Effect of Marjoram Leaves Extract on Performances, Blood Indices, Digestive Enzymes, Immunity, Antioxidant and Microbial Population of Growing Japanese Quails

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ABSTRACT

The purpose of this study was to investigate the effects of adding marjoram leaves extract (MLEx) to growing Japanese quails diets on the gut microbiota, serum parameters, immunity, and growth performance. A total of 320 birds, were randomly assigned to four groups each consisting of four replicates of 20 birds (4 ×4×20). A MLEx-free control diet was given to the first group. The control diet plus 50, 100, and 200 mg of MLEx/kg diet were given to the second, third, and fourth groups, respectively. The study found that the groups fed a diet with 200 mg MLEx/kg diet had the best performance index, body weight, body weight gain, feed conversion ratio and faster growth rate when compared to the control group. Moreover, quails treated with MLEx consumed less feed than control group. With the exception of TG, the group that was fed a diet containing 200 mg of MLEx/kg diet had the lowest numbers of Salmonella, Escherichia coli, lipid profiles, blood glucose and liver enzymes (ALT and AST), with the highest levels of IgG, IgA, IgM and trypsin in comparison to the control group (p < 0.01). Furthermore, quails given 100 mg MLEx/kg diet showed the highest numbers of lactobacilli, amylase, and lipase, while TG and TBARS values were lowest in these birds. To sum up, growth performance, antioxidant activity, serum biochemical and immunity indices, and gastrointestinal bacteria were enhanced by the addition of MLEx at 200 and 100 mg/kg in diets of growing Japanese quails.

Keywords: Marjoram leaves extract, growth, antioxidant, immunity, quail.

INTRODUCTION

The utilization of antibiotics as a supplement in poultry nutrition for the purpose of enhancing performance has resulted in an escalation in pathogen resistance and the build-up of antibiotic residues in birds tissues (Azeem et al., 2014). Recently, there has been a surge in interest in extracting antioxidants from botanical sources for incorporation into animal diets as a substitute for antibiotics, where act as growth promoters, combat microbes and as natural antioxidants in poultry farming (Hernandez et al., 2004). Additionally, these botanical extracts have exhibited promise as potential replacements for antibiotics, while also demonstrating growth-enhancing effects comparable to those of antibiotics (Windisch et al., 2008). Herbal botanicals play a crucial role in enhancing feed efficiency, promoting body weight gain, and positively influencing the health of poultry (Yildirim et al., 2018). OriganumMajoranaL., (OM) belonging to the Lamiaceae family (also known as Majoranahortensis Moench), is indigenous to the Mediterranean region and commonly referred to as sweet marjoram and have many beneficial effects for human and animal consumption, whether in form oil or powdered leaves (Charles, 2013). Additionally, OM contain a wide range of active ingredients, including thymol and carvacrol (Bina and Rahimi, 2017). Many researchers showed that the multifaceted effects for OM may attributed to its antioxidative, antibacterial, anticoagulant, anti-inflammatory, and anti-cocciidal properties (Erenler et al., 2016; Deuschle et al., 2018; Mohamed et al., 2021). Habib et al. (2012) found that birds fed a diet supplemented with 2 or 4 g/kg of OM powder have highest feed intake with best immunological parameters. Additionally, Ali (2014) documented that incorporating OM powder at a concentration of 1.5% into the diet of broiler chickens led to improved productivity by augmenting weight gain and feed intake with best FCR. Moreover, Shawky et al. (2020) administered that broilers treated by 2% marjoram powder in the diet improved FCR and body weight gain. Marjoram was well-known to the ancient civilizations of Greece, Rome, and Egypt (Tainter and Grenis, 1993). Marjoram, recognized as a therapeutic plant, is renowned for its antifungal and antibacterial properties (Deans and Svoboda, 1990). Moreover, the aqueous extracts of marjoram exhibit potent antioxidant effects (Juliani and Simon, 2002). Thymol, an essential component of marjoram, is acknowledged for its health-promoting attributes (Shoji and Nakashimis, 2004), displaying antimicrobial and antifungal properties as well (Manou et al., 1998). Marjoram also plays a role in augmenting metabolism (Ahmed et al., 2009), which contains over 30 predominantly phenolic antioxidant compounds that displaying antimicrobial and anti-inflammatory characteristics (Alma et al., 2003). The supplementation of oregano has demonstrated positive impacts on productivity, mortality rates, modulation of gastrointestinal microflora, pathogen suppression, and immune system enhancement in poultry. Limited research exists on the effects of MLEx on quail performance. Therefore, this investigation was conducted to evaluate the potential implications of incorporating MLEx as feed additives on the growth performance, hematological parameters, immune function, antioxidant status, and caecal microbial count in growing Japanese quail.

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MATERIALS AND METHODS

The current experiment occurred at the Poultry Research Center, Faculty of Agriculture at Fayoum University in Egypt. The live animal research was conducted in accordance with the protocols approved by the Egyptian Institutional Animal Care and Use Committee at Fayoum University (Code No. of the research proposal: AEC 2360).

Experimental design and diets:
A total 500 1-day-old Japanese quail chicks were raised in electrically heated batteries and provided with a basal diet containing 24% crude protein and 2900 Kcal ME/kg of diet from 1-10 days, as recommended by the National Research Council (NRC, 1994). Upon reaching day ten, 320 unsexed quail chicks were randomly allocated to four uniform groups, each consisting of 80 birds divided into four replicates of 20 birds per group. The initial group was given a basal diet devoid of MLEx (control), while the subsequent three groups were administered the basal diet supplemented with 50, 100, and 200 mg MLEx/kg diet, respectively. The newly hatched chicks were tagged with small plastic bands at the age of ten days and housed in cages measuring 40 × 60 × 25 cm, with individual weighing of the quail chicks. The temperature within the rearing facility was maintained at 34–35°C in the first ten days and gradually reduced by approximately 2°C weekly until it reached 30-31°C by the third week, following which the birds were subjected to the standard environmental conditions at Fayoum University Poultry Farms. The birds were exposed to 23 hours of light per day, while feed and water were provided ad libitum throughout the duration of the study. Marjoram leaves in powdered form were procured from a local market in Egypt, soaked in 80% ethanol, and agitated on a magnetic stirrer. Subsequently, the dry extract utilized in the experiment was obtained through filtration and evaporation of the solvent, and the Marjoram leaves extract (MLEx) was preserved frozen until required. The MLEx was manually integrated into the diets using a small portion of feed, gradually increasing the amount with thorough mixing until achieving uniformity, following which the mixture was stored in sealed and labeled bags corresponding to each treatment to ensure the efficacy of the additives. The detailed composition of the basal diet is outlined in Table1.

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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>33.5</td>
</tr>
<tr>
<td>Soybean meal (44 %)</td>
<td>30.5</td>
</tr>
<tr>
<td>Corn gluten meal (60%)</td>
<td>9.5</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>1.5</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>0.5</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.20</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.30</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin and mineral premix*</td>
<td>0.50</td>
</tr>
<tr>
<td>Lime-stone</td>
<td>2.00</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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, 12.000IU; vitaminD3, 2.4000 IU; vitamin E, 30mg; vitaminK3, 4mg; vitaminB1, 3mg; vitaminB2, 7mg; vitaminB6, 5mg; vitaminB12, 15µg; nicacin, 25 mg, Fe, 80 mg; folc. acid, 1mg; pantothenic acid, 10mg; biotin, 45mg; choline, 125,000mg; Cu, 5mg; Mn, 80mg; Zn, 60 mg; Se, 150µg.

**According NRC, 1994.

Results:

- Growth performance:
  The live weight of each quail (LBW) was measured individually, and the amount of feed consumed by each pen was tracked weekly (FI). The calculation for body weight gain (BWG, g) was determined using the equation: BWG10 – BWG10 = BWG10. The feed conversion ratio (FCR) was assessed by the formula: FI (g)/BWG (g). Additionally, the performance index (PI) was computed in accordance with the methodology outlined by North (1981) as: PI = BW (kg) / FCR. Furthermore, the growth rate was determined based on the approach established by Brody (1945) as: GR = (LBW38 – LBW10) / 0.5 (LBW10 + LBW38).

- Blood biochemical, anti-oxidant and immunity:
  At the culmination of the study period (38 days of age), blood samples obtained from the slaughtered quails, where selected two birds from each replicate (one ♂ with one ♀). Quails initially weighed, after then slaughtered by cutting the Jugular vein (in accordance with Islamic guidelines). Subsequently, 32 individual blood samples were collected in dry, sterile centrifugal tubes, followed by centrifugation at a force of 755 rpm for a duration of 15 minutes to isolate the serum, which was then preserved at –20°C in Eppendorf tubes until further analysis. The quantitative assessment encompassed the determination of total cholesterol (TC), triglycerides (TG), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT). The various blood biochemical parameters were assessed calorimetrically utilizing commercially available diagnostic kits (manufactured by Spectrum Diagnostics Company, Egypt). Amylase and lipase enzyme levels were evaluated following the methodology proposed 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) activity was determined calorimetrically as per the procedure outlined by Paglia and Valentine (1967), whereas the measurement of thiobarbituric acid-reactive substances (TBARS) was conducted according to the methodology described by Yagi (1998) utilizing diagnostic kits from Cayman Chemical Company (USA). For the analysis of chicken Immunoglobulins Isotypes IgG, IgM, and IgA via Sandwich ELISA, the protocol established by Erhard et al. (1992) was followed, with absorbance readings taken using an ELISA plate reader set at a wave length of 450nm.

Microbial analysis:
Following the slaughter process, the contents of the intestines were promptly gathered in sterile glass receptacles, while the digesta was discharged and blended. The hermetically sealed containers were stored in the laboratory at 4°C until the enumeration of microbial populations. Subsequently, samples consisting of 1g the combined fresh mass were introduced into sterile test tubes, diluted at a ratio of 1:10 in sterile 0.1% peptone solution, and homogenized using a Stomacher homogenizer for a duration of 3 minutes. Serial dilutions, increasing tenfold up to 10^4 for each sample, were prepared in nine milliliters of 0.1% sterile peptone solution. Enumeration of Salmonella spp, Escherichia coli (E.coli), and Lactobacillus spp was conducted. A volume of one milliliter from the serial dilution was cultured on sterile Petridishes and sealed with an appropriate medium. The colony count of Lactobacillus spp. was ascertained by
employing MRS agar (Biokar Diagnostic, France) following an incubation period in an anaerobic environment at 37°C for 24 hours. Colonies of Salmonella and E.coli were enumerated on brilliant green agar plates and incubated at 37°C for 24 hours. Post-cultivation in Petri dishes, the total colony count for Lactobacilli, Salmonella, and E.coli was calculated as the number of colonies per the reciprocal of the dilution. The microbial counts were quantified as colony forming units (cfu) per gram of the sample.

**Statistical analysis**

The outcomes were examined by statistical techniques, analysis of variance, using Infostat software developed by Di Rienzo, 2017. The model is presented as follows:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]

Where: \(Y_{ij}\): observation of traits, \(\mu\): overall mean, \(T_i\): treatment effect, \(e_{ij}\): random error. All means were compared using multiple range test (Duncan, 1955). At significance level of 0.05.

**RESULTS AND DISCUSSION**

**Results**

**Growth performance**

The results displayed in Table 2 demonstrated that the nutritional supplements had a substantial influence (\(p < 0.001\)) on the growth performance during study period. Where, quails fed diets supplemented with 200 mg MLEX/kg diet showed the best PI 10-38, LBW38d, BWG10-38, FCR10-38, and faster GR10-38 in comparison to control treatment. Additionally, birds fed diets containing 200 mg MLEX/kg diet demonstrated a substantially lower feed intake value (\(p < 0.001\)) compared to the control group.

**Blood biochemistry**

Results presented in Table 3 demonstrated that the consumption of MLEX had a significant impact (\(p < 0.001\)) on all serum biochemical parameters. Quails that were fed diets supplemented with 200 mg MLEX/kg diet exhibited decreased levels of glucose, total cholesterol, LDL, VLDL, AST, ALT, along with increased HDL and trypsin compared to those on an un-supplemented diet. Additionally, birds that received diets with 100 mg MLEX/kg diet displayed the lowest TG levels and the highest amylase and lipase levels in comparison to the control diet.

**Antioxidants and immunity**

As presented in Table 4, MLEX significantly (\(p < 0.001\)) affected immunity indices (IgM, IgA and IgG) and antioxidant parameters (TBARS and GPx). When compared to the control, birds that received diets containing MLEX have the highest levels of IgM, IgA, IgG, and GPx, with the lowest levels of TBARS. More specifically, group supplemented with 200 mg MLEX/kg diet showed significantly improvements in GPx, IgG, IgA, and IgM. While, birds fed diets containing 100 mg MLEX/kg diet showed the lowest levels of TBARS when compared to control and other treatment groups. Overall, as MLEX levels in the diet increased, so did the immune indices (IgM, IgA, and IgG) and GPx levels.

**Intestinal bacteria**

The effects of dietary MLEX on intestinal bacteria in growing Japanese quail were demonstrated in Table 5. In comparison to the control group, the addition of MLEX to quail diets recorded a significant (\(p < 0.001\)) increasing in beneficial intestinal bacteria Lactobacillus population, with decreasing in harmful bacteria Salmonella and E. coli. In this respect, when MLEX was added to the diet, the number of Lactobacilli increased and the number of Salmonella and E. coli decreased noticeably in the intestines.
Table 4. Effect of dietary marjoram on antioxidant parameters and immune response in growing Japanese quail.

<table>
<thead>
<tr>
<th>Items / Treat.</th>
<th>Control</th>
<th>MLEx 50mg/kg</th>
<th>MLEx 100mg/kg</th>
<th>MLEx 200mg/kg</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH-PX (mmol/min/mg)</td>
<td>1807.75a</td>
<td>1991.25a</td>
<td>1889.25b</td>
<td>1995.00a</td>
<td>27.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>TBARS (nmol/μmol)</td>
<td>1.40a</td>
<td>0.90b</td>
<td>0.90b</td>
<td>0.90b</td>
<td>0.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>Immune Indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>955.59b</td>
<td>1021.74a</td>
<td>1035.33a</td>
<td>1037.91a</td>
<td>8.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>91.37c</td>
<td>96.16b</td>
<td>98.89a</td>
<td>100.05b</td>
<td>0.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>182.33c</td>
<td>189.15b</td>
<td>189.83b</td>
<td>194.83a</td>
<td>1.41</td>
<td>0.0001</td>
</tr>
</tbody>
</table>


**a**: Means within the same row with different superscript

Discussion

Quails fed a diet containing 200 mg MLEx have the best LBW 38d, BWG 10-38, and PI 10-38, with faster GR 10-38 values in comparison to the control cohort. In this respect, marjoram contains numerous bioactive compounds like thymol, carvacrol, and essential oil, which function as antioxidants, antibacterial, and antifungals. The enhancement in growth factors could be ascribed to thymol and carvacrol's potential to boost digestion efficiency by elevating digestive morphology, enhancing nutrient absorption and production in the intestine, potentially playing a crucial role in promoting growth (Ahmadifar et al., 2011 and Zhao et al., 2023). Furthermore, these active constituents have the potential to boost digestion efficiency by elevating digestive enzymes, saliva production, enhancing appetite, preventing intestinal infections, alleviating diarrhea and constipation (Baj et al., 2020, Hao et al., 2021, Khorsand et al., 2022, Ili´c et al., 2022, and Kolypetri et al., 2023). Bahakaim et al. (2020) noted that chickens fed with 600 μl marjoram displayed higher BW (P<0.05) in comparison to those fed with 300 μl marjoram and the control diet. Skoufos et al. (2016) proposed that this outcome might be due to the performance enhancement of broilers' diets supplemented with marjoram, potentially influencing the height of intestinal villi, limiting intestinal pathogens, increasing absorptive surface area, and enhancing nutrient digestion and absorption. The findings align with previous studies by Ampode and Mendoxa (2021), and Umar, et al., (2023), which found that the inclusion of marjoram leaves in broiler diets led to improvements in overall growth performance parameters. Similarly, research by Zaaaza, et al., (2022) and Salama, et al., (2023) showed that supplementing broiler diets with marjoram extract and essential oil resulted in increased body weight gain. Shawky, et al., (2020) observed a significant enhancement in growth factors such as BW and BWG, as well as an improved FCR with the addition of 2% marjoram to the diet compared to the control group. Furthermore, feeding higher levels of marjoram led to better performance indices values and improved protein efficiency ratio, as reported by Abd El-Hack et al., (2016). Moreover, Ri et al., (2017) found that incorporating marjoram powder in diets increased average DWG throughout the growth period. Additionally, studies by Gül, et al., (2020), Gao, et al., (2022), and Johnson, et al., (2022) showed that broilers and layers receiving marjoram-supplemented diets exhibited significantly higher LBW and BWG. Roofchaeae et al. (2011) discovered that supplementing broiler diets with 600 mg/kg of marjoram during the grower period significantly increased BWG. Ruan, et al., (2021) demonstrated that broilers supplemented with marjoram essential oil displayed better growth performance than those receiving antibiotics or no supplementation. Essential oils derived from marjoram, such as thymol and carvacrol, were also effective in enhancing broiler growth performance, as highlighted by (Li, et al., 2022 and 2023). Furthermore, combining carvacrol and thymol may improve intestinal morphology, enhancing nutrient absorption and production performance in laying hens, according to Wang, et al. (2022). Studies by Ezz El-Arab (2008), Osman et al. (2010), Ali (2014), Abdel-Wahab (2019), Zhang et al. (2021) and Amer et al. (2021) indicated that broilers supplemented with 1.0 and 1.5% marjoram powder exhibited significantly higher average daily weight gain compared to control birds. Ku, et al., (2015) illustrated the protective effects of a dietary supplement containing thymol and carvacrol from marjoram against pathogenic bacteria in broilers, relieving adverse effects and improving growth performance. Moreover, Johnson et al. (2022) found that adding essential oil of marjoram to diets increased body weights. Conversely, the supplementation of oregano essential oil in broiler diets at levels of 50, 100, 150, 300, and 1,000 mg/kg did not yield any advantageous outcomes on growth performance, as indicated by Botsoglou et al. (2002). Several studies have also demonstrated the negligible influence of marjoram and its constituents on bird's growth (Barreto et al., 2008; Avila-Ramos et al., 2012 and Kirkpinar et al., 2014). Furthermore, earlier investigations have suggested the absence of a beneficial impact on growth performance across all oregano concentrations (Karimi et al., 2010). Correspondingly, Lewis et al. (2003) and Cross et al. (2007) highlighted that the incorporation of marjoram in chick diets did not bring about any significant changes in broiler performance.

Regarding FI and FCR, birds that were provided diets treated with 200 mg MLExs exhibited the best FCR (10-38 days of age), accompanied by the lowest FI values when compared to the control cohort. In this respect, by increasing saliva and digestive enzymes secretion, calming the stomach and digestive tract, enhancing appetite, treating or preventing simple intestinal infections, and relieving diarrhea and constipation, marjoram may improve digestion process while minimizing feed intake. Additionally, the advantages that
growth-promoting feed additives provide for birds stem from modulating the gut ecology by decreasing harmful bacteria. Also, natural additives have active components and pharmacologically potent substances such as thymol and carvacrol that are enhancing the performance of poultry from stimulate digestive process (Ertas et al., 2005 and Alagawany et al., 2015). Furthermore, Lee et al. (2003a) established that certain bioactive constituents of carvacrol enhanced FCR in broilers, suggesting that the impact of carvacrol on FCR might be linked to increment feed utilization efficiency. There is empirical evidence indicating that herbal essential oils possess properties that stimulate appetite and digestion (Hernandez et al., 2004). Additionally, essential oils can significantly affect FCR and the modulation of microbial populations, which enhance nutrient absorption (Alagawany et al., 2018). In line with our investigation, Roofchaeet al. (2011) showed that the addition of 600 and 1200 ppm of marjoram essential oil significantly (P<0.05) improved the FCR of broiler chicks in comparison to untreated group. Additionally, Abdel-Wareth (2011) illustrated that the feed conversion ratio was positively influenced by the addition of 15 or 20 g marjoram/kg to broiler chicks. Mansoub (2011) identified that the most optimal (P<0.05) FCR for broiler chicks were observed in the group receiving 200 ppm of marjoram, while the lowest (P>0.05) daily feed intake result was noted in the group receiving 150 ppm of marjoram. Studies by Ezz El-Arab (2008), Osman et al. (2010), Ali (2014), Khattab et al. (2018), Abd El-Wahab (2019), and Shawky et al. (2020) showed that broilers fed a diet supplemented with 0.5, 1.0, 1.5, and 2 % marjoram had a preferable influence on feed consumption, in comparison to the untreated group (control). Where the supplementation groups had the best-feeding conversion rate (FCR) with the lowest feed intake (P<0.05). Furthermore, the total FI of the control group was significantly higher (P ≤ 0.05) than that of the treated groups, according to a recent study by (Salama et al., 2023). Conversely, Bahakaim et al. (2020) who mentioned that adding 300 μl and 600 μl of oregano essential oils (OEO) to the kg diet didn’t affect feed intake in either group. Furthermore, feeding broiler chickens with 150 mg/kg of marjoram powder increased their feed intake, according to (Ri et al., 2017). Moreover, Symeon et al. (2010) concluded that adding marjoram essential oil to broiler feed substantially negative influence on birds eating and drinking habits. Recently, Feng et al. (2021) reported that birds fed a diet supplemented with marjoram essential oil did not affect feed intake. Additionally, Abo Ghanima et al. (2020) showed the same results in layer hens.

In relation to random blood sugar (glucose), quails that were provided with diets containing 200 mg MLEX/kg exhibited decreased levels of glucose. Our results, in contrast to Ali (2014), who demonstrated that the addition of marjoram to broiler diets did not elicit any significant impact on glucose levels. Likewise, research by Ezz El-Arab (2008) and Osman et al. (2010) indicated that the supplementation of basal diets with marjoram did not result in any noticeable changes in glucose concentration in blood of broiler chicks.

Concerning the lipid profile, quails that were provided with diets supplemented with 200 mg MLEX/kg exhibited decreased levels of total cholesterol, LDL, and VLDL, coupled with elevated HDL levels compared to those fed an un-supplemented diet. Furthermore, quails fed diets containing 100 mg MLEX/kg displayed the lowest levels of TG compared to control treatment. Researchers Shad et al. (2016), Abo Ghanima et al. (2020) and Saleh et al. (2021) showed that thymol and carvacrol the main bioactive ingredients in marjoram essential oil (MEO), inhibited the formation of cholesterol by restricting the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase, an enzyme that contributes to cholesterol synthesis. Additionally, reduces pancreatic and gastric lipase activity, resulting in a significant reduction in gastric lipase, inhibiting lipid digestion and potentially reducing fat absorption and increased excretion of fecal bile acid cholesterol (Deng et al., 1998 and Yang and Koo, 2000). As mentioned by Dehghani et al. (2019), another possibility might be the effect of MEO on restricting enzymes needed for the mechanism of lipogenesis. Birds consuming dietary MEO exhibited notably higher plasma HDL concentrations compared to control treatment (Moghroovy et al., 2019). Furthermore, the decrease in triglycerides and total cholesterol was attributed to the cholesterol-lowering effect of carvacrol on HMG-CoA reductase, the key enzyme in cholesterol synthesis (Case et al., 1995). Furthermore, Abd-El-Wahab (2019) found that broilers given diets including marjoram had higher levels of HDL and significantly lower levels of cholesterol, LDL, and triglycerides in their serum when compared to the control treatment. Furthermore, birds given a diet with 10 mg marjoram/kg showed a substantial decline in serum TG levels, indicating that marjoram supplementation may increase bird's antioxidant capacity (Abou-Elkhair et al., 2014). Conversely, Bolukbas et al. (2006) indicated that dietary thyme oil increased plasma concentrations of TG, LDL, and HDL in broilers. According to reports from Ezz El-Arab (2008) and Ali (2014), they showed that the incorporation of marjoram did not substantially change cholesterol, TG, LDL and HDL levels.

Concerning to liver enzymes ALT and AST, marjoram significantly affected the levels of ALT and AST in broilers blood. Where the incorporation of marjoram to broiler diets resulted in a significant reduction of the liver enzymes (AST and ALT). In this respect, Abd El-Ghany and El-Metwally (2010) and Alagawany et al., (2015) suggested that the active substances in marjoram such carvacrol may safeguard the liver from damage, which would then lower the activity of the liver enzymes. The positive impact on liver function is probably due to the therapeutic properties of marjoram. According to our research, taking MLEX supplements lowered ALT and AST levels. This may be because MLEX has antioxidant properties that protect cells from damage to DNA, as mentioned by Abo Ghanima et al. (2020) in their investigation of laying hens fed marjoram active ingredients. Similarly, Oladokun et al. (2021) observed similar results in quails. Recent research by Johnson et al. (2022) demonstrated that the ALT and AST levels in groups that used oregano essential oil were significantly lower than control group. Conversely, Reis et al. (2018) found no variations in AST or ALT activities in broilers supplemented with a phytogenic additive containing carvacrol, thymol, and cinnamaldehyde.
Concerning digestive enzymes (amylase, lipase, and trypsin), quails that were fed diets supplemented with 200 mg MLEEx/kg exhibited elevated trypsin levels. Additionally, birds that consumed diets supplemented with 100 mg MLEEx/kg displayed the highest levels of amylase and lipase in compare to control treatment. The pharmacologically active ingredients and bioactive substances found in marjoram serve as growth enhancers, encouraging the release of gastrointestinal fluid and digestive enzymes (lipase and amylase) in broilers. According to Lee et al. (2003a) and Lovkova et al., (2001), this stimulation promotes feed digestion, stops bacteria adhesion in intestinal tract, and preserves the equilibrium of microorganisms in the gastrointestinal tract. Consequently, the significance of these plants is growing due to their antimicrobial properties and their positive impact on the bird's digestive system (Osman et al., 2005). In alignment with the current research, the commercial product CRINA, which contains 29% active components such as thymol, notably increased trypsin activity in broilers (Lee et al., 2003b). Hashemipour et al. (2013) found that gastrointestinal trypsin, lipase, and protease actions were significantly (P < 0.05) higher in birds fed diets complemented with marjoram than in the control treatment. Additionally, comparable to the untreated group, marjoram resulted in a linear increase in trypsin, lipase, and protease secretion from pancreas. It has been demonstrated that involving marjoram active ingredients to broilers feed will increase the production of α-amylase and trypsin from pancreas (Jang et al., 2007).

Concerning to antioxidant parameters, including TBARS and GPx, were substantially influenced by MLEEx (p < 0.001), where diets treated by MLEEx have the highest GPx levels and the lowest TBARS levels compared to the control group. Where group treated with 200 mg MLEEx have significantly the highest GPx. While, birds consuming diets supplemented with 100 mg of MLEEx exhibited the lowest TBARS levels in compare to untreated group. In this respect, Shan et al. (2005), Hashemipour et al., (2013), Park et al., (2014) and Gadde et al. (2017) showed that the active constituents in marjoram such (carvacrol and thymol), demonstrate robust antioxidant characteristics that potentially bolster the bird's defense mechanisms toward oxidative damage. Additionally, GSH-Px function as natural scavengers and enzymes that facilitate the conversion of hydrogen peroxide and peroxide radicals to inactive compounds (Fanucchi, 2014). Ri et al. (2017) noted that the addition of oregano powder improved the antioxidant profile primarily by diminishing MDA content and augmenting T-AOC levels in the broilers serum. According to Yanishlieva et al. (1999), phenolic OH groups present in thymol act as hydrogen donors for the proxy radicals produced during the first stage of lipid oxidation, which delays the production of hydroxyl peroxide. Our findings align with Abdel-Wahab (2019), who highlighted a substantial increase (P<0.001) in GSH-Px activity in the broiler blood-serum of groups treated with marjoram in compare to untreated. Marjoram, recognized for its robust antioxidant properties (Badee et al., 2013), potentially owes its efficacy to the carvacrol molecule, which acts as a natural antioxidant by mitigating lipid peroxidation, consequently preventing oxidative harm to cellular membranes (Yanishlieva et al., 1999). Consequently, the heightened GSH-Px concentrations resulting from marjoram supplementation may enhance the scavenging of free reactive radicals in broilers. Animals receiving a diet enriched with carvacrol exhibited elevated levels of SOD and GSH-Px, along with higher concentrations of polyunsaturated fatty acids (PUFA) in brain phospholipids compared to the un-supplemented control (Yoadim and Deans, 2000). Recent research by Johnson et al. (2022) unveiled that hens fed a marjoram-supplemented diet displayed significantly elevated TAC, SOD, and GSH-Px levels in comparison to control hens. Previous research has demonstrated an augmentation in TAC levels upon the administration of marjoram oils to poultry, as shown by Reshadi et al. (2020) and Zhang et al. (2021). Moreover, similar enhancements in GSH-Px levels have been noted in studies involving laying hens and quail, as reported by Herve et al. (2018) and Yu et al. (2019).

With regard to immunological parameters, diets containing MLEx were found to enhance the levels of IgM, IgA, and IgG in comparison to the control group. Notably, the most significant improvements were observed in the group administered with MLEEx at a dosage of 200 mg for IgG, IgA, and IgM. This phenomenon is further supported by the notion that herbs abundant in flavonoids, like thyme, can prolong the efficacy of vitamin C, function as antioxidants, and potentially augment immune responses (Acamovic and Brooker, 2005). Additionally, essential oils derived from oregano exhibit immune-boosting properties that lead to heightened antibody titers in broilers. Specifically, supplementation of oregano essential oil has been linked to increased levels of intestinal serum IgA (Ruan et al., 2021) as well as serum IgE and IgG (Li et al., 2023). Furthermore, the inclusion of a thymol and carvacrol mixture in the diet has been shown to elevate serum IgA levels in broilers (Hashemipour et al., 2013 and Awaad et al., 2014) owing to the antibacterial, antiviral, and antioxidant attributes associated with thymol and carvacrol, which are anticipated to enhance immune responses in chicks (Botsoglou et al., 2002). Acamovic and Brooker (2005) also noted the immune-boosting effects of the polyphenol fraction of thymol and oregano essential oil on the mononuclear phagocyte system, cellular, and humoral immunity. Similarly, Toghyani et al. (2010) highlighted the positive impact of marjoram extract supplementation on immunity, aligning with the findings of Osman et al. (2010) who emphasized the superior immunological performance associated with higher levels of marjoram supplementation. Moreover, recent research by Shawky et al. (2020) proposed that the incorporation of Marjoram at 2% level led to a significant increase (P<0.05) in gamma globulin, signifying its potential as an immune enhancer for broiler chicks. Additionally, Ezz El-Arab (2008) demonstrated that experimental diets containing marjoram improved immune status compared to control diets, potentially attributed to the higher mineral content of marjoram influencing oxygen transport essential for enhanced hemoglobin synthesis in the blood (Jones and Bark, 1979). Hong et al. (2012) indicated that oregano supplementation significantly boosted antibody levels in broiler chickens, acting as a stimulant for the immune system. Furthermore, Silva-Vázquez et al. (2018) observed elevated leukocyte and lymphocyte counts in broilers treated with two types of Mexican oregano oil. Lastly, Namkung et al. (2004) reported that the concentration of IgG in serum was heightened in mice.
and pigs fed diets supplemented with oregano extract compared to those on control diets.

The introduction of MLEx into the diet led to a noteworthy rise in the population of beneficial Lactobacilli in the intestines, accompanied by a substantial reduction in the numbers of intestinal E. coli and Salmonella. The antimicrobial characteristics of marjoram extract can be ascribed to its bioactive chemical constituents, including carvacrol as the primary element, sabine, terpinene, γ-cymene, α-terpineol, linalool, and other phenolic compounds. In this regard, Cross et al. (2007) illustrated that adding 1 g of marjoram/ kg of broiler diet resulted in a decline in the prevalence of E. coli. Our results are consistent with the research of Abdel-Wareth (2011), suggesting that marjoram boosted the population of lactobacilli in the small intestine of broilers. Nevertheless, Penalver et al. (2005) noted a significant antibacterial effect of marjoram essential oil against poultry-origin E. coli strains, accrediting this potent activity to thymol and carvacrol as the primary active constituents. Helander et al. (1998) carried out a study on the antibacterial mechanism of carvacrol and thymol, the two main components of marjoram essential oil, against E. coli, indicating their capacity to disrupt the bacterial membrane, resulting in the liberation of membrane-associated substances into the external milieu. They postulated that thymol and carvacrol could infiltrate bacteria, potentially influencing their proliferation. Abdel-Moneim et al. (2015) documented a marked decrease in overall intestinal bacteria in chickens that received a diet supplemented with MPEx as a natural growth enhancer, compared to the control cohort. This result was expected; as prior studies have corroborated the efficacy of marjoram extracts in managing pathogenic intestinal microflora through direct antimicrobial activities. Roochae et al. (2011) explored the influence of incorporating marjoram into broiler diets at different concentrations and noted that levels of 300 and 600 mg/kg diet led to a significant reduction in E. coli in contrast to the control group, without displaying notable augmentation in lactic acid bacteria.

**CONCLUSION**

The productive performance, physiological characteristics, lipid profile, antioxidant activity, immunity and gastrointestinal bacteria of quails can all be improved by adding MLEx to their diet at levels of 200 and 100 mg/kg/diet. For, growing Japanese quail, MLEx can therefore be used as a growth promoter and a health status enhancer.

**REFERENCES**


Abdel-Kader, I. A. et al.,


