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### Effect of Thyme Aqueous Extract on Reproductive Performance, Blood Constituents, Antioxidant Status, and Immunity of Gimmizah Chicken

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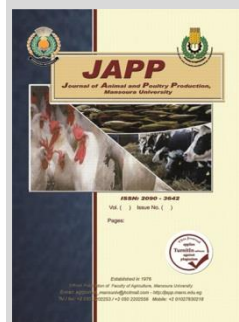


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#### ABSTRACT

The present study aimed to assess the effect of thyme leaf aqueous extract (TLAE) on productive and reproductive performance of Gimmizah chickens. Total of 48 males and 48 females aged 38 wk were divided into three groups (16 of each sex in each) fed on diet (16% CP and ME of 2750 Kcal/kg). Birds in G1 were controls, while those in G2 and G3 were drank water with 2 and 4 ml of 20% TLAE /10 L for 60 days. Results showed that TLAE (4ml) improved ( $P<0.05$ ) Hb, Ht, glucose, creatinine, and cholesterol in both sexes, basophils in males, albumin and calcium in females. Also, 4 ml-TLAE decreased ( $P<0.05$ ) plasma AST, ALT, MDA, while increased ( $P<0.05$ ) T<sub>3</sub>, IgG, IgM, and TAC in both sexes. 4 ml-TLAE increased ( $P<0.05$ ) weight, tail, width, Haugh unit, and shape of eggs; weight, height, and index of albumen; and height and index of yolk. Sperm motility parameters, morphological features, velocity, and kinetics were increased by 4 ml-TLAE. Also, egg yield, hatched chick weight, and chick-ovo index were increased ( $P<0.05$ ) by ml-TLAE, while fertility and hatchability of eggs were not affected. In conclusion, thyme administration of thyme in drinking water (4 ml/10 L) can be used to achieve beneficial impacts on reproductive and productive performance of males and females of Gimmizah local strain.

**Keyword:** Gimmizah chicken, thyme, egg, reproduction, blood.



#### INTRODUCTION

In the last years, there is a prohibition on resistance of different strains of bacteria through their using in poultry industrial and production (Rahimi *et al.*, 2012; Gholami-Ahangaran *et al.*, 2021). Non-antibiotic compounds, such as organic substances, enzymes, and phytobiotics with potential (Da Silveira Deminicis *et al.*, 2021) are considered effective alternatives to antibiotics (Ghasemian *et al.*, 2021). There are bioactive substances named photobiotics it a broad subset from plants. There are phenolic compounds (flavonoids, tannins and saponins), and essential oils in photogenic herbs (Yadav *et al.*, 2016).

Phytobiotics supplementation to the animal diets was achieved to increase the productive and reproductive performance by improving feeding values to promote the animal production and enhancing the products quality of livestock (Gholami-Ahangaran *et al.*, 2021). There are antioxidant properties of phytochemicals could be useful in increasing the keeping quality of animal products (Yadav *et al.*, 2016) by improving the activity of digestive enzymes, and absorptive ability (Mohammadi and Kim, 2018).

Thyme (*Thymus vulgaris*), as phytobiotics, contain phenolic components manly, thymol (5-methyl-1-2-isopropyl phenol) and the carvacrol (5-isopropyl-2-methyl phenol). Thyme has special functions such as antimicrobial, antioxidant, expectorant, antispasmodic and antiseptic (Abu-Darwish *et al.*, 2009). Thymol is one of the vital compounds in different species of thyme (Bahmani *et al.*, 2014; Attia *et al.*, 2017), possessing multiple therapeutic effects due to its antioxidant (Attia *et al.*, 2018), anti-hyperlipidemic, and anti-inflammatory actions (Gholami-Ahangaran *et al.*, 2020). Dietary thyme supplementation for

its contents from carvacrol or thymol can be used for inhibition of lipid oxidation as compared to the synthetic antioxidant in production of animals and poultry for increasing the health and animal performance (Alma *et al.*, 2003; Luna *et al.*, 2018).

In broiler chickens, dietary supplementation with thyme essential oil improved growth performance (Cross *et al.*, 2007). Thymol extract administration enhanced the productive performance (Hernandez *et al.*, 2004), feed conversion ratio (Lee *et al.*, 2003), and feed utilization (Gopi, 2014). Thymol and carvacrol in thyme, as sources of flavonoids could improve the immune functions (De Cassia Da Silveira *et al.*, 2013).

Several benefits, including improvement in hematological parameters in broilers fed diets contained thyme and cinnamon oils (Al-Kassie, 2009), and in protein and lipid profiles of hens fed diet supplemented by thyme (Abdel-Wareth, 2013). Thyme had positive impacts on yield and quality of eggs (Khan *et al.*, 2012; Ghanima *et al.*, 2020). Several reports (Bozkurt *et al.*, 2012; Abdel-Wareth *et al.*, 2018) found a significant augmentation in weight, thymol content, and the production rate of eggs by essential oil or by thyme powder (Abdel-Hack and Alagawany, 2015; Alagawany *et al.*, 2017).

In males, TLAE in drinking water increased the ejaculate volume, sperm massive movement, and reduced sperm abnormality of male broilers breeder ROSS308 (Shanoon and Jassim 2012) and rabbits (Kandeil *et al.*, 2019). Supplementation of thyme oil (Abdel-Wareth *et al.*, 2020; Ahmed *et al.*, 2020) or dietary thyme leaves supplementation (Ezzat *et al.*, 2020) showed positive impacts on rabbit male fertility, by improved semen volume,

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function and the sperm cells morphology. Also, thyme treatment improved sperm concentrations, total sperm outputs, total sperm fractions, normality, viability, and acrosome reaction in rabbit semen (El-Gindy, 2022).

Therefore, the current study aimed to explore the impact of TLAE administration in drinking water on growth, hematological, biochemical, and mineral parameters, blood enzyme activity, immunity, and antioxidant status of males and females of Gimmizah local strain. Also, egg production, quality, and hatchability of laying hens as well as motility parameters, normality, and velocity parameters of sperm cells in cocker semen was evaluated.

## MATERIALS AND METHODS

The experimental work of this study was performed at El-Gimmizah research station belonging to Animal production research Institute, agricultural research center, Egypt.

### Birds:

Total number of 96 birds (48 males and 48 females) from Gimmizah local strain (38 wk of age) were divided into three experimental groups for each sex (16 males and 16 females in each group, and 4 replicates for each sex. All experimental birds were housed on floor in one ventilated building under the same conditions. The daily light period was 16 h light/8 h dark throughout the experimental period of 60 days from 48 to 56 wk of age. Birds in each group were fed their diets *ad libitum* (16% CP and 2750 Kcal/kg) according to NRC (1994), while drinking water was available at all times. Composition and chemical analysis of the diet are presented in Table 1.

**Table 1. Composition and calculated analysis of bird diet.**

Ingredient	(%)	Calculated chemical analysis	
Yellow corn	62.41	Crude protein (%)	16.52
Soybean meal (44%CP)	22.25	Crud fibers (%)	3.84
Wheat bran	4.43	Ether extract (%)	2.91
Limestone	7.60	Calcium (%)	3.37
Di-calcium phosphate	1.31	Available phosphorus (%)	0.37
DL-Methionine	0.15	Lysine (%)	0.87
Vegetable oil	1.25	Methionine (%)	0.38
Premix	0.30	ME (Kcal/kg diet)	2700
NaCl	0.30	-	-

### Experimental groups:

All birds in the experimental groups were fed the same diet. Birds in the first group (G1) did not receive any treatment, while the second (G2) and third (G3) groups were treated daily with 2 and 4 ml of 20% thyme extract per 10 liters in the drinking water.

Birds were individually weighed at the beginning and end of experimental period, then the total body weight gain was calculated. Throughout the experiment period, egg production of hens and semen quality of cockers were evaluated, and blood samples were collected for analytical measurements.

### Egg quality parameters

At the end of the experiment, the external and internal quality parameters of eggs were estimated for 8 fresh eggs in each experimental group. Egg weight was recorded then their measurements (tail and width) were determined using caliper, then it broken on a smooth and flat surface to measure height and width then weights of albumen and yolk using tripod micrometer for height diameter and caliper for width one. The

egg shell thickness was measured using caliper. Egg shape index was measured according to Romanoff and Romanoff (1949). Albumen (Heiman and Carver, 1936), and yolk (Wells, 1968) indexes, and Haugh unit (Haugh, 1937) were calculated as the following:

$$\text{Egg shape index} = \text{width/tail} \times 100$$

$$\text{Albumen index} = \text{height/width} \times 100$$

$$\text{Yolk index} = \text{height/width} \times 100$$

The score of Haugh unit (HU) for each egg was determined as the following:

$$\text{HU} = 100 \log (H + 7.57 - 1.7W^{0.37})$$

**Where:** H is albumen height (mm) and W is egg weight (g).

### Hatchability measurements

Rate of fertile and hatched eggs were determined as the following:

$$\text{Fertility rate} = (\text{fertile eggs} / \text{total eggs}) \times 100.$$

$$\text{Hatchability rate} = (\text{hatched chick} / \text{fertile eggs}) \times 100.$$

On the day of hatch, also, hatched chick weights were recorded.

### Blood constituents

At the end of the experimental period, 5 ml of blood samples were collected randomly from the brachial vein from 4 hens and 4 cockers in each group into sterilized heparinized tubes. Each blood sample was divided into two portions the first was for the hematological parameters in the whole blood samples, while the second was for analytical procedures.

In the 1st blood samples, count of white (WBCs) and red (RBCs) blood cells, concentration of hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were determined immediately. The second portion of blood samples were centrifuged at 3000 rpm for 15 minutes to obtain the blood plasma that transferred into Eppendorf tubes and stored at -20°C until further analysis. In blood plasma samples, the concentrations of total protein, albumin, glucose, cholesterol, calcium, T3, and phosphor were determined using spectrophotometer and commercial diagnostic kits (Biodiagnostic Co. Giza, Egypt) as described by manufacturer procedure. Liver enzymes activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) were measured using commercial kits (Bio-Merieux, Egypt). Globulin was calculated by subtracting albumin from total protein. According to the manufacturers' instructions total antioxidant capacity (TAC) and malondialdehyde (MDA), as a lipid peroxidation marker, were assayed by chemical kits using a spectrophotometer (Shimadzu, Japan). Concentration of different types of immunoglobulins (IgG, and IgM) were estimated by ELISA procedure according to the manufacturer's using kits (Bethyl Laboratories, Montgomery, TX, USA).

### Semen evaluation by Computer Assisted Semen Analysis (CASA):

CASA (SPERMOLAB®, Cairo, Egypt) was used in analyze fresh cocker semen. Semen of different treatments (5 µL) was placed on a warmed slide (dis-posable Leja) and was allowed to settle on heating stage at 37 °C. The following sperm variables were analyzed motility parameters percentages including:

#### Types of spermatozoa motility:

Percentages of progressive sperm motility (PSM%), Non-progressive motility (NPSM%), Total sperm motility

(TSM%), Rapid sperm motility (RPSM%), Slow sperm motility (SPSM%), and Sperm immotility (IMS%)

**Where:** TSM = PSM + NPSM; PSM = RPSM + SPSM; IMS= 100 minus TSM.

#### Sperm morphological features:

Using CASA analysis, percentages of normality, abnormalities in head, neck, and the tail as well sperms with mono, dual, and tri deformation were evaluated.

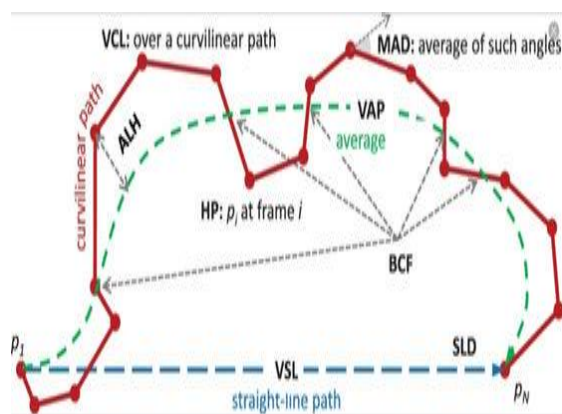
- Sperm with normal forms (%)
- Sperm deformity index (SDI)
- Sperm with abnormal head (%)
- Sperm with mono deformation (%)
- Sperm with abnormal neck (%)
- Sperm with dual deformation (%)
- Sperm with abnormal tail (%)
- Sperm with tri deformation (%)

$$SDI = (H+N+T)/n$$

**Where:** H = sperms with abnormal head, N = sperms with abnormal neck, T= sperms with abnormal tail and n = counted sperms number.

#### Sperm velocity measurements (µm/s):

In addition, sperm kinetic properties, in terms of sperm velocity and kinetic indices were also evaluated. According to the following diagram, different types of sperm velocity were measured.



- **VCL:** The curve linear of velocity: average sperm velocity within its real track.
- **VSL:** The straight linear of velocity: average sperm velocity via straight line from the first to the last track position.
- **VAP:** The average path of velocity: average sperm velocity through its average trajectory.

**Table 2. Effect of thyme treatment on live body weight of males and females of Gimmizah strain.**

Item	G1 (control)	G2 (THY-2ml)	G3 (THY-4ml)	P-value
Males:				
Initial live body weight (g)	2022.50±30.81	2027.49±36.73	2055.00±35.05	0.773
Final live body weight (g)	2135.00±29.55	2136.87±38.07	2166.89±35.56	0.768
Chang in live body weight (g)	112.50±3.13	109.38±3.59	111.87±2.49	0.756
Females:				
Initial live body weight (g)	1737.50±29.50	1756.25±37.13	1768.75±35.28	0.810
Final live body weight (g)	1880.00±29.99	1905.63±35.95	1918.75±36.76	0.723
Chang in live body weight (g)	142.50±3.27	149.38±3.33	150.00±2.98	0.209

#### Hematological parameters:

Results in Table 3 showed that thyme at a level of 2 ml increased ( $P<0.05$ ) hematological parameters including Hb concentration, Ht percentage, WBCs count, and basophil percentage in males as well as only Ht and WBCs in females

#### Sperm kinetic indices:

- **LIN (linearity, %):** The straightness of sperm track.

$$LIN\% = VSL/VCL (\mu m/s)$$

- **STR (straightness, %):** The righteousness of motion.

$$STR\% = VSL/VAP (\mu m/s)$$

- **WOB (wobble, %):** The degree of the sperm head actual path oscillation in relationship to VAP.

$$WOB\% = VAP/VCL (\mu m/s)$$

#### Statistical analysis:

Homogeneity and normality of distribution of all numerical data have been checked using Lieven's test and Shapiro-Wilk test, respectively. Data were statistically analyzed as a complete randomized design to study the effect of thyme level (0, 1, and 2), on different parameters studied. The model used was as the following:

$$Y_{ij} = \mu + T_i + e_{ij}$$

**where:**  $Y_{ij}$  = observed item,  $\mu$  = the overall mean,  $T_i$  = thyme effect, and  $e_{ij}$  = the random error.

The computer program of SAS (2007) was used. Duncan's test was used for separating the significant differences at  $P<0.05$  (Duncan, 1955). Data were presented as mean  $\pm$  SE.

## RESULTS AND DISCUSSION

#### Change in growth rate:

Live body weight of male and female of Gimmizah strain was not affected significantly by thyme treatment (Table 2). In agreement with our results, Lee *et al.* (2003) found no effect on growth performance of broilers fed diet supplemented with 200 mg of carvacrol and thymol/kg. On the other hand, live body weight chicks fed diets with thyme oil was increased ( $P<0.05$ ) by the supplementation of thyme oil compared to control group (Hassan 2019). Similarly, broilers fed diets contained leaves of thyme (Abdel-Wareth *et al.*, 2012). Addition of thyme oil to the diet has positive impacts on the stability of population of microorganisms, enhancing the absorptive ability, enzyme activation, and digestion as well as improving live body weight (Youssef *et al.*, 2021).

The absent of positive effects on LBW of males and females as affected by thyme treatment in drinking water in our study may be related to the level, method, and period of treatment as well as breed and age stage of chickens. In this respect, Toghyani *et al.* (2010) indicated an improved in the growth performance of broilers fed diets supplemented with thyme at a level of 10 g/kg as compared to the low level (5 g/kg).

as compared to controls (G1). Increasing level of thyme to 4 ml in G3 improved ( $P<0.05$ ) Hb concentration, Ht percentage in males and females, and increased basophils only in males as compared to controls (G1). On the other hand, MCV, MCH,

MCHC, eosinophils, heterophils, lymphocytes, and monocytes were not affected by thyme treatment.

These results revealed beneficial impacts of both thyme levels on hematological parameters of males and females, being better with the higher level of thyme. This improvement in hematological parameters was reported in broilers fed diets supplemented with thyme and cinnamon oils, in terms of increased Hb, Ht, RBCs, and WBCs (Al-Kassie, 2009). Concentrations of Hb was higher by thyme powder (30g/kg diet) than that of the control, indicating a positive effect of thyme on the health condition of heat-stressed broilers, in terms of increasing WBCs (Attia *et al.*, 2018). Inclusion of thyme oil also increased Hb and Ht, but did not affect RBCs and WBCs counts in rabbit does (Abdelnour *et al.*, 2022). Thyme oil showed a beneficial effect on health status of rabbits especially in hot environments (Placha *et al.*, 2013; Attia *et al.*, 2017; Abdel-Wareth *et al.*, 2018). The dietary essential oils can enhance the digestion with a specific antimicrobial property which can promote the general condition of health (Hippenstiel *et al.*, 2011; Bozkurt *et al.*, 2014). This impact of thyme on hematological parameters may be due to the improvement in iron utilization and the nutrient digestibility related to thyme antimicrobial (Masek *et al.*, 2014) and antioxidant effects (Hashemipour *et al.*, 2013; El-Faham *et al.*, 2015). In rainbow trout, the addition of thymol to diet did not affect significantly in MCV, MCH or MCHC, but can increase lymphocyte (Ahmadifar *et al.*, 2011). On the other hand, the powder of thyme at 1 g/kg diet had no effect on leukocyte fractions including lymphocyte, monocyte, and eosinophil percentages in blood of broiler (Demir *et al.*, 2008). Generally, it clears that the essential oils inclusion in diet could enhance the digestion with antimicrobial specific activity which promotes the general condition of animal health (Hippenstiel *et al.*, 2011; Bozkurt *et al.*, 2014).

#### Biochemical and mineral profiles:

Results in Table 3 showed that both thyme level increased ( $P<0.05$ ) plasma glucose in males and each of albumin, glucose, and calcium in females, while decreased ( $P<0.05$ ) creatinine and cholesterol in both sexes as compared to their controls. The higher thyme level (4 ml) further increased ( $P<0.05$ ) plasma phosphorus in both sexes, while decreased ( $P<0.05$ ) cholesterol in males as comparing with controls, and the lower level of thyme. These results showed positive effects of thyme (4 ml) on metabolism of carbohydrates and proteins, kidney function, mineral concentration (Ca and P), and lipid profile of laying hens and cockers.

In accordance with increasing glucose level and decreasing creatinine level in both sexes by both thyme levels in our study, no significant differences were seen in the blood serum protein (Yalcin *et al.*, 2020). Al-Mashhadani *et al.* (2011) found that serum glucose concentration in broilers was increased by dietary addition of thyme oil (200 mg/kg). Hassan (2019) reported that serum glucose was higher in chicks fed diet supplemented with thyme oil than in control. Thyme extracts (100 mg/kg) significantly decreased creatinine in rabbits (Abdel-Gabbar *et al.*, 2019). Inclusion of thyme oil (150, 300 and 450 mg/kg) had reduced serum creatinine concentration in quails compared to control group (Gümüç *et al.*, 2017). In rabbits, Abdelnour *et al.* (2022) reported the lowest blood creatinine

concentration by thyme essential oil (100 mg/kg diet) for 30 days when compared with control under heat stress condition. Also, thyme oil significantly lowered the serum levels of creatinine in rabbits (Abdel-Wareth *et al.*, 2020). Serum creatinine decreased by dietary supplementation with thyme leaves compared to the controls (Ezzat *et al.*, 2020). Feeding rabbits on diets supplemented with thyme oil (at rates of 60, 120, and 180 mg/kg) decreased creatinine level compared with control (Abdel-Wareth and Metwally, 2020). In rats fed on diets supplemented with leaves of thyme (2.5%) there is a significant decrease found in blood creatinine concentrations (Salem, 2015).

**Table 3. Effect of thyme treatment on hematological parameters of males and females of Gimmizah strain.**

Item	G1 (control)	G2 (THY-2ml)	G3 (THY-4ml)	P- value
Males:				
Hb (g/dl)	10.28±1.13 <sup>b</sup>	14.93±1.06 <sup>a</sup>	15.90±1.48 <sup>a</sup>	0.023
RBCs (x10 <sup>6</sup> /μL)	4.60±0.50 <sup>b</sup>	5.75±0.48 <sup>ab</sup>	6.98±0.63 <sup>a</sup>	0.038
Hct (%)	31.53±2.39 <sup>b</sup>	39.68±2.80 <sup>a</sup>	43.05±2.18 <sup>a</sup>	0.024
MCV (fL)	69.75±4.82	69.50±3.80	69.80±6.52	0.998
MCH (pg)	25.12±8.76	27.75±7.67	26.87±6.27	0.970
MCHC (g/dl)	34.50±10.16	39.52±10.53	37.52±6.40	0.928
WBC (x10 <sup>3</sup> /μL)	6.40±0.27 <sup>b</sup>	7.58±0.33 <sup>a</sup>	8.25±0.25 <sup>a</sup>	0.004
Basophil (%)	1.30±0.04 <sup>b</sup>	1.60±0.04 <sup>a</sup>	1.73±0.05 <sup>a</sup>	0.000
Eosinophil (%)	9.25±0.40	9.15±0.46	9.18±0.31	0.982
Heterophil (%)	34.90±1.14	34.20±1.46	35.48±1.29	0.791
Lymphocyte (%)	42.80±1.07	41.90±1.15	40.33±1.22	0.347
Monocyte (%)	11.75±0.44	13.15±1.14	13.30±2.05	0.692
Females:				
Hb (g/dl)	10.90±1.79 <sup>b</sup>	15.75±1.36 <sup>ab</sup>	17.40±1.63 <sup>a</sup>	0.046
RBCs (x10 <sup>6</sup> /μL)	4.48±0.46 <sup>b</sup>	5.93±0.55 <sup>ab</sup>	6.58±0.49 <sup>a</sup>	0.041
Hct (%)	35.25±1.89 <sup>b</sup>	41.25±1.97 <sup>a</sup>	44.90±1.71 <sup>a</sup>	0.016
MCV (fL)	82.03±6.06	70.88±5.65	69.27±5.35	0.521
MCH (pg)	24.32±5.90	28.19±6.39	27.49±5.26	0.886
MCHC (g/dl)	32.20±8.83	38.46±5.89	39.50±7.03	0.758
WBC (x10 <sup>3</sup> /μL)	5.95±0.30 <sup>c</sup>	7.83±0.41 <sup>b</sup>	9.63±0.23 <sup>a</sup>	0.000
Basophil (%)	1.20±0.11	1.18±0.14	1.35±0.10	0.546
Eosinophil (%)	9.10±0.56	9.08±0.51	9.10±0.50	0.999
Heterophil (%)	35.38±1.25	34.50±1.32	34.75±1.65	0.905
Lymphocyte (%)	40.88±1.20	41.00±1.08	42.50±1.32	0.587
Monocyte (%)	13.45±1.13	14.25±1.07	12.30±1.54	0.632

<sup>a,b,c</sup> Mean in the same row was different significantly at  $P<0.05$ .

Regarding the observed decrease in total cholesterol level in males by thyme (4 ml) and females by 2 and 4 ml thyme, it was observed that thyme treatment (0.1, 0.15 and 0.2 ml/liter drinking water, Moomivand *et al.*, 2015) or thyme extract (0.2, 0.4, and 0.6% to drinking water, Abdulkarimi *et al.* (2011) decreased serum total cholesterol in broiler chickens. Moreover, Rahimi *et al.* (2011) revealed reduced serum total cholesterol that dietary addition of thyme extract (0.1 %) had in broilers. Recently, a reduction in serum total cholesterol of broilers fed diet contained 100 mg/kg of thyme oil was reported by Moustafa *et al.* (2020) and Hassan (2019). In the same way, Khafar *et al.* (2019) found reduced total cholesterol by feeding diets with thyme oil (150 and 200 mg/kg) in broilers. Shamma *et al.* (2019) showed that 0.4 ml of thyme oil per kg of diet reduced ( $P<0.05$ ) serum total cholesterol in broiler chickens. Also, total cholesterol was lower in serum of quails by thyme supplementation in the diet (300 or 450 mg/kg) compared to the controls (Gümüç *et al.*, 2017). Serum level of cholesterol in broilers was decreased by feeding diets with thyme oil (200 mg) per kg (Al-Mashhadani,

*et al.*, 2011). Similarly, many reports showed that the supplementation of thyme and other phytogetic feed additive decreased poultry blood cholesterol (El-Ghousein and Al-Beitawi 2009; Manafi *et al.*, 2016). The total cholesterol reduction in blood may be attributed to the increased in secretion of digestive enzymes (Jang *et al.*, 2004) and/or improved the release of bile acids (Amad *et al.*, 2011). Carvacrol in thyme has hypocholesterolemic and antilipidemic effects on HMG-CoA (3-hydroxy-3-methylglutaryl) reductase which could reduce the absorption of fat from the gut or the lipid catabolism for gluconeogenesis (Abdulkarimi *et al.*, 2011; El-Ghousein and Al-Beitawi, 2009; Khan *et al.*, 2012). Higher secretion of bile and digestive enzymes increase lipid digestibility which might have resulted in low serum cholesterol (Manafi, 2015). This trend showed that thyme could have antioxidative and hypolipidemic action in laying hens (Yalcin *et al.*, 2020).

Increasing plasma Ca and P contents in females, and P content in males may be attributed to increasing the absorption

rate through the intestinal mucosa by thyme treatment. Thyme oils extract affect broiler's digestive tract, so the absorption rate of minerals may be increased and consequently the chicks feeding increase as well (Botsoglou *et al.*, 2002; Alçiçek *et al.*, 2004; Hernandez *et al.*, 2004; Al-Kassie, 2009).

Similar to our results, Attia *et al.* (2018) found a significant positive effect of dietary dried thyme powder on protein metabolites in heat stressed Arbor Acres broilers during 1–28 days of age. Kucukgul Gulec *et al.* (2013) found that the supplementation of diet with essential oils of herbals containing thyme increased albumin and some electrolytes parameters. Greater plasma total protein, but variations in plasma albumin, globulin were reported. On the other hand, concentrations of blood total protein and its fraction (albumin) were reduced due to thyme powder addition (Hosseini *et al.*, 2013). In broilers, dry thyme powder (1.5 and 3 g/kg diet) had no significant effect on blood protein levels and liver function markers (El-Faham *et al.*, 2015).

**Table 4. Effect of thyme treatment on biochemical and mineral profiles in plasma of males and females of Gimmizah strain.**

	Item	G1 (control)	G2 (THY-2ml)	G3 (THY-4ml)	P-value
Males	Total protein (mg/dl)	5.58±0.38	6.68±0.42	6.78±0.53	0.163
	Albumin (mg/dl)	2.53±0.17	2.65±0.22	2.93±0.28	0.472
	Globulin (mg/dl)	3.05±0.27	4.03±0.28	3.85±0.37	0.113
	Glucose (mg/dl)	219.5±3.2 <sup>c</sup>	237.4±4.9 <sup>b</sup>	253.3±4.2 <sup>a</sup>	0.001
	Cholesterol (mg/dl)	132.3±7.2 <sup>a</sup>	133.9±6.2 <sup>a</sup>	105.9±5.6 <sup>b</sup>	0.021
	Creatinine (mg/dl)	0.98±0.05 <sup>a</sup>	0.76±0.02 <sup>b</sup>	0.71±0.04 <sup>b</sup>	0.002
	Calcium (mg/dl)	8.38±0.56	9.95±0.48	9.78±0.52	0.115
	Phosphor (mg/dl)	5.13±0.52 <sup>b</sup>	6.30±0.40 <sup>ab</sup>	6.85±0.44 <sup>a</sup>	0.067
Females	Total protein (mg/dl)	5.50±0.44	6.70±0.49	6.93±0.45	0.115
	Albumin (mg/dl)	2.10±0.26 <sup>b</sup>	2.95±0.21 <sup>a</sup>	3.13±0.25 <sup>a</sup>	0.033
	Globulin (mg/dl)	3.15±0.27	4.03±0.28	3.85±0.37	0.164
	Glucose (mg/dl)	201.2±5.8 <sup>b</sup>	247.8±4.2 <sup>a</sup>	262.1±4.9 <sup>a</sup>	0.000
	Cholesterol (mg/dl)	143.5±6.4 <sup>a</sup>	121.2±7.1 <sup>b</sup>	100.4±6.5 <sup>b</sup>	0.005
	Creatinine (mg/dl)	0.94±0.02 <sup>a</sup>	0.67±0.03 <sup>b</sup>	0.61±0.02 <sup>b</sup>	0.000
	Calcium (mg/dl)	10.1±0.52 <sup>b</sup>	12.0±0.58 <sup>a</sup>	12.8±0.54 <sup>a</sup>	0.018
	Phosphor (mg/dl)	5.03±0.43 <sup>b</sup>	6.45±0.53 <sup>ab</sup>	7.03±0.44 <sup>a</sup>	0.038

<sup>abc</sup> Mean in the same row was different significantly at P<0.05.

#### Enzyme activity and thyroid function:

Results in Table 5 showed that both thyme levels decreased (P<0.05) activity of AST and ALT, and increased T<sub>3</sub> concentration in blood plasma of males and females, as compared to their controls.

These impacts of thyme administration were reported by several authors on different sources and levels

of thyme. In this line, activity of AST and ALT in blood serum in broilers was reduced by thyme at a level of 100 mg/kg diet (Moustafa *et al.*, 2020), dietary thyme powder (1, 1.5 and 2 g per kg) supplementation as compared with controls (Attia *et al.*, 2017), or thyme oil (Saleh *et al.*, 2014).

**Table 5. Activity of transaminases (AST and ALT) and thyroid hormone (T<sub>3</sub>) in plasma of males and females of Gimmizah strain as affected by thyme.**

	Item	G1 (control)	G2 (THY-2ml)	G3 (THY-4ml)	P-value
Males	ALT (IU/L)	28.10±1.87 <sup>a</sup>	18.45±2.82 <sup>b</sup>	17.48±2.09 <sup>b</sup>	0.018
	AST (IU/L)	43.25±1.03 <sup>a</sup>	36.90±1.14 <sup>b</sup>	34.25±1.31 <sup>b</sup>	0.001
	T <sub>3</sub> (ng/ml)	2.40±0.20 <sup>b</sup>	3.28±0.26 <sup>a</sup>	3.45±0.29 <sup>a</sup>	0.036
Females	ALT (IU/L)	24.60±1.73 <sup>a</sup>	17.60±1.50 <sup>b</sup>	15.70±1.97 <sup>b</sup>	0.013
	AST (IU/L)	40.35±2.55 <sup>a</sup>	32.80±2.12 <sup>b</sup>	29.25±2.13 <sup>b</sup>	0.046
	T <sub>3</sub> (ng/ml)	2.53±0.23 <sup>b</sup>	3.40±0.25 <sup>a</sup>	3.63±0.31 <sup>a</sup>	0.037

<sup>abc</sup> Mean in the same row was different significantly at P<0.05. T<sub>3</sub>: Triiodothyronine.

In rabbits, blood AST and ALT activities were reduced by thyme extracts at 100 mg/kg (Abdel-Gabbar *et al.*, 2019), thyme oil (100 g/kg diet) treated for 30 days under heat stress condition (Abdelnour *et al.*, 2022) and normal conditions (Abdel-Wareth and Metwally, 2020; Abdel-Wareth *et al.*, 2020), dietary supplementation with thyme leaves (Ezzat *et al.*,

2020), or aqueous thyme extracts (Abu Raghif *et al.*, 2015). In general, El-Ratel *et al.* (2020) reported that the activity of AST and ALT of rabbits was remarkably enhanced by oral administration of phytochemicals as compared to the control. In comparable with the obtained results, serum ALT in broiler was not affected by 1 g thyme powder supplemented per kg



diet (Ragaa *et al.*, 2016; El-Faham *et al.*, 2015; Tayeb *et al.*, 2019). The activity of serum ALT increased and AST did not change in broilers fed thyme-diets (Zhu *et al.*, 2014). However, dietary dry thyme powder supplementation decreased plasma AST and increased ALT activity in rabbits in compare with controls (Attia *et al.*, 2018).

The increase in T3 concentration of males and females under the effect of thyme treatment was reported by Hassan (2019), who found significant increase in T3 concentration by thyme treatment (100 mg/kg) in 96 day-old boiler chicks. Also, Osman *et al.* (2019) indicated that thyme extract ameliorates T3. Moreover, flavonoids in thyme have an ability to enhance iodide uptake and sodium-iodide symporter expression and thyroperoxidase (the key enzyme in thyroid hormones biosynthesis) as reported by Lima *et al.* (2006).

**Table 6. Effect of thyme treatment on concentration of immunoglobulins (IgG and IgM) in plasma of males and females of Gimmizah strain.**

Item		G1(control)	G2(THY-2ml)	G3(THY-4ml)	P-value
Males	IgG (ng/ml)	48.65±1.46 <sup>b</sup>	61.80±1.25 <sup>a</sup>	62.55±1.11 <sup>a</sup>	0.000
	IgM (ng/ml)	11.23±1.10 <sup>b</sup>	14.55±1.15 <sup>a</sup>	14.93±1.04 <sup>a</sup>	0.050
Females	IgG (ng/ml)	34.40±1.54 <sup>b</sup>	42.78±1.90 <sup>a</sup>	44.90±1.72 <sup>a</sup>	0.005
	IgM (ng/ml)	11.10±0.99 <sup>b</sup>	13.95±0.83 <sup>a</sup>	14.10±0.95 <sup>a</sup>	0.028

<sup>ab</sup> Mean in the same row was different significantly at P< 0.05.

In agreement with the present results of immunoglobulins, Abdelnour *et al.* (2022) reported an increase in immunoglobulins level (IgG and IgM) by 18 and 21%, respectively, in rabbit does receiving thyme oil (100 mg per kg). Thymol administration can rise serum ELISA antibody titers against infectious bursal disease (IBD) (Hashemipour *et al.*, 2013; Almremdhy and Al-khafaji, 2020) and bursa percent (Hashemipour *et al.*, 2013). Thyme oil addition (at 150 and 200 mg per kg) in the diet of broiler had been impacted immune response (Khafar *et al.*, 2019) and this level is important tool to increase immunity (Khafar *et al.*, 2019). Many reports had showed improvements in the immunity by thymol (Acamovic and Brooker, 2005; Hashemipour *et al.*, 2013; Abd El-Hack *et al.*, 2016), and dietary thyme (1g/kg) had been enhanced the immunity of heat-stressed broilers (Fallah and Mirzaet 2016; Attia *et al.*, 2017). Thyme can extend the activity of vitamin C, thus, act as antioxidants and consequently improve immune functions (Acamovic and Brooker, 2005) and feeding birds on diets contain Thymol can improve IgG and the response of primary and secondary against the sheep red blood cells

#### Immune response:

Regarding the immune response (Table 6), both thyme levels increased (P<0.05) plasma concentration of IgG and IgM in males and females. These results indicated a positive effect on immunity of laying hens and cockers.

The immune condition of host play major role in its infections resistance. It is of possible to improve the broiler chickens phagocyte system, humoral and their cellular immunity response by feeding diet with essential oils addition that increase the defense system ability to cope the infectious organisms in addition to that, the activity of immune-stimulating by thymol polyphenol fraction and essential oil of oregano (Perez-Roses *et al.*, 2015).

(SRBC) antigen (Hashemipour *et al.*, 2013). In this context, herbs containing high levels of flavonoids (thymol) could enhance the activation of immunity via their action as antioxidants and increasing vitamin C activity (De Cassia Da Silveira *et al.*, 2013). The activities of thymol as antibacterial, antiviral, and antioxidant it could improve the response of chicks immunity (Botsoglou *et al.*, 2002). In rabbits, dietary inclusion with thyme essential oil (0.5 g/kg) improved immunity and health (Placha *et al.*, 2019). Dietary thyme addition (5 g/kg) improved the immunity of broilers (Hassan and Awad, 2017).

#### Antioxidant status:

Results shown in Table 7 revealed that both thyme levels increased (P<0.05) plasma level of TAC, while decreased (P<0.05) MDA level in blood plasma of males and females. These results reflected higher antioxidant balance in males and females in treatment groups (G2 and G3) than in control one (G1), indicating an improvement in lipid peroxidation and antioxidant status of laying hens and cockers by both thyme levels.

**Table 7. Effect of thyme treatment on level of total antioxidant capacity (TAC) and malondialdehyde (MDA) in plasma of males and females of Gimmizah strain.**

Item		G1(control)	G2(THY-2ml)	G3(THY-4ml)	P-value
Males	TAC (mmol/L)	0.73±0.05 <sup>b</sup>	0.92±0.02 <sup>a</sup>	0.96±0.02 <sup>a</sup>	0.002
	MDA (mmol/L)	2.38±0.09 <sup>a</sup>	1.73±0.11 <sup>b</sup>	1.70±0.10 <sup>b</sup>	0.001
	Antioxidant balance	36.67	56.17	56.47	-
Females	TAC (mmol/L)	0.62±0.05 <sup>b</sup>	0.82±0.03 <sup>a</sup>	0.89±0.04 <sup>a</sup>	0.001
	MDA (mmol/L)	2.95±0.30 <sup>a</sup>	2.05±0.33 <sup>ab</sup>	1.93±0.25 <sup>b</sup>	0.050
	Antioxidant balance	21.01	40.00	46.11	-

<sup>ab</sup> Mean in the same row was different significantly at P< 0.05.

The levels of TAC are valuable indication of reducing blood ROS, so TAC level in blood is important to neutralization of ROS, generated via different pathways of oxidation (Abdelnour *et al.*, 2020; Sheiha *et al.*, 2020). In harmony with our results, thyme essential oil treatment could be effective in reducing level of MDA to increase antioxidant enzyme activities and to eliminate lipid

peroxidation and ROS generation (Nazar *et al.*, 2019; Alagawany *et al.*, 2021). Also, thyme oil has a great defensive effect to protect the peroxidation of lipids within the cells through decreasing MDA levels (Bacova *et al.*, 2020). Diet incorporation of thymol in broiler diets could decrease fatty acid oxidation by reducing the level of MDA in mucosa of duodenum (Placha *et al.*, 2014). Dry thyme

powder exhibited a lower MDA level than control broilers (Attia *et al.*, 2017). oxidative stress was decreased, antioxidant activity was increased, and immunity was improved by using thyme oil (Nazar *et al.*, 2019; Toschi *et al.*, 2020). In rats, thyme essential oil treatment decreased the oxidative damage and enhanced the antioxidant activity (Güvenç *et al.*, 2018). Also, level of MDA was reduced in rats treated with thymol (10 or 20 mg/kg diet) in compared to controls (Güvenç *et al.*, 2018).

In our study, diet supplemented with both thyme levels has similar effectiveness to inhibit lipid peroxidation in Gimmizah strain and is considered to be better than the synthetic antioxidants in poultry industry to improve the productivity and health (Alma *et al.*, 2003; Luna *et al.*, 2018). In rabbit dose, thyme oils can enhance the redox status by improving the reproduction. Thus, thymol in thyme extract increases the antioxidant activity and ameliorates the oxidative stress (Yu *et al.*, 2016) and thyme can stimulate the antioxidant activity by neutralizing the free radicals (Salem, 2015).

#### Egg quality:

Results of egg quality parameters of laying hens (Table 8) showed that weight, tail, wide, Haugh unit, and shape index of eggs as well as weight and height of albumen were improved ( $P<0.05$ ) by both thyme levels. Further improvement ( $P<0.05$ ) was recorded in albumen index along with yolk height and index by increasing thyme level from 2 to 4 ml in G3. However, the effect of thyme was not significant on albumen width, yolk weight, and shell weight and thickness.

Based on these results, both levels of thyme exhibited pronounced improvement in egg quality of laying in comparing with controls, being better for hens treated with thyme at a level of 4 ml than those fed diet treated with thyme (2 ml).

In the same way of our results, dietary supplementation of 150 mg/kg of essential oils (8% thymol and 4.9% carvacrol) improved the external and internal quality of the eggs of brown laying hens at 70-week-old (Ramirez *et al.*, 2021). On the same line, other authors (Bölükbaşı *et al.*, 2010; Olgun, 2016) observed that addition of essential oil to diet (100-mg/kg) increased the average egg weight in Hy-Line hens (He *et al.*, 2017) as well as egg weight and egg mass. Dietary thyme supplementation and essential oils improved egg weight (Ghasemi *et al.*, 2010; Arpášová *et al.*, 2015; Ding *et al.*, 2017; Çufadar 2018) and Haugh unit values (Vakili and Majidzadeh Heravi, 2016). Improving egg quality by thyme, in terms of increasing egg weight and egg mass at interval of 68 to 72 weeks of age was reported by Abdel-Wareth (2013). Thyme, as a feed supplement, was found to have beneficial impacts on the quality of eggs (Khan *et al.*, 2012), and the addition of thymol and carvacrol to diet in an essential oil mixture increased the egg weight (Bozkurt *et al.*, 2012). Essential oil mixture boosted egg weight (Ozek *et al.*, 2011). As proved in our study, no significant alterations were observed in terms of eggshell properties in response to the supplementation of dietary essential oils (Wang *et al.*, 2022). Addition of thyme 0, 1 and 2% did not significantly affect shell thickness, albumen index, yolk index without affecting hen day egg production and egg weight (Yalcin *et al.*, 2020). Dietary addition of thyme had no effect on shell thickness (Ali *et al.*, 2007; Manafi *et al.*, 2016; Çufadar, 2018), shell weight (Ali *et al.*, 2007; Vakili and Majidzadeh Heravi, 2016; He *et al.*, 2017; Çufadar, 2018), and yolk index (Vakili and Majidzadeh Heravi, 2016). Additionally, thyme had no significant effect on shell thickness in laying Japanese Quail (Shahryar *et al.*, 2011) and shell thickness and yolk index of laying hens (Abdel-Wareth, 2013).

**Table 8. Effect of thyme treatment on egg quality parameters of Gimmizah hens.**

	Item	G1(control)	G2(THY-2ml)	G3(THY-4ml)	P-value
Egg	Weight (g)	47.03±0.76 <sup>c</sup>	49.33±0.03 <sup>b</sup>	52.45±0.73 <sup>a</sup>	0.000
	Tail (mm)	52.93±0.30 <sup>c</sup>	56.60±0.31 <sup>b</sup>	60.35±0.29 <sup>a</sup>	0.000
	Width (mm)	40.30±0.15 <sup>c</sup>	41.78±0.18 <sup>b</sup>	43.23±0.13 <sup>a</sup>	0.000
	Shape index	76.16±0.69 <sup>a</sup>	73.82±0.65 <sup>b</sup>	71.63±0.53 <sup>c</sup>	0.002
Albumen	Haugh unit	78.76±0.73 <sup>b</sup>	81.49±0.74 <sup>a</sup>	83.34±0.91 <sup>a</sup>	0.009
	Weight (g)	24.79±0.43 <sup>c</sup>	26.68±0.58 <sup>b</sup>	29.29±0.29 <sup>a</sup>	0.000
	Height (mm)	5.60±0.14 <sup>c</sup>	6.13±0.11 <sup>b</sup>	6.58±0.13 <sup>a</sup>	0.001
	Width (mm)	7.67±0.27	7.87±0.35	8.26±0.17	0.340
	Index	73.14±1.73 <sup>b</sup>	78.11±2.28 <sup>ab</sup>	79.60±0.97 <sup>a</sup>	0.050
Yolk	Weight (g)	15.64±0.14	16.08±0.27	16.60±0.91	0.499
	Height (mm)	17.10±0.04 <sup>b</sup>	18.03±0.37 <sup>ab</sup>	18.88±0.58 <sup>a</sup>	0.034
	Width (mm)	41.55±0.59	42.91±0.51	43.62±0.98	0.176
	Index	41.18±0.50 <sup>b</sup>	42.00±0.47 <sup>ab</sup>	43.25±0.38 <sup>a</sup>	0.030
Shell	weight (g)	6.60±0.48	6.57±0.35	6.57±0.21	0.998
	Thickness (mm)	0.90±0.01	0.87±0.01	0.88±0.01	0.348

<sup>a,b,c</sup> Mean in the same row was different significantly at  $P<0.05$ .

The observed improvement in egg quality by drinking water supplemented with thyme extracts was mainly attributed to that thyme contains carvacrol, which has several bioactive actions against bacteria, oxidants, inflammation, fungi, and viruses. It also has hypocholesterolemic, antiseptic, expectorant, antitussive, immunomodulatory and chemopreventive properties (Luna *et al.*, 2018; Hashemipour *et al.*, 2013; Bravo *et al.*, 2014; Alagawany *et al.*, 2015).

#### Semen quality:

##### Sperm motility parameters:

Sperm motility parameters of cockers illustrated in figure 1 revealed that thyme (2ml) improved motility parameters in terms of increasing ( $P<0.05$ ) progressive, rapid and total motilities, and decreasing ( $P<0.05$ ) immotility percentages.

Further improvements by increasing level of thyme to 4 ml was observed in all motility parameters, in terms of increasing ( $P<0.05$ ) progressive, total, and rapid motilities,

and reducing ( $P<0.05$ ) non-progressive motility and immotility percentages. However, sperm slow motility percentage was not affected ( $P\geq 0.05$ ) by thyme levels, in comparing with untreated group.

#### Sperm morphological features (%)

Thyme treatment at a level of 4 ml increased ( $P<0.05$ ) normal forms percentage, and decrease ( $P<0.05$ ) abnormality percentages in head, neck, and tail as well as dual deformation percentages of spermatozoa, in comparing with the control. However, thyme (2 ml) showed positive impact only on decreasing ( $P<0.05$ ) mono deformation and increasing ( $P<0.05$ ) tri deformation of sperm cells. This results were reflected in the lowest ( $P<0.05$ ) deformity index value by thyme at a level of 4 ml as compared to thyme (2 ml) and untreated group.

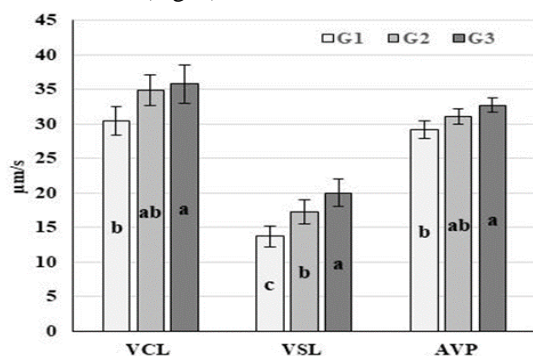
**Table 9. Effect of thyme treatment on sperm morphological features (%) in semen of Gimmizah cockers.**

Item	G1 (Control)	G2 (THY-2ml)	G3 (THY-4ml)	P-value
Normal forms	47.06±4.11 <sup>b</sup>	48.07±3.96 <sup>b</sup>	71.79±4.33 <sup>a</sup>	0.006
Abnormal head	41.18±3.46 <sup>a</sup>	37.22±3.08 <sup>a</sup>	14.00±3.19 <sup>b</sup>	0.004
Abnormal neck	19.61±1.33 <sup>a</sup>	20.30±1.18 <sup>a</sup>	04.76±1.07 <sup>b</sup>	0.009
Abnormal tail	19.61±1.24 <sup>a</sup>	18.07±1.34 <sup>a</sup>	05.13±1.15 <sup>b</sup>	0.008
Mono deformation	25.49±1.52 <sup>a</sup>	21.70±1.40 <sup>b</sup>	23.08±1.31 <sup>a</sup>	0.006
Dual deformation	21.57±0.46 <sup>a</sup>	15.00±0.51 <sup>a</sup>	00.37±0.10 <sup>b</sup>	0.003
Tri deformation	05.88±0.53 <sup>b</sup>	15.23±0.61 <sup>a</sup>	04.76±0.47 <sup>b</sup>	0.004
Deformity index	00.80±0.15 <sup>a</sup>	00.76±0.19 <sup>a</sup>	00.24±0.11 <sup>b</sup>	0.007

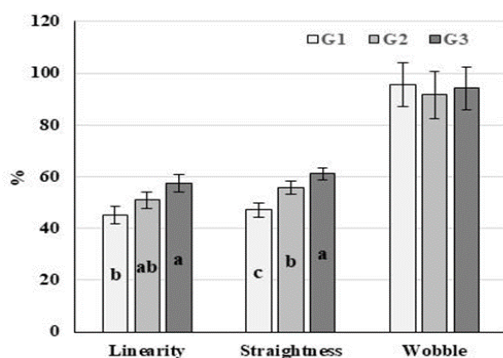
<sup>ab</sup> Mean in the same row was different significantly at  $P<0.05$ .

#### Sperm velocity and kinetic indexes:

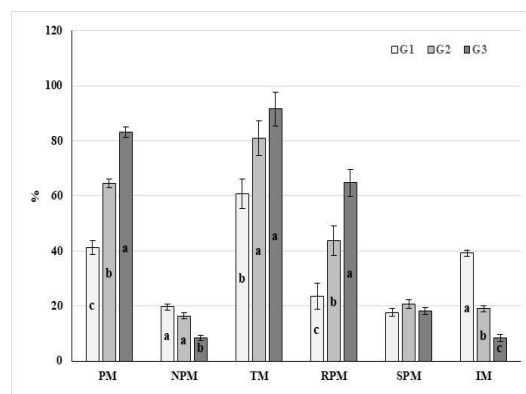
Sperm velocity parameters including VCL, VSL, and VAP were the highest ( $P<0.05$ ) in 4 ml-thyme group (G3) than in control one (G1). However, 2 ml-thyme group increased ( $P<0.05$ ) only VSL (Fig. 2). However, sperm kinetic indexes including LIN and STR were increased by both thyme levels, but WOB was not affected ( $P\geq 0.05$ ) by thyme treatment (Fig. 3).



**Fig. 2. Effect of thyme treatment on sperm velocity parameters.**



**Fig. 3. Effect of thyme treatment on sperm kinetic indexes.**



**Fig. 1. Effect of thyme treatment on sperm motility parameters in semen of Gimmizah cockers.**

In accordance with the present results, ATLE improved ( $P<0.05$ ) the ejaculate volume, concentration of sperm cells, and motility, viability, and morphological normality of spermatozoa in male broilers breeder ROSS308 (Shanoon and Jassim 2012). Supplemented feeds with thyme powder (1%) significantly increased sperm motility, sperm density, and viability in kabir rooster (Toukala *et al.*, 2020). In rabbits, feeding diets with 50 or 100 g of thyme treatment for 60 days significantly shortened buck reaction time, reduced percentage of non-viable and abnormal sperm, and increased ejaculation frequency, forward-moving sperm percentages, and total sperm output compared to the control group (El-Gindy, 2022). In rabbits, dietary thyme leaf supplementation significantly increased the volume of ejaculates, and viability and forward motility of sperm cells, while decreased abnormal sperm, under hot climate (Ezzat *et al.*, 2020). Moreover, thyme essential oil increased sperm motility, sperm livability, and ejaculate volume, while decreased sperm abnormality in rabbits compared with control group (Abdel-Gabbar *et al.*, 2019; Abdel-Wareth and Metwally, 2020; Younan *et al.*, 2021).

Improving motility parameters, morphological features, and velocity of cocker spermatozoa in our study could be due to thyme's antioxidant properties for protecting and neutralizing reactive oxygen species (ROS) in semen and thereby improving sperm parameters (Dragsted, 2003). Thyme treatment increased antioxidant levels in seminal plasma (Agarwal *et al.*, 2005). Thyme treatments significantly elevated the capacity of total antioxidant and levels of glutathione peroxidase, while decreased malondialdehyde level in plasma of semen as compared to the control group (El-Gindy, 2022). Also, thyme treatment improves some reproductive parameters and testes weight in response to feeding on thyme leaves in rabbits (Ezzat *et al.*, 2020; Kandeil *et al.*, 2019) and semen quality was improved by thyme components (Ezzat *et al.*, 2020). Thymol action



may be in three paths 1) reducing the hurtful microbiota, 2) strengthening the antioxidant system due to the polyphenols components (Abdulkarimi *et al.*, 2011), 3) improving feed utilization and immunity (Ghazalah and Ali, 2008).

#### **Yield, fertility and hatchability of eggs:**

Egg yield was increased by both thyme levels, being the highest ( $P<0.05$ ) in G3 (4 ml thyme) and insignificantly

higher in G2 (2 ml thyme), in comparing with G1 (control). Both thyme levels of thyme increased ( $P<0.05$ ) egg weight and hatched chick weight, reflecting the highest chick-ovo index in G3, moderate in G2, and the lowest in G1. However, fertility and hatchability of eggs were not affected by thyme treatment (Table 10).

**Table 10. Effect of thyme treatment on yield, fertility, hatchability, and hatched check weight of Gimmizah hens.**

Item	G1(Control)	G2(THY-2ml)	G3(THY-4ml)	P-value
Egg production (eggs/hen)	30.51±0.65 <sup>b</sup>	32.74±0.84 <sup>b</sup>	33.89±0.69 <sup>a</sup>	0.012
Egg fertility rate (%)	85.38±1.03	86.63±1.05	87.88±1.01	0.253
Egg hatchability (%)	85.81±0.99	86.33±1.37	86.48±1.23	0.920
Hatched chick weight (g)	32.98±0.53 <sup>c</sup>	38.58±0.25 <sup>b</sup>	42.61±0.33 <sup>a</sup>	0.000
Chick-ovo index	70.1	78.2	81.2	-

<sup>a,b,c</sup> Mean in the same row was different significantly at  $P<0.05$ .

The beneficial effect of thyme (4 ml) on egg production in our study was reported by many authors. In this line, Ramirez *et al.* (2021) found that dietary supplementation with 150-ppm essential oil (thymol and carvacrol) increased the yield and mass of eggs produced from Isa Brown laying hens at 70-week of age.

Ghanima *et al.* (2020) reported the greatest egg yield of layers receiving thymol. Egg production increased by the dietary thyme leaves (Ghasemi *et al.*, 2010), essential thyme oil (Arpášová *et al.*, 2015; Ding *et al.*, 2017; Çufadar, 2018). Dietary supplementation of mixed essential oils and thyme oil (Bölükbaşı *et al.*, 2008) or dietary phytogetic feed additive containing thymol (Manafi *et al.*, 2016) increased the egg production. Abdel-Wareth (2013) reported that dietary thyme supplementation improved hen-day-egg production. Also, thyme, as a feed additive, exhibited positive impacts on the production of eggs (Ali *et al.*, 2007; Khan *et al.*, 2012) and the dietary addition of thymol and carvacrol enhanced the production rate of eggs in hens (Bozkurt *et al.*, 2012). In comparable with our results, thyme extract treatment had no effects on egg production, but improved weight and mass of eggs (Vakili and Majidzadeh Heravi, 2016). Improving the egg yield may be attributed to increasing nutrients digestibilities of the diet. Also, thyme improved the digestive ability to induce the available nutrients in the intestine for the benefits of the body in animals and poultry (Windisch *et al.*, 2008). Also, essential oils may increase the ovarian activity and the intestinal digestibility of nutrients leading to an increase in weight and mass of eggs produced by laying hens (Olgun, 2016).

## **CONCLUSION**

Considering the above findings, the levels of 2 and 4 ml thyme aqueous extract/10 L of drinking water can promote beneficial impacts on the hematological, biochemical parameters, liver and kidney function, immunological indicators, antioxidant capacity, semen quality, yield and quality of eggs, and weight of hatched chicks, without adverse effects on fertility and hatchability of Gimmizah local strain.

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## تأثير المستخلص المائي للزعتر على الأداء التناسلي، مكونات الدم، مضادات الأكسدة والمناعة لدجاج الجيزة

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### الملخص

تهدف هذه الدراسة لتقييم تأثير إضافة المستخلص المائي لأوراق الزعتر (TLAE) على الأداء الإنتاجي والتناسلي لدجاج الجيزة. تم تقسيم 48 ذكر و 48 أنثى بعمر 38 أسبوعاً إلى ثلاث مجموعات (16 من كل جنس في كل منها) تمت تغذيتهم على نظام غذائي (16٪ بروتين خام و طاقة ممثلة 2750 سعر حراري/كجم). كانت الطيور في المجموعة الأولى هي المجموعة الضابطة، بينما كانت الطيور في المجموعة الثانية والثالثة تشرب الماء به 2 و 4 مل من 20٪ مستخلص الزعتر / 10 لتر لمدة 75 يوماً. أظهرت النتائج أن المعاملة بـ 4 مل مستخلص الزعتر تحسن ( $P < 0.05$ ) نسبة الهيموجلوبين والهيماتوكريت والجلوكوز والكرياتينين والكرياتينول في كلا الجنسين، والخلايا القاعدية في الذكور، والألبومين والكالسيوم في الإناث. كما خفضت المعاملة بـ 4 مل مستخلص الزعتر نشاط انزيمات AST و ALT، ومستوى MDA في البلازما في حين زاد تركيز  $\text{IgM}$ ،  $\text{IgG}$ ،  $\text{T}_3$  ( $P < 0.05$ )، و TAC في كلا الجنسين. زاد وزن وطول وعرض ووحدة Haugh ومعامل الشكل للبيض، وزن، ارتفاع ومعامل البيض وكذلك ارتفاع ومعامل الصفار. زاد قياس الأنواع المختلفة من حركة الحيوانات المنوية والصفات المورفولوجية والسرعة وبالمعاملة بـ 4 مل مستخلص الزعتر ( $P < 0.05$ ). كما زاد محصول البيض ووزن الكتاكيت المفقس ومعامل وزن الفقس لوزن البيض (chick-ovo) بالمعاملة بـ 4 مل مستخلص الزعتر ( $P < 0.05$ )، في حين لم تتأثر خصوبة البيض ومعامل الفقس. الخلاصة: تستنتج من ذلك أن إضافة مستخلص أوراق الزعتر في ماء الشرب (4 مل / 10 لتر) يمكن الاستفادة منه لتحقيق تأثيرات مفيدة على الأداء التناسلي والإنتاجي لذكور وإناث سلالة الجيزة المحلية.