J. of Animal and Poultry Production, Mansoura Univ., Vol . 15 (6):99 - 109, 2024

Journal of Animal and Poultry Production

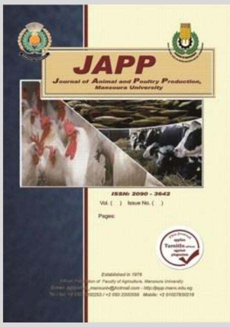
Journal homepage & Available online at: www.jappmu.journals.ekb.eg

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

Abdel-Kader, I. A.[1]; A. A. Abdel-Wahab[1*]; A. M. Abdelsalam[3]; Marwa M. Bahnas[1] and Rasha A. M. Somida[2]

Cross Mark

[1]Poult. Prod. Dep., Fac. of Agric., Fayoum Uni., 63514 Fayoum, Egypt.
[2]Animal Production Department, Faculty of Agriculture, Fayoum University.
[3]Agricultural Research Center, Animal Production Research Institute, Giza, Egypt



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-coccidial 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 Nakashims, 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.

*** Corresponding author.**
E-mail address: aaa16@fayoum.edu.eg
DOI: 10.21608/jappmu.2024.286901.1116

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 34–35ºC during the first ten days and gradually reduced by approximately2ºCweekly until it reached 30-31ºC 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 overnight. 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.

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
Lime-stone	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 + Cystine %	0.89

***- Premix provided per kg of diet: vitamin A, 12.000IU; vitaminD3, 2.400IU; vitamin E, 30mg; vitaminK3, 4mg; vitaminB1, 3mg; vitaminB2, 7mg; vitaminB6, 5mg; vitaminB12, 15µg; niacin,25 mg, Fe,80 mg; folic acid, 1mg; pantothenic acid, 10mg; biotin, 45mg; choline, 125,000mg; Cu,5mg; Mn,80mg; Zn,60 mg; Se,150µg.**
****AccordingNRC,1994.**

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 –38 = BW38–BW10. 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 asper the procedure outlined by Paglia and Valentine (1967), whereas the measurement of thiobarbaturic 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 1gofthe 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^{-7} for each sample, were prepared in nine milliliters of 0.1% sterile peptone solution. Enumeration of *Salmonella spp, Escherichia coli (E.coli), and Lactobacilli 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 for72hours. Colonies of *Salmonella and E.coli* were enumerated on brilliant green agar plates and incubatedat37°Cfor 24hours.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:

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

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.

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 200mg 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.

Table 2. Effect of dietary marjoram on growth performance in growing Japanese quail.

Items / Treat.	Control	MLEx 50mg/kg	MLEx 100mg/kg	MLEx 200mg/kg	SE	P-value
Initial LBW(g)	59.77	60.91	60.67	60.29	0.65	0.6185
LBW38d (g)	221.50^{d}	227.57^{c}	231.96^{b}	240.72^{a}	1.51	0.0001
BWG10-38 (g)	161.72^{d}	166.66^{c}	171.28^{b}	180.44^{a}	1.42	0.0001
FI 10-38 (g)	623.66^{a}	601.95^{b}	604.01^{b}	565.44^{c}	1.28	0.0001
FC 10-38 (g/g)	3.90^{a}	3.63^{b}	3.54^{c}	3.15^{d}	0.03	0.0001
GR_{10-38}	1.15^{c}	1.16^{bc}	1.17^{b}	1.20^{a}	0.01	0.0001
PI_{10-38}	5.78^{d}	6.32^{c}	6.60^{b}	7.72^{a}	0.10	0.0001

Abbreviations: MLEx: Marjoram Leaves Extract, LBW: Live Body Weight, BWG: Body Weight Gain, FI: Feed Intake, FC: feed conversion, GR: Growth rate (GR= (LBW_{38}– $LBW_{10)}$ /0.5 (LBW_{10} + $LBW_{38)}$), PI: Performance index (PI = BW_{kg}/FCR), SE: Standard Error, $^{a-c}$: Means within the same row with different superscript.

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.

Table 3. Effect of dietary marjoram on lipid profile, liver functions and digestive enzymes in growing Japanese quail.

Items / Treat.	Control	MLEx 50mg/kg	MLEx 100mg/kg	MLEx 200mg/kg	SE	P-value
RBS	127.45^{a}	119.60^{b}	106.00^{c}	105.93^{c}	1.52	0.0001
lipids profile						
Total chol. mg/dL	154.88^{a}	150.25^{b}	149.88^{b}	149.75^{b}	1.16	0.0107
HDL mg/dL	79.75^{b}	81.28^{ab}	82.63^{a}	82.73^{a}	0.76	0.0316
LDL mg/dL	48.01^{a}	45.08^{b}	44.96^{b}	44.93^{b}	0.36	0.0001
VLDL	27.11^{a}	22.50^{b}	22.29^{b}	21.60^{b}	0.38	0.0001
TG , mg/dL	101.53^{a}	75.28^{b}	74.05^{b}	74.10^{b}	1.24	0.0001
liver functions						
ALT, IU/L	45.75^{a}	36.88^{b}	33.70^{c}	32.88^{c}	0.70	0.0001
AST, IU/L	209.85^{a}	186.25^{b}	183.88^{b}	182.25^{b}	1.85	0.0001
Digestive Enzymes						
Amylase U/L	538.50^{b}	609.75^{a}	612.00^{a}	611.00^{a}	8.21	0.0001
Lipase U/L	71.40^{b}	75.38^{ab}	76.30^{a}	76.20^{a}	1.38	0.0568
Trypsin U/L	72.50^{b}	87.75^{a}	87.00^{a}	87.75^{a}	1.02	0.0001

Abbreviations: MLEx: Marjoram Leaves Extract, RBS: Random Blood Sugar, Total Chol: Total Cholesterol, TG: triglycerides, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, SE: Standard Error, $^{a-c}$: Means within the same row with different superscript

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.

Items / Treat.	Control	MLEx 50mg/kg	MLEx 100mg/kg	MLEx 200mg/kg	SE	P-value
Antioxidant Parameters						
GSH-PX (nmol/min/ml)	1807.75[c]	1991.25[a]	1889.25[b]	1995.00[a]	27.71	0.0001
TBARS (nmol /ml)	1.40[a]	0.96[b]	0.90[b]	0.99[b]	0.03	0.0001
Immune Indices						
IgG (mg/dl)	955.59[b]	1021.74[a]	1035.33[a]	1037.91[a]	8.82	0.0001
IgA (mg/dl)	91.37[c]	96.16[b]	98.89[a]	100.05[a]	0.59	0.0001
IgM (mg/dl)	182.33[c]	189.15[b]	189.83[b]	194.83[a]	1.41	0.0001

Abbreviations: MLEx: Marjoram Leaves Extract, GSH-PX: Glutathione Peroxidase TBARS: Thiobarbaturic Acid- Reactive Substances, IgG: Immunglobin G, IgA: Immunglobin A, IgM: Immunglobin M, SE: Standard Error
[a-c]: Means within the same row with different superscript

Table 5. Effect of dietary marjoram on intestinal bacteria and intestinal environment in growing Japanese quail.

Items / Treat.	Control	MLEx 50mg/kg	MLEx 100mg/kg	MLEx 200mg/kg	SE	P-value
E.coli log 10 cfug	6.86[a]	5.89[b]	5.89[b]	5.78[b]	0.13	0.0001
Salamonella log 10 cfug	7.05[a]	6.02[b]	6.08[b]	6.03[b]	0.07	0.0001
Lactobacillus log 10 cfug	5.41[b]	5.78[a]	5.92[a]	5.87[a]	0.11	0.0087

Abbreviations: MLEx: Marjoram Leaves Extract, SE: Standard Error, [a-b]: 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 presence, which positively affect intestinal flora and possess antibacterial properties, thereby enhancing digestion and absorption 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 compare 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 Mendoza, (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 Zaazaa, *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 index 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. Roofchaee *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. Du, *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 MLEx 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, Roofchaee *et al*. (2011) showed that the addition of 600 and 1200 ppm of marjoram essential oil significantly ($P \leq 0.05$) 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 ($P \leq 0.05$) FCR for broiler chicks were observed in the group receiving 200 ppm of marjoram, while the lowest ($P \leq 0.05$) 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-feed conversion rate (FCR) with the lowest feed intake ($P<0.05$). Furthermore, the total FI of the control group was significantly higher ($P \leq 0.05$) 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-hydrox-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 (Moghrovyan *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, Abdel-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, Bolukbası *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 MLEx/kg exhibited elevated trypsin levels. Additionally, birds that consumed diets supplemented with 100 mg MLEx/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 MLEx ($p < 0.001$), where diets treated by MLEx have the highest GPx levels and the lowest TBARS levels compared to the control group. Where group treated with 200 mg MLEx have significantly the highest GPx. While, birds consuming diets supplemented with 100 mg of MLEx 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 ($P \leq 0.001$) 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 (Youdim 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 MLEx 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, sabinene, 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. Roofchaee *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 and100 mg/kg/diet. For, growing Japanese quail, MLEx can therefore be used as a growth promoter and a health status enhancer.

REFERENCES

Abd EI-Ghany, M.A. and El-Metwally, N.Y. (2010). Effect of marjoram leaves on injured liver in experimental rats. Report and Opinion 2(12):181-191.

Abd El-Hack, M.Z., Alagawany, M., Ragab Farag, M., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J. and Adel, M. (2016). Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *Journal of Essential Oil Research*, *28*(5), 365-382.

Abdel - Moneim, M. A., Hammady, G. A., Hassanin, M. S., El-Chaghaby, G.A. (2015). The effect of using Marjoram Extract as natural growth promoter on the performance and intestinal bacterial of broiler chicken. *Journal of Animal and Poultry Production*. 6, (11): 647 – 656.

Abdel-Wahab, A.A. (2019). Effect of adding Marjoram Powder to broiler chicks diet on performance, blood and antioxidant enzyme activity. *Egyptian Journal of Nutrition and Feeds. 22*(3):611-625.

Abdel-Wareth, A.A.A., (2011). Effect of thyme, oregano and their major active components on performance and intestinal microbial populations of broilers. Ph.D. Faculty of Agriculture, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany.

Abo Ghanima, M. M., Alagawany, M., Abd El-Hack, M. E., Taha, A., Elnesr, S. S., Ajarem, J., *et al*. (2020). Consequences of various housing systems and dietary supplementation of thymol, carvacrol, and euganol on performance, egg quality, blood chemistry, and antioxidant parameters. Poultry Sci. 99 (9), 4384–4397.

Abou-Elkhair, R., Gaafar, K. M. and Elbahy, N. M. (2014). Bioactive effect of dietary supplementation with essential oils blend of oregano, thyme and garlic oils on performance of broilers infected with eimeria species. Global Veterinaria. 13(6):977–985.

Acamovic, T., and J. D. Brooker. (2005). Biochemistry of plant secondary metabolites and their effects in animals. Proc. Nutr. Soc.64:403–412.

Ahmadifar E, Falahatkar B and Akrami R, (2011). Effects of dietary thymolcarvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, Oncorhynchus mykiss. J Appl Ichthyol, 27: 1057-1060.

Ahmed LA, Ramadan RS and Mohamed RA, (2009). Biochemical and histopathological studies on the water extracts of marjoram and chicory herbs and their mixture in obese rats. Pak J Nutr, 8: 1581-1587.

Alagawany, M., El-Hack, M.E.A., Farag, M.R., Tiwari, R. & Dhama, K., (2015). Biological effects and modes of action of carvacrol in animal and poultry production and health – A review. Adv. Anim. Vet. Sci. 3 (2), 73-84.

Alagawany, M., Abd El-Hack, M. E., Farag, M. R., Shaheen, H. M., Abdellatif, M. A., Noreldin, A. E., *et al*. (2018). The usefulness of oregano and its derivatives in poultry nutrition. World's poultry Sci. J. 74 (3), 463–474.

Ali, A. H. H. (2014). Productive performance and immune response of broiler chicks as affected by dietary Marjoram leaves powder. *Egyptian Poultry Science Journal*. 34(1):57-70.

Alma, M. H., A. Mavi, A. Yildirim, M. Digrak, and T. Hirata. (2003). Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in turkey. Biol. Pharm. Bull. 26:1725-1729.

Amer, S. A., Tolba, S. A., Al Sadek, D. M. M., Abdel Fattah, D. M., Hassan, A. M., and Metwally, A. E. (2021). Effect of supplemental glycerol monolaurate and oregano essential oil blend on the growth performance, intestinal morphology, and amino acid digestibility of broiler chickens. BMC Veterinary Res. 17 (1), 312.

Ampode, K.M. and Mendoza, F.C. (2021). Oregano (Origanum vulgare Linn.) powder as phytobiotic feed additives improves the growth performance, lymphoid organs, and economic traits in broiler chickens. Adv. Anim. Vet. Sci., 10, 434–441.

Avila-Ramos F, A. Pro-Martinez, E. Sosa-Montes, J.M. Cuca-Garcia, C.M. Becerril-Perez, J.L. Figueroa-Velasco and C. Narciso-Gaytan (2012). Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. Poult. Sci., 91: 505–511.

Awaad, M.H.H.; Elmenawey, M. and Ahmed, K.A. (2014). Effect of a specific combination of carvacrol, cinnamaldehyde, and capsicum oleoresin on the growth performance, carcass quality and gut integrity of broiler chickens. Vet. World, 7, 284–290.

Azeem, T., Zaib-Ur-Rehman., Umar, S., Asif, M., Arif, M., Rahman, A. (2014). Effect of *Nigella Sativa* on poultry health and production: A Review. *Science Letter Journal*, (2):76-82.

Badee, A.Z.M., R.K. Moawad, M.M.Elnoketi and M.M. Gouda (2013). Improving the quality and shelf-life of refrigerated chicken meat by marjoram. J. of Appl Sci. Res. 9: 5718–5729.

Bahakaim, A. S. A.; Abdel-Halim, H. A. H.; Mousa, S. M. M. and Fadl, A. (2020). Effect of dietary oregano supplementation on productive, physiological and immunological performance of broiler chicks. *Egyptian Poultry Science Journal*, *40* (2), 507-524.

Baj, T.; Biernasiuk, A.; Wróbel, R. and Malm, A. (2020). Chemical composition and in vitro activity of Origanum vulgare L., Satureja hortensis L., Thymus serpyllum L. and Thymus vulgaris L. essential oils towards oral isolates of candida albicans and candida glabrata. Open Chem., 18, 108–118.

Barreto M. S. R., Menten J. F. M., Racanicci A. M. C., Pereira P. W. Z., Rizzo P. V., (2008). Plant extracts used as growth promoters in broilers. Brazilian Journal of Poultry Science 10(2):109-115.

Bina, F. and Rahimi, R. (2017). Sweet Marjoram: A Review of Ethnopharmacology, Phytochemistry, and Biological Activities. Journal of Evidence-Based Complementary & Alternative Medicine, 22,(1):175-185.

Bolukbası SC., Erhan MK., and Ozkan A., (2006). Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers South Afric. J. Anim. Sci. 36(3):189-196.

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J. and Spais, A.B., (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br. Poult. Sci., 43:223-230.

Brody, S. (1945): Bioenergetics and Growth. New York: Reinhold Publishing Co.

Case, G.L., He, L., Mo, H. and Elson, C.E., (1995). Induction of geranyl pyrophosphatase activity by cholesterol - suppressive isoprenoids. Lipids, 30: 357-359.

Charles, D.J. (2013). *Marjoram Sweet*. In: Antioxidant Properties of Spices. Herbs and other Sources. New York, Springer,393-399.

Cross, DE., McDevitt, RM., Hillman, K. and Acamovic, T., (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br. Poult. Sci., 48: 496-506.

Deans SG and Svoboda KP, (1990). The antimicrobial properties of marjoram (Origanummajorana L.) volatile oil. Flav Frag J, 5: 187-190.

Dehghani, N., Afsharmanesh, M., Salarmoini, M., and Ebrahimnejad, H. (2019). In vitro and in vivo evaluation of thyme (Thymus vulgaris) essential oil as an alternative for antibiotic in quail diet1. J. Anim. Sci. 97 (7), 2901–2913.

Deng, Z.Y.; Teo, B.Y.; Li, X.L.; He, J.M.; Cheni, Y.F. and Chu, F. (1998). Effect of tea on blood glucose, blood lipid and antioxidants activity in old rats. J. of tea- Sci. 18: 1, 74-77.

Deuschle, R.A.N., Deuschle, V.C.,Azzolin, G.B., Oliveira, J.S.,Sostisso, Q.C., Goulart, J.S., Mayer, M.S., Horn, R.C.,Golle, D.P. (2018). Phytochemical screening and antioxidant activity of Origanum Majorana against oxidative stress biomarkers. *Journal of Agricultural Science,* Vol. 10, No. (12):1916-9752.

Di Rienzo, J. A. (2017). InfoStat versión 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Argentina. http://www.Infos tat. Com.Ar

Du, E.; Gan, L.; Li, Z.; Wang, W.; Liu, D. and Guo, Y. (2015). In vitro antibacterial activity of thymol and carvacrol and their effects on broiler chickens challenged with Clostridium perfringens. J. Anim. Sci. Biotechnol., 6, 58–69.

Duncan, D.B., (1955). Multiple range and multiple F-tests. Biometrics, 11:1-42.

Erenler, R., Sen, O., Aksit, H. (2016). Isolation and identification of chemical constituents from *Origanum Majorana* and investigate on of anti-proliferative and antioxidant activities. *Journal of Science Food Agriculture*, 2016(96):822-836.

Erhard, M. H., Von Quistorp, I., Schranner, I., Jüngling, A., Kaspers, B., Schmidt, P. and R. Kühlmann, (1992). development of specific enzyme-linked immunosorbent antibody assay systems for the detection of chicken immunoglobulins g, m, and a using monoclonal antibodies. Poult. Sci. Volume 71, Issue 2, 1 February 1992, Pages 302–310.

Ertas, O.N., Guler, T., Ciftci, M., Dalkilic, B. and Simsek, U.G., (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Int. J. Poult. Sci. 4, 879-884.

Ezz El-Arab, W. F. (2008). Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. M. Sc. Thesis Faculty of Agriculture, Alexandria University, Egypt.

Fanucchi, M. V. (2014). "Chapter 11 - Development of Antioxidant and Xenobiotic Metabolizing Enzyme Systems," in The Lung, 2nd ed.. Eds. R. Harding and K. E. Pinkerton (Boston: Academic Press), 223–231.

Feng, J., Lu, M., Wang, J., Zhang, H., Qiu, K., Qi, G., *et al.* (2021). Dietary oregano essential oil supplementation improves intestinal functions and alters gut microbiota in late-phase laying hens. J. Anim. Sci. Biotechnol. 12 (1), 72.

Friedman, R. B. and D. S. Young (2005). Effects of disease on clinical laboratory tests. Columbia University press

Gadde, U., Kim, W. H., Oh, S. T., and Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18 (1), 26–45.

Gao, F.; Zhang, L.; Li, H.; Xia, F.; Bai, H.; Piao, X.; Sun, Z.; Cui, H. and Shi, L. (2022). Dietary oregano essential oil supplementation influences production performance and gut microbiota in late-phase laying hens fed wheat-based diets. Animals, 12, 3007.

Gül, M.; Yilmaz, E.; Sezmi¸s, G.; Yildirim, B.A.; Kaya, A. and Önel, S.E. (2020). Effect of oregano (Oreganum syriacum L.) essential oil and cage density on performance parameters, egg quality criteria, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology in laying hens. GSC Biol. Pharm. Sci., 13, 136–145.

Habib, A.S., Iman, A.M., Majid, T. (2012). Effect of marjoram powder (*Origanum Majorana* L.) as antibiotic growth promoter substitutions on performance and immunity of broiler chicks. *Clinical Pharmacology and Drug Therapy*, 1683-4100.

Hao, Y.; Li, J. and Shi, L. (2021). A carvacrol-rich essential oil extracted from oregano (Origanum vulgare "hot & spicy") exerts potent antibacterial effects against Staphylococcus aureus. Front. Microbiol., 12, 741861.

Hashemipour, H.; Kermanshahi, H.; Golian, A. and Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poult. Sci., 92, 2059–2069.

Helander, IM., Alakomi, HL., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, EJ., Gorris, LGM. and Von Wright, A., (1998). Characterization of the action of selected essential oil components on gram negative bacteria. J. Agric. Food Chem., 46: 3590- 3595.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Magias, MD, (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci., 83:169-174.

Herve, T., Raphaël, K. J., Ferdinand, N., Laurine Vitrice, F. T., Gaye, A., Outman, M. M., *et al*. (2018). Growth Performance, Serum Biochemical Profile, Oxidative Status, and Fertility Traits in Male Japanese Quail Fed on Ginger (Zingiber officinale, Roscoe) Essential Oil. Veterinary Med. Int. 2018, 7682060.

Hong, J.C., Steiner, T., Aufy, A. & Lien, T.F., (2012). Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livest. Sci. 144, 253-262.

Ili´c, Z.; Stanojevi´c, L.; Milenkovi´c, L.; Šuni´c, L.; Milenkovi´c, A.; Stanojevi´c, J. and Cvetkovi´c, D. (2022). The yield, chemical composition, and antioxidant activities of essential oils from different plant parts of the wild and cultivated oregano (Origanum vulgare L.). Horticulture, 8, 1042.

Jang, I. S., Y. H. Ko, S. Y. Kang, and C. Y. Lee. (2007). Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. Technol. 134:304–315.

Johnson, A.M.; Anderson, G.; Arguelles-Ramos, M. and Ali, A.A.B. (2022). Effect of dietary essential oil of oregano on performance parameters, gastrointestinal traits, blood lipid profile, and antioxidant capacity of laying hens during the pullet phase. Front. Vet. Sci., 3, 1072712.

Jones E. A., and P. D. Bark (1979). Chemical Diagnosis of disease. Brown, S. S., F. L. Mitchell and D. S. Young (Eds), elsevier, Biomedical Press, Amsterdam, New York, Oxford, P: 325-363.

Juliani HR and Simon JE, (2002). In: Janick J and A Whipkey (ed.), Antioxidant Activity of Basil. Trends in New Crops and New Uses. pp: 575–9. Proceedings of the Fifth National Symposium, Atlanta, Georgia, USA, 10-13 November, 2001.

Karimi A, Yan F, Coto C, Park JH, Min Y, Lu C, Gidden JA, Lay JO, Jr, Waldroup PW. (2010). Effects of level and source of oregano leaf in starter diets for broiler chicks. J Appl Poult Res. 19:137–145.

Khattab, M.A., Roshdy, A.R., Ali A.M. (2018). Effect of some medicinal plants on broiler performance. SINAI *Journal of Applied Sciences*,6, 2314-6079.

Khorsand, G.J.; Morshedloo, M.R.; Mumivand, H.; Bistgani, Z.E.; Maggi, F. and Khademi, A. (2022). Natural diversity in phenolic components and antioxidant properties of oregano (Origanum vulgare L.) accessions, grown under the same conditions. Sci. Rep., 12, 5813.

Kirkpinar F, Unlu HB, Serdaroglu M, Turp GY. (2014). Effects of dietary oregano and garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory quality of broiler meat. Br Poult Sci. 55:157–166.

Kolypetri, S.; Kostoglou, D.; Nikolaou, A.; Kourkoutas, Y. and Giaouris, E. (2023). Chemical composition, antibacterial and antibiofilm actions of oregano (Origanum vulgare subsp. Hirtum) essential oil against Salmonella typhimurium and Listeria monocytogenes. Foods, 12, 2893.

Lee, KW., Everts, H., Kappert, HJ., Frehner, M., Losa, R. and Beynen, AC., (2003a). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci. 44: 450-457.

Lee, KW., Everts, H., Kappert, HJ., Yeom, K.H. and Beynen, A.C., (2003b). Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res., 12: 394-399.

Lewis, MR., Rose, SP., Mackenzie, AM. and Tucker, LA., (2003). Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. Br. Poult. Sci., 44: S43-S44.

Li, Z.; Jin, X.; Wu, Q.; Long, L.; Li, Y.; Zhang, Q.; Liu, A.; Chen, X.; Geng, Z. and Zhang, C. (2022). Effects of encapsulated thymol and carvacrol mixture on growth performance, antioxidant capacity, immune function and intestinal health of broilers. Ital. J. Anim. Sci., 21, 1651–1659.

Li, L.; Chen, X.; Zhang, K.; Tian, G.; Ding, X.; Bai, S. and Zeng, Q. (2023). Effects of thymol and carvacrol eutectic on growth performance, serum biochemical parameters, and intestinal health in broiler chickens. Animals, 13, 2242.

Lovkova, M.Y., Buzuk, G.N., Sokolova, S.M. and Kliment'eva, N.I. (2001). Chemical features of Medicinal Plants (Review). Appl. Biochem. Microbiol. 37, 229-237.

Manou I, Bouillard L, Devleeschouwer MJ, *et al*., (1998). Evaluation of the preservative properties of Thymus vulgaris essential oil in topically applied formulations under a challenge test. J Appl Micro, 84: 368-376.

Mansoub, N.H., (2011). Performance, carcass quality, blood parameters and Immune System of broilers fed diets supplemented with oregano oil (*Origanum* sp.). Annals of Biological Research, 2011, 2 (6):652-656.

Moghrovyan, A., Sahakyan, N., Babayan, A., Chichoyan, N., Petrosyan, M., and Trchounian, A. (2019). Essential Oil and Ethanol Extract of Oregano (Origanum vulgare L.) from Armenian Flora as a Natural Source of Terpenes, Flavonoids and other Phytochemicals with Antiradical, Antioxidant, Metal Chelating, Tyrosinase Inhibitory and Antibacterial Activity. Curr. Pharm. design 25 (16), 1809–1816.

Mohamed, E.R.A., Elazab, M.F., El-Habashi, N., Elhawary, N.,Mokhbatly, A.A.(2021). Anticoccidial effect of Origanum Majoranum aqueous extract on Eimeria tenella-infected chicken. *Tropical Biomedicine*,38 (1): 62-72.

Namkung H, Li M, Gong J, Yu H, Cottrill M, de Lange CFM (2004). Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Can. J. Anim. Sci. 84: 697- 704.

National Research Council, NRC, (1994). Nutrient Requirements of Poultry, 10th ed. Washington, DC, National Academy Press.

North, M.O (1981). Commercial chicken production manual, 2nd Edition. AVI Publishing Company Inc, USA.

Oladokun, S., MacIsaac, J., Rathgeber, B., and Adewole, D. (2021). Essential Oil Delivery Route: Effect on Broiler Chicken's Growth Performance, Blood Biochemistry, Intestinal Morphology, Immune, and Antioxidant Status. Animals 11, 3386.

Osman, M., Yakout, H. M., Motawe, H. F., & El-Arab, W. E. (2010). Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. *Egyptian Poultry Science Journal*, *30*(1), 25-53.

Osman, N., Talat, G., Mehmet, C., Bestami, D. and Simsek, G., (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Intern. J. Poult. Sci., 4: 879-884.

Paglia, D.E. and W.N. Valentine (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The J. of laboratory and clinical medicine, 70(1), 158-169.

Park, J. H., Kang, S. N., Shin, D., and Shim, K. S. (2014). Antioxidant Enzyme Activity and Meat Quality of Meat Type Ducks Fed with Dried Oregano (Origanum vulgare L.) Powder. Asian-Australas J. Anim. Sci. 28 (1), 79–85.

Penalver, P., Huerta, B., Borge, C., Astorga, R., Romero, R. and Perea, A., (2005). Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS, 113:1-6.

Reis, J.H., Gebert, R.R., Barreta, M., Baldissera, M.D., Santos, I.D., …, Silva, A.S., (2018). Effects of phytogenic feed additive based on thymol, carvacrol and cinnamic aldehyde on body weight, blood parameters and environmental bacteria in broilers chickens. Microb. Pathog. 125,168-176.

Reshadi, H., Torki, M., and Mohammadi, H. (2020). Changes in performance, egg quality and blood parameters of laying hens fed selenium and oregano oil. Anim. Production Sci. 60 (13), 1620–1629.

Ri, C. S., Jiang, X. R., Kim, M. H., Wang, J., Zhang, H. J., Wu, S. G., Bontempo, V. & Qi, G.H. (2017). Effects of dietary oregano powder supplementation on the growth performance, antioxidant status and meat quality of broiler chicks. *Italian Journal of Animal Science*, *16*(2), 246-252.

Roofchaee, A., Irani, M., Ebrahimzadeh, M.A. and Akbari, M.R., (2011). Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. African J. of Biotechnology Vol. 10(32):6177-6183.

Ruan, D.; Fan, Q.; Fouad, A.M.; Sun, Y.; Huang, S.; Wu, A.; Lin, C.; Kuang, Z.; Zhang, C. and Jiang, S. (2021). Effects of dietary oregano essential oil supplementation on growth performance, intestinal antioxidative capacity, immunity, and intestinal microbiota in yellow-feathered chickens. J. Anim. Sci., 99, 1–11.

Salama, A. M., Belih, S. S., & Khedr, N. E. (2023). Influence of dietary oregano plant extract supplementation on growth performance and economic efficiency of broiler chicks. Benha Med. J., 44, 15–19.

Saleh, A. A., Hamed, S., Hassan, A. M., Amber, K., Awad, W., Alzawqari, M. H., *et al*. (2021). Productive Performance, Ovarian Follicular Development, Lipid Peroxidation, Antioxidative Status, and Egg Quality in Laying Hens Fed Diets Supplemented with Salvia officinalis and Origanum majorana Powder Levels. Animals 11 (22), 3513.

Shad, A. A., J. Bakht, H. U. Shah and Y. Hayat (2016). Antioxidant activity and nutritional assessment of under-utilized medicinal plants. Pak. J. Pharm. Sci, 29(6), 2039-2045.

Shan, B., Cai, Y. Z., Sun, M., and Corke, H. (2005). Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. J. Agric. Food Chem. 53 (20), 7749–7759.

Shawky, S. M., Orabi, S. H., and Dawod, A. (2020). Effect of marjoram supplementation on growth performance and some immunological indices in broilers. *International Journal of Veterinary Science*, *9*(2), 297-300.

Shoji Y and Nakashima H, (2004). Nutraceutics and delivery systems. J Drug Targ, 12: 385-391.

Silva-Vázquez R., Duran-Meléndez L. A., Hernández-Martínez C. A., Gutiérrez-Soto J. G., Hume M. E., Méndez-Zamora G., (2018). Effects of two sources of Mexican oregano oil on performance, blood profile, carcass variables, and meat of broilers. Revista Brasileira de Zootecnia 47:e20170198.

Skoufos I, Giannenas I, Tontis D, Bartzanas T, Kittas C, Panagakis P and Tzora A (2016). Effects of oregano essential oil and attapulgite on growth performance, intestinal microbiota and morphometry in broilers. South African Journal of Animal Science 46, 77–88.

Symeon, G.K., C. Zintilas, N. Demitris, L.A. Bizelis and S.G. Deligeorgis (2010). Effect of oregano essential oil dietary supplementation on the feeding and drinking behavior as well as the activity of broilers. Int. J. of Poult. Sci., 9: 401-405.

Tainter DR and Grenis AT, (1993). Spices and Seasonings: A Food Technology Handbook. VCH, Publishers, Inc, New York.

Toghyani, M., Mousavi, S.K. and Modaresi, M., (2010). Effect of water extract of marjoram (*Origanummajorana* L.) as an alternative to antibiotic growth promoter on immunity and serum lipid profile of broiler chicks. In 2010 2nd International Conference on Chemical, Biological and Environmental Engineering pp: 314-316. IEEE.

Umar, A.A.; Kware, A.A.; Abdurrahman, B. and Alhaji, B.A. (2023). Evaluation of oregano (Origanum vulgare) leaf powder on performance indices of finisher broiler chickens in Sokoto, Nigeria. Biomed. J. Sci. Technol. Res., 52, 43263–43274.

Wang, Y.; Wang, Y.; Su, C.; Wang, L.; Lv, X.; Cui, G.; Ji, L.; Huang, Y.; Zhang, H. and Chen, W. (2022). Dietary cinnamaldehyde with carvacrol or thymol improves the egg quality and intestinal health independent of gut microbiota in post-peak laying hens. Front. Vet. Sci., 9, 994089.

Windisch, W., Shedle, K. and Kroismayr, A., (2008). Use of phytogenic products as feed additives for swine and poultry. J. Anim Sci., 86: E140-E148.

Yagi, K. (1998). Simple assay for the level of total lipid peroxides in serum or plasma 460 *Free radical and antioxidant protocols* (pp. 101-106): Springer.

Yang, T. T. C. and M. W. L. Koo, (2000). Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. Life Sci. 66 (5): 477-423.

Yanishlieva, N.V., E.M. Marinova, M.H. Gordon and V.G. Raneva (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem., 64: 59 - 66.

Yildirim,B.A., Tunc, M.A., Gül, M., Yildirim, F.,Yıldız, A.(2018). The effect of Rosemary (Rosmarinus officinalis L.) extract supplemented into broiler diets, on performance and blood parameters. *GSC Biological and Pharmaceutical Sciences*, 2018; 2 (3):1-9.

Youdim, K. A., and S. G. Deans. (2000). Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. Br. J. Nutr. 83:87–93.

Yu, C., Guo, Y., Yang, Z., Yang, W., and Jiang, S. (2019). Effects of star anise (Illicium verum Hook.f.) essential oil on nutrient and energy utilization of laying hens. Anim. Sci. J. 90 (7), 880–886.

Zaazaa, A.; Mudalal, S.; Alzuheir, I.; Samara, M.; Jalboush, N.; Fayyad, A. and Petracci, M. (2022). The impact of thyme and oregano essential oils dietary supplementation on broiler health, growth performance, and prevalence of growth-related breast muscle abnormalities. Animals, 12, 3065.

Zhang, L. Y., Peng, Q. Y., Liu, Y. R., Ma, Q. G., Zhang, J. Y., Guo, Y. P., *et al.* (2021). Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. Poultry Sci. 100 (7), 101163.

Zhao, B.C.; Wang, T.H.; Chen, J.; Qiu, B.H.; Xu, Y.R.; Zhang, Q.; Li, J.J.; Wang, C.J.; Nie, Q.F. and Li, J.L. (2023). Effects of dietary supplementation with a carvacrol–cinnamaldehyde–thymol blend on growth performance and intestinal health of nursery pigs. Porc. Health Manag., 9, 24–33.

تأثير مستخلص أوراق البردقوش على الأداء الإنتاجي ومقاييس الدم والإنزيمات الهاضمة والمناعة ومضادات الأكسدة والتجمعات الميكروبية لطيور السمان الياباني النامي

إبراهيم عبد التواب عبد القادر[1]، عبدالوهاب عبدالله عبد الوهاب[1]، عادل محمد عبد السلام[3]، مروة محمد بهنس[1] و رشا عبدالحميد محمود صميده[2]

[1]قسم إنتاج الدواجن، كلية الزراعة، جامعة الفيوم، 63514 الفيوم، مصر.
[2]قسم الإنتاج الحيواني، كلية الزراعة، جامعة الفيوم.
[3]مركز البحوث الزراعية، معهد بحوث الإنتاج الحيواني، الجيزة، مصر.

الملخص

هدفت هذه الدراسة إلى دراسة تأثير إضافة مستخلص أوراق البردقوش (MLEx) إلى علائق طيور السمان الياباني النامى على الأداء الإنتاجى وعدد بكتيريا الأمعاء وبعض مقاييس الدم والمناعة. في هذه الدراسة تم توزيع عدد 320 طائرا عشوائياً على أربع مجموعات بحيث تكونت كل مجموعة من أربع مكررات في كل مكرر 20 طائر (4 × 4 × 20). حيث تم إعطاء نظام غذائي خالٍ من مستخلص أوراق البردقوش للمجموعة الأولى (كنترول). بينما المجموعات الثانية والثالثة والرابعة على التوالي تم إعطائها عليقة الكنترول مضافا إليها 50 و 100 و 200 ملجم من مستخلص أوراق البردقوش لكل كجم عليقة. أوضحت نتائج الدراسة أن المجموعة التى تلقت مستخلص أورق البردقوش بمعدل 200 ملجم لكل كجم عليقة كانت الأفضل من حيث مؤشر الأداء، ووزن الجسم الحي، ومعدل الزيادة في وزن الجسم، ونسبة تحويل العلف، مع معدل نمو أسرع مقارنةً بمجموعة الكنترول .علاوة على ذلك، فقد أستهلكت طيور السمان التى تلقت مستخلص أوراق البردقوش كمية أقل من العلف مقارنة بالكنترول. باستثناءTG ، كانت المجموعة التي تم تغذيتها بنظام غذائي يحتوي على 200 ملجم من/MLEx كجم من العليقة لديها أقل أعداد من السالمونيلا والإشريشيا كولاى ومقاييس الدهون وجلوكوز الدم وإنزيمات الكبد ALT وAST، مع أعلى مستويات من مقاييس المناعة IgGو IgAو IgM والتربسين مقارنة بالكنترول (P <0.01) .علاوة على ذلك، أظهرت طيور السمان الذي تم إعطائها 100 ملجم من /MLExكجم من العليقة أعلى أعداد من العصيات اللبنية والأميليز والليبيز، مع أقل قيم TG وTBARS. خلاصة النتائج، تم تعزيز أداء النمو، ونشاط مضادات الأكسدة، ومقاييس الدم والمناعة ، والبكتيريا المعوية عن طريق إضافة مستخلص أوراق البردقوش MLEx بمعدل 200 و 100 ملغم / كجم في علائق طيور السمان الياباني النامي.