harmful microorganisms that colonize these animals'

digestive systems. The current findings are consistent with those reported by Polzonetti, *et al.* (2004), who found that birds fed a diet supplemented with oleuropein increased pepsin activity. This may be because olive leaf extract can increase digestive enzymes, stimulate animals' appetites and food consumption, have antimicrobial and anti-fungal impacts, protect against diseases, and improve the animals' performances.

Either all antioxidant parameters or all immunological indices were significantly affected ($p \leq 0.001$) by all OLEx treatments. Where, diets supplemented by OLEx increased immune responses, glutathione peroxidase, SOD and depressed thiobarbaturic acid-reactive substances and plasma MDA as compared to the control diet. Furthermore, group treated by OLEx 600 ppm showed the best effect for GPx, TBARS, SOD, plasma MDA, IgG, IgA, and IgM. In this regard, OLEx has been demonstrated to have a variety of biological properties including anti-inflammatory, anti-oxidant and anti-cancer features (Visioli, *et al.*, 1998, Owen, *et al.*, 2000, Visioli, *et al.*, 2002 and Carluccio, *et al.*, 2003). Our results are consistent with those of El-Damrawy (2011), who found that olive leaf extract substantially enhanced plasma SOD activity and reduced thiobarbaturic acid reactive compounds, the substantial amounts of SOD found could be a result to significantly improving in oxidative status and overall health of Mandarah chicks. According to El-Damrawy, *et al.* (2013), they showed that chicks fed a diet supplemented with olive leaves have greatly improved performance, oxidative status and immune response. Oleuropein has reportedly been shown to have strong antioxidant effects, including the inhibition of low-density lipoprotein (LDL) and to scavenge free radicals (Visioli, *et al.*, 2002). Additionally, Oke, *et al.* (2017) observed that birds treated by 15 mL was equal to those given 10 mL of OLEx in plasma SOD, however better than those received 5 mL of OLEx and untreated group (control). In addition, birds fed a diet with 15 mL OLEx had lower serum MDA concentration, while the highest MDA concentration was observed in control group. Furthermore, the ability of OLEx to provide the birds with sufficient antioxidant defense towards peroxidation of lipids throughout heat stress may be the cause of a lower amount of MDA in the poultry given 10 and 15 mL OLEx. Recently, Xie, *et al*., (2022) reported that chicks treated by OLEx at 0.3 % increased serum T-SOD levels while decreased serum MDA. According to Mahmoud, *et al.* (2013), supplementing olive oil caused the enzymes glutathione peroxidase and super oxide dismutase to rise dramatically ($P < 0.05$). In addition, olive oil's phenolic components reduce the oxidation of LDL (Zhou, *et al.*, 2009). According to Lee and Lee (2010) and Hayes, *et al.* (2011), the antioxidants found in the extract of olive leaves are thought to function as eliminating free radicals by stopping the free radical chemical reaction. Also, treatments given various amounts of oleuropein had substantially higher superoxide dismutase (SOD) compared to control. Moreover, treatments that receiving the highest amount of oleuropein had the substantially higher SOD compared to other treatments. According to Younan, *et al*. (2018), they noticed that by increasing the amount of OLEx in the diet, TAC greatly ($P<0.05$) increased with substantially ($P<0.05$) decrement in MDA. Also, Ahmed, *et al.* (2017) showed that laying chickens fed a diet with 50, 100 and 150 ppm of oleuropein have a considerable ($P<0.01$) betterment in SOD and TAC levels, with substantially reduction in MDA level. This indicate that, OLEx and olive leaf is a source of numerous polyphenols that were thought to be possible sources of antioxidants (Jemai, *et al.*, 2008). According to Agah, *et al.* (2019), chicks fed OLEx at 200 ppm had an improvement in blood GPx levels. In addition, the blood level of MDA was substantially lower in the birds given OLEx diet at 400 ppm in compared to control and other treatments. The lower serum MDA levels in supplemented birds compared to the control group's birds suggested that OLEx reduced lipid peroxidation by enhancing antioxidant action. Moreover, OLE's antioxidant properties in lowering both ROS

and lipid peroxidation appear to be the cause of the drop in serum MDA level. Xie, *et al.*, (2022) showed that birds treated by OLEx at 0.3% have dramatically lower MDA with higher T-SOD, GSH and GSH-Px levels in breast muscle. In this context, OLEx demonstrates significant antioxidant capabilities in attenuating reactive oxygen species and lipid peroxidation. Consequently, the enhanced antioxidant potential of OLEx on the pectoral muscle of the broiler chickens may have arisen from these collective endeavors, such as the synergistic efficacy of bioactive polyphenols. Furthermore, SOD is also considered as the primary antioxidant defense mechanism against assaults from free radicals. Thus, in order to eliminate or mitigate the oxidative toxicity caused by free radicals, GSH is catalyzed by GSH-Px with H_2O_2 to convert lipid peroxide into water and fatty alcohols. Consequently, the CAT activity, which functions as an H_2O_2 resistance mechanism, was considerably heightened and led to an improved antioxidant performance (Osman, *et al*,. 2020).According to the study conducted by Parsaei, *et al.* (2014), broilers that were fed a diet supplemented with OL at a concentration of 0.75% exhibited a significant increase in IgY levels, while there was no effect on total Ig and IgM levels compared to the control group. Olive leaves contain flavonoids, which have been shown to enhance IgY levels and improve immune function (Christaki, *et al*, 2004). Moreover, flavonoids possess antiviral, anti-inflammatory, and anti-allergic properties (Cushnie and Lamb, 2005). Certain plant extracts have the ability to enhance immunity by promoting antibody production and influencing gastrointestinal mucosal lymphocytes (Shams-Ghafarokhi, *et al*, 2003). Furthermore, *in vitro* studies have demonstrated the potent inhibitory effects of compounds derived from olive leaves against viruses (Micol, *et al*, 2005), as noted by Renis (1969). El-Damrawy, *et al.* (2013) asserted that the inclusion of olive leaf powder in the diets of broiler chickens led to a noteworthy increase in white blood cells (WBC's), heamaaglutination inhibition (HI), and superoxide dismutase (SOD). Furthermore, Parsaei (2014) provided evidence that the supplementation of broiler chickens' diets with olive leaf powder yielded beneficial effects on their immune system. In a study conducted by Ahmed, *et al.* (2017), it was found that the counts of white blood cells, percentage of lymphocytes, and the activity of total antioxidant capacity showed no significant differences between the groups that were provided with 100 and 150 mg oleuropein/kg diet. However, these groups displayed significantly higher levels of these parameters compared to the other experimental groups ($p<0.01$). On the other hand, the percentage of heterophils, the ratio between heterophils and lymphocytes (H/L), and the concentration of malondialdehyde (MDA) were significantly reduced in the groups that received varying levels of oleuropein compared to the group that did not receive any supplementation.

In the current investigation, the inclusion of dietary OLEx in the diet of quails yielded a significant improvement ($p \leq 0.001$) in gut ecology. This improvement was characterized by an increase in the population number of beneficial intestinal bacteria, specifically *Lactobacillus*. Additionally, there was an increase in viscosity and a decrease in intestinal pH. Conversely, there was a decrease in the population number of harmful intestinal bacteria, namely *Salmonella and E. coli*, compared to the control group. Notably, the quails that were fed diets supplemented with OLEx at concentrations of 300 and 600 ppm exhibited the lowest population number of *E. coli and Salmonella*, while showcasing the highest population number of *Lactobacilli*. Furthermore, these quails displayed increased viscosity and intestinal pH. In light of these findings, it is worth mentioning that olive leaf and olive leaf extract are abundant in phytochemicals, making them potential sources of antioxidants (Silva, *et al.,* 2006 and Jemai, *et al.,* 2008). Moreover, the use of herbs and phytogenic products in the poultry industry has been found to have antimicrobial effects, which help control and limit the growth and colonization of various pathogenic and non-pathogenic bacteria in the gut of poultry

(Toghyani, *et al.,* 2011). It is worth noting that olive leaf has been reported to exhibit inhibitory effects on several gram-negative and gram-positive bacteria, as well as yeast and parasites (Elliott, *et al.,* 1969 and Markin, *et al.,* 2003). Erener, *et al.* (2020) discovered that, apart from the OLEx 300 group, the tested doses of OLEx led to a reduction in the count of E. coli in comparison to the control group. Notably, the treatments did not affect the beneficial microorganisms, *Lactobacillus spp.*, although all levels of OLEx exhibited a significant antibacterial impact on the populations of *Clostridium spp. and S. aureus* when compared to the control group. In light of previous *in vitro* (Markin, *et al.,* 2003 and Sudjana, *et al.,* 2009) and *in ovo* (Ahmed, *et al.,* 2014 and Jabri, *et al.*, 2017) studies, as well as the present study, it can be suggested that OLEx has the potential to be included in the category of non-antibiotic growth promoters. Jabri, *et al.*, (2017) observed that the aqueous extract of olive leaves possessed antimicrobial activity against certain cecal pathogenic bacteria (total germs and coliforms) at a dosage of 10ml/l, while also significantly stimulating the growth of *lactobacillus*.

In an early study by Aziz et al. (1998), they established that oleuropein and other phenolic compounds completely hinder the progression of *Escherichia coli.* Moreover, Sudjana, *et al.* (2009) indicated that oleuropein plays a role in regulating the composition of gastric flora by selectively reducing the levels of *Campylobacter jejuni*, *Helicobacter pylori*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Xie, *et al.* (2022) observed a decrease in the intensity of *Escherichia coli* and a significant increase in the intensity of *Lactobacillus* and *Bifidobacterium* in the OLEx 0.3 group. These factors have the potential to significantly enhance the gut health of broilers, thereby making a substantial contribution to the enhancement of meat quality (Chen, *et al.* 2019; Dev, *et al*., 2020). The reduction in the abundance of *Escherichia* and *Shigella* in the broiler caeca (Eeckhaut, *et al.* 2016) holds promise for improving the gut health of broilers and reducing the occurrence of woody breast development (Zhang, *et al,.* 2021). Additionally, certain cell surface proteins derived from *Lactobacillus* have the ability to immobilize *Escherichia coli* and impede its proliferation (Tsou, *et al.* 2017). The inhibitory effects of secoiridoids, flavonoids, and simple phenols present in OLEx on *Escherichia coli* are attributed to their influence on the cytoplasmic membranes and the cell wall (Rodrıguez, *et al.* 2009), resulting in a significant decrease in *Escherichia coli* abundance. However, the presence of different compounds in this extract can also have additive or synergistic effects, making it difficult to distinguish and correlate each activity to specific compounds (Vezza, *et al.* 2019). Previous studies have shown that OLEx exhibits significantly higher antioxidant activity compared to vitamin C and E. This is due to the synergistic effects of flavonoids, oleuropeosides, and substituted phenols (Benavente-Garcı, *et al,.* 2000). In the context of this study, oleuropein and hydroxytyrosol play a critical role in adjusting the composition of gut microbiota and enhancing gut integrity (Sarica and Urkmez, 2016). One possible explanation for the inhibitory effect of polyphenols on the growth of *E. coli* is their ability to bind to bacterial cell membranes, thereby disrupting membrane function. This includes changes in permeability and the proton motive force through the loss of Hþ-ATPase and membrane-related functions (Lin, *et al.,* 2005 and Cardona, *et al*., 2013). Another potential mechanism by which phenols affect the growth of bacteria such as *Lactobacillus and Bifidobacterium* is by utilizing these phenols as substrates. *Lactobacilli* have the capacity to metabolize polyphenols during their growth process, with polyphenols providing energy for the cells in return. Additionally, these polyphenols can enhance nutrient consumption, particularly carbohydrates (Garcıa-Ruiz, *et al.,* 2008). In summary, the addition of polyphenols in poultry can modulate the species and levels of caecal microbiota.

5 | CONCLUSION

From the findings of the current investigation, it is conceivable to deduce the subsequent inference: the addition of OLEx in the diet at concentrations of 300 and 600 ppm exhibited enhanced efficacy in terms of productive performance, antioxidant capacity, blood biochemical and immunological indicators, as well as intestinal microbiota in quails. Consequently, OLEx may be employed as a stimulant for growth and a promoter of health status in the context of rearing Japanese quail.

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%)	9.5
Wheat Bran	1.5
Vegetable oil	0.5
DL-methionine	0.20
L-Lysine HCl	0.30
Salt (NaCl)	0.50
Vitamin and mineral premix*	0.50
Limestone	1.00
Di calcium phosphate	2.00
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 + Cystine	0.89

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

**According NRC, 1994.

Table (2): Effect of dietary olive leaves extract on growth performance in growing Japanese quail.

Items / Treat.	Control	OLEx 150 ppm	OLEx 300 ppm	OLEx 600 ppm	SE	P-value
Initial LBW(g)	60.10	60.52	60.64	60.58	0.59	0.9143
$LBW_{38}d$ (g)	228.91^{b}	241.88^{a}	242.77^{a}	243.49^{a}	1.32	0.0001
BWG_{10-38} (g)	168.82^{b}	181.36^{a}	182.13^{a}	182.90^{a}	1.08	0.0001
FI_{10-38} (g)	598.07^{a}	598.17^{a}	588.70^{b}	588.97^{b}	0.19	0.0001
FC_{10-38} (g/g)	3.56^{a}	3.30^{b}	3.24^{c}	3.23^{c}	0.02	0.0001
GR_{10-38}	1.17^{b}	1.20^{a}	1.20^{a}	1.20^{a}	0.01	0.0001
PI_{10-38}	6.48^{c}	7.35^{b}	7.53^{ab}	7.58^{a}	0.08	0.0001

Abbreviations: LBW: Live Body Weight, BWG: Body Weight Gain, FI: Feed Intake, FC: feed conversion, SE: Standard Error, OLE**x**: Olive Leaves Extract,

$^{a-c}$: Means within the same row with different superscript are significantly different ($P \leq 0.05$).

Table (3): Effect of dietary olive leaves extract on lipid profile, liver functions and digestive enzymes in growing Japanese quail.

Items / Treat.	Control	OLEx 150 ppm	OLEx 300 ppm	OLEx 600 ppm	SE	P-value
lipids profile						
Total chol. mg/dL	210.83^{a}	182.00^{b}	188.17^{b}	178.83^{b}	3.69	0.0001
TG , mg/dL	150.00^{a}	104.33^{b}	101.50^{b}	108.17^{b}	2.87	0.0001
HDL, mg/dL	32.83^{b}	30.00^{b}	39.67^{a}	33.00^{b}	1.72	0.0023
LDL, mg/dL	142.83^{a}	127.33^{b}	121.50^{b}	123.50^{b}	2.83	0.0001
VLDL, mg/dL	35.17^{a}	24.67^{bc}	27.00^{b}	22.33^{c}	1.47	0.0001
liver functions						
ALT, U/L	2.56^{a}	2.44^{ab}	2.39^{ab}	2.28^{b}	0.07	0.0601
AST, U/L	294.00^{a}	270.33^{ab}	261.50^{b}	247.67^{b}	10.40	0.0344
Liver MDA (nmol /ml)	1.47	1.46	1.48	1.47	0.01	0.5849
HSI	1.55^{b}	1.70^{a}	1.74^{a}	1.80^{a}	0.05	0.0032
Digestive Enzymes						
Amylase U/L	492.17^{c}	639.50^{b}	842.67^{a}	729.00^{b}	35.88	0.0001
Lipase U/L	67.05^{b}	67.13^{b}	98.95^{a}	101.42^{a}	2.40	0.0001
Trypsin U/L	69.33^{b}	91.67^{a}	93.17^{a}	96.17^{a}	3.94	0.0001

Abbreviations: Total Chol: Total Cholesterol, TG: triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine Aminotransaminase, AST: Aspartate Aminotransaminase, MDA: Malondialdehyde, HIS: Hepatosomatic Index (HSI) (g liver/ g final body *weight*), SE: Standard Error, OLEx: Olive Leaves Extract,

$^{a-c}$: Means within the same row with different superscripts are significantly different($P \leq 0.05$).

Table (4): Effect of dietary olive leaves extract on antioxidant parameters and immune response in growing Japanese quail.

Items / Treat.	Control	OLEx 150 ppm	OLEx 300 ppm	OLEx 600 ppm	SE	P-value
Antioxidant Parameters						
GSH-PX (nmol/min/ml)	454.00^{c}	512.00^{b}	512.33^{b}	526.67^{a}	3.63	0.0001
TBARS (nmol /ml)	1.57^{a}	1.18^{b}	1.25^{b}	1.03^{c}	0.05	0.0001
SOD	1091.67^{b}	1211.00^{a}	1223.33^{a}	1231.67^{a}	8.35	0.0001
Plasma MDA (nmol /ml)	0.84^{a}	0.57^{b}	0.54^{bc}	0.51^{c}	0.01	0.0001
Immune Indices						
IgG (mg/dl)	927.75^{c}	1023.92^{b}	1063.52^{ab}	1085.25^{a}	14.01	0.0001
IgA (mg/dl)	91.90^{c}	95.36^{bc}	99.12^{ab}	101.91^{a}	1.69	0.0008
IgM (mg/dl)	178.15^{b}	182.09^{b}	192.24^{a}	196.85^{a}	2.64	0.0001

Abbreviations: GSH-PX: Glutathione Peroxidase TBARS: Thiobarbaturic Acid- Reactive Substances, IgG: Immunglobin G, IgA: Immunglobin A, IgM: Immunglobin M, SE: Standard Error, OLE**x**: Olive Leaves Extract,
$^{a-c}$: Means within the same row with different superscript are significantly different (P≤0.05).

Table (5): Effect of dietary Olive leaves extract on intestinal bacteria and intestinal environment in growing Japanese quail.

Items / Treat.	Control	OLEx 150 ppm	OLEx 300 ppm	OLEx 600 ppm	SE	P-value
Gut Ecology						
E.coli log 10 cfug	7.17^{a}	6.42^{b}	5.66^{c}	5.70^{c}	0.12	0.0001
Salamonella log 10 cfug	6.91^{a}	5.91^{b}	5.28^{c}	5.70^{bc}	0.17	0.0001
Lactobacillus log 10 cfug	5.22^{b}	5.75^{a}	5.94^{a}	5.82^{a}	0.08	0.0001
Viscosity	2.27^{c}	2.47^{b}	2.53^{b}	2.79^{a}	0.03	0.0001
Intestines pH	6.71^{b}	6.71^{b}	6.71^{b}	6.80^{a}	0.03	0.0565

Abbreviations: SE: Standard Error, OLEx: Olive Leaves Extract, $^{a-c}$: Means within the same row with different superscript are significantly different (P≤0.05).,

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الملخص العربى

تأثير التغذية على علائق مضاف إليها مستخلص أوراق الزيتون (Olea europaea) على الأداء الإنتاجي ومقاييس الدهون وإنزيمات الهضم والمحتوى الميكروبي ومؤشرات مضادات الأكسدة والاستجابات المناعية للسمان الياباني النامى

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كان الهدف من الدراسة الحالية هو دراسة تأثير مستخلص أوراق الزيتون (Olea europaea) (OLEx) كإضافات أعلاف طبيعية في علائق طيور السمان الياباني النامية على الأداء الإنتاجي، وإنزيمات الهضم، ومقاييس الدهون، ووظائف الكبد، ومؤشرات مضادات الأكسدة، والمناعة، وبيئة القناة الهضمية. تم تقسيم 360 كتكوت إلى أربع معاملات، كل معاملة تحتوي على ستة مكررات كل مكرر يتكون من 15 طائر .غذيت المجموعة الأولى على نظامًا غذائيًا قياسيًا خاليًا من (OLEx) كعليقة كنترول، بينما تم إعطاء المجموعات من الثانية إلى الرابعة عليقة الكنترول مضافا إليها 150 و300 و600 جزء في المليون من مستخلص أوراق الزيتون على التوالي. أظهرت النتائج أن طيور السمان التي تم تغذيتها على عليقة مضاف إليها 300 و600 جزء في المليون من OLEx سجلت أعلى وزن للجسم وزيادة في وزن الجسم وأفضل نسبة تحويل ومعدل نمو أسرع مع أفضل مؤشر أداء معنويا ($p\leq 0.001$)، مقارنة مع مجموعة الكنترول. علاوة على ذلك، فإن طيور السمان التي تمت معاملتها بـ OLEx بمعدل 300 و600 جزء في المليون استهلكت كمية علف أقل مقارنة بمجموعة الكنترول. طيور السمان التي تم تغذيتها على علائق تحتوي على OLEx بمستويات 300 و600 جزء في المليون أظهرت بشكل ملحوظ ($p \geq 0.001$) أدنى مستويات للدهون باستثناءHDL ، ومالونديالدهيد البلازما، والمواد المتفاعلة مع حمض الثيوباربا توريك، والسالمونيلا وبكتيريا القولون وإنزيمات الكبد ALT وAST ، مع إرتفاع مؤشرات مضادات الأكسدة GSH-PX وSOD، ومؤشرات المناعة IgG ، IgA، IgM وبكتيريا حامض اللاكتيك مقارنة بمجموعة الكنترول. من خلال نتائج هذه الدراسة يمكن استنتاج أن إضافة OLEx بنسبة 300 و600 جزء في المليون أدت إلى تحسين الأداء الإنتاجي، والقدرة المضادة للأكسدة، والمؤشرات البيوكيميائية والمناعية للدم، والميكروبات المعوية في طيور السمان اليابانية النامية.