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RESEARCH ARTICLE

Thyme (*Thymus vulgaris* L.) essential oil supplementation: impact on performance, egg quality, serum, and faecal mineral content in laying partridges

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Abstract

Aim of study: This study evaluated the impact of thyme (*Thymus vulgaris* L.) essential oil (TEO) supplementation on performance, egg quality, and serum and faecal mineral composition in chukar partridges (*Alectoris chukar*).

Area of study: Türkiye.

Material and methods: Ninety-layer partridges, aged 32 weeks, were randomly assigned to five groups. Within each group, there were six replicates with three birds/replicate. The experimental design included a control group receiving a basal diet (0 mg/kg TEO) and four treatment groups with diets supplemented with TEO at 50, 100, 150, and 200 mg/kg over 56 days.

Main results: The results showed no significant differences in performance parameters among the groups ($p>0.05$). However, egg production and mass were significantly higher in the 50 mg/kg TEO group than in the control group ($p<0.01$). Additionally, eggshell thickness and yolk index were improved in the 50 mg/kg group compared to the 100 mg/kg group ($p<0.05$). Serum and faecal mineral compositions did not differ significantly among the experimental groups ($p>0.05$).

Research highlights: These results suggest that incorporating TEO at 50 mg/kg into the diet of chukar partridges may be optimal, as higher concentrations could potentially interfere with nutrient absorption and negatively affect partridge attributes. Nevertheless, further research is needed to elucidate the mechanisms of action of TEO´s active ingredients in partridge feed.

Keywords: Avian production, chukar partridge, egg quality, mineral concentration, thyme essential oil.

Suplementación con aceite esencial de tomillo (*Thymus vulgaris* L.): Impacto en el desarrollo, en la calidad de los huevos y en el contenido mineral en heces y suero sanguíneo de perdices de puesta

Resumen

Objetivo del estudio: Este estudio evaluó el impacto de la suplementación dietética con aceite esencial de tomillo (*Thymus vulgaris* L.) (TEO) en la dieta de perdices de puesta (*Alectoris chukar*) a nivel del desarrollo productivo, de la calidad de los huevos, y del contenido mineral en heces y suero sanguíneo.

Área de estudio: Turquía.

Material y métodos: Noventa perdices ponedoras de 32 semanas se dividieron aleatoriamente en cinco grupos; dentro de cada grupo había seis réplicas con tres aves cada una. El diseño experimental incluyó un grupo de control que recibió una dieta basal (0 mg/kg de TEO) y cuatro grupos de tratamiento con dietas suplementadas con TEO a 50, 100, 150 y 200 mg/kg durante 56 días.

Resultados principales: Los resultados no mostraron diferencias significativas en los parámetros de desarrollo productivo entre los grupos experimentales (p>0,05). Sin embargo, la producción de huevos y la masa de huevos fueron significativamente mayores en el grupo que había recibido 50 mg/kg de TEO en comparación con el control (p<0,01). Además, el grosor de la cáscara del huevo y el índice de yema mejoraron en el grupo de 50 mg/kg en comparación con el de 100 mg/kg (p<0,05). Las composiciones minerales sérica y fecal no difirieron significativamente entre los grupos experimentales (p>0,05).

Aspectos destacados de la investigación: Estos resultados sugieren que la incorporación de TEO a 50 mg/kg en la dieta de perdices puede ser óptima, ya que concentraciones mayores podrían interferir potencialmente con la absorción de nutrientes y afectar negativamente a los rendimientos y las características de los huevos de las perdices. No obstante, se necesitan más investigaciones para determinar los mecanismos de acción de los ingredientes activos de la TEO en la alimentación de las perdices.

Palabras clave: Aceite esencial de tomillo, calidad de los huevos, concentración mineral, perdices, producción avícola.

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Introduction

In recent years, heightened concerns regarding the detrimental effects of antibiotic growth promoters (AGP) in livestock diets have led to a transition towards AGP-free animal production. This shift aims to curb the emergence of antibiotic resistance and the transmission of resistance among bacterial populations. (Gholami-Ahangaran et al., 2022; Noruzi et al., 2022). However, removing AGP from diets could negatively impact growth performance, particularly in intensive farming, such as poultry systems (Yarmohammadi Barbarestani et al., 2020; Ramirez et al., 2021). Given the health concerns and regulatory restrictions associated with antibiotic use, researchers are exploring various alternatives to AGPs. These alternatives are designed to significantly boost animal health and productivity, lower expenses, and mitigate the risks of antimicrobial resistance and antibiotic residues in livestock products (Ramirez et al., 2021; Gül et al., 2023).

In response to the challenge of AGP, there is a growing interest in natural feed additives (Sarmiento-García et al., 2024). Among these, therapeutic herbs and their essential oils (EO) have emerged as promising candidates due to their safety and lack of residues in poultry products (Ding et al., 2017; Abo Ghanima et al., 2020; Ramirez et al., 2021; Gholami-Ahangaran et al., 2022; Noruzi et al., 2022). Essential oils also referred to as ethereal or volatile oils, are aromatic oily liquids derived from plant materials. They contain a complex blend of molecules, including terpenes, alcohols, aldehydes, and ketones (Ding et al., 2017; Bajac et al., 2022). Previous research suggests that EOs may enhance the immune system and improve avian performance due to their appetizing, digestion-stimulating properties, and antimicrobial effects. These characteristics position EO as a promising alternative to AGP (Ding et al., 2017; Abo Ghanima et al., 2020; Noruzi et al., 2022; Puvača et al., 2022). Among the wide range of medicinal plants, thyme (*Thymus vulgaris* L.) stands out. This therapeutic and aromatic plant from the Lamiaceae family contains essential oils such as thymol (40%) and carvacrol (15%), which play an important role in avian health and growth performance. Thymol exhibits a broad spectrum of pharmacological activities, including anti-spasmodic, antioxidant, antimicrobial, anticancer, antiviral, anti-inflammatory, and growth-enhancing properties (Wade et al., 2021; Gholami-Ahangaran et al., 2022; Noruzi et al., 2022).

Given the promising properties of EOs and the specific benefits of thyme, together with the need to find alternatives to AGPs that ensure production profitability, including thyme in the poultry diet could be an excellent solution. Chukar partridges (*Alectoris chukar*) have gained recognition as a valuable poultry source for human consumption, owing to the high market value of their meat and eggs, as well as their important role in wildlife conservation efforts (Loponte et al., 2017). The intensive rearing of chukar partridges has increased, highlighting the need for optimized nutrition to ensure their health, and development while reducing costs (Mohtashami et al., 2021; Çam et al., 2022). Despite the burgeoning interest in those species, research on their nutrition remains relatively scarce compared to conventional avian species with existing data often lacking precision. To our knowledge, no studies investigated the effects of thyme essential oil (TEO) on chukar partridge. In base on the aforementioned information, this study aims to investigate the effects of TEO supplementation on various aspects of chukar partridge production and health. By evaluating performance parameters, egg quality, mineral metabolism, and overall health outcomes at different TEO concentrations, the current research seeks to not only ascertain the potential benefits of TEO supplementation but also determine the optimal dose for enhancing production efficiency and improving the quality of chukar partridge products.

Material and methods

Experimental design and animal housing

The research was carried out following a completely randomized design using ninety 32-week-old healthy female chukar partridges (*Alectoris chukar*) at the Directorate Partridge Breeding Unit of Bahri Dağdaş International Agricultural Research Institute (Konya, Türkiye). Before starting the study, female chukar were individually weighed (540 ± 10 g) and immediately randomly assigned to one of five dietary groups, with each group comprising six replicates, each containing three females All enclosures were uniformly sized at 30 × 45 cm, and maintained in well-ventilated, hygienic, and sanitised conditions. The room temperature was set at 22°C (± 2.0) with a 16-hour light cycle. Each enclosure had its feeders and waterers, providing *ad-libitum* access throughout the eight weeks.

Throughout the 8-week study, all chukar partridges had the same basal diet consisting of corn and soybean meal. This diet was characterized by a crude protein (CP) content of 170.2 g/kg and a metabolizable energy (ME) of 2,904 kcal/kg, as detailed in Table 1. Thyme essential oil was procured from a local company (AKSUVITAL Natural Products Food Co, Beylikdüzü, Istanbul, Türkiye). The primary active ingredients of the TEO were carvacrol and the proportions of active ingredients were as follows: 1.00% α-pinene, 0.50% β-myrcene, 1.18% gamma-terpinene, 2.99% p-cymene, 0.20% terpineol, 1.16% caryophyllene, 1.65% borneol, 90.8% carvacrol as provided by the manufacturer. The TEO was then incorporated into the basal diet, replacing corn, at inclusion rates of 50, 100, 150, and 200 mg/kg TEO to ensure that all diets maintained isoproteic and isoenergetic profiles. The mash-formulated basal diet was designed to achieve the nutritional requirements of chukar partridge as recommended by the National Research Council (1994). The chemical composition of the diet was evaluated using the methods prescribed by the Association of Official Analytical Chemists (2006). This included determining ash content via incineration and drying (method 942.05), protein and fat levels using the Kjeldahl (method 990.03) and Soxhlet methods (method 2003.06), and moisture content by drying at 105°C (method 2001.12), which are shown in Table 1.

Table 1. Ingredients and chemical composition of the basal diet of chukar partridges.

Ingredients	g/kg	Nutrient composition	g/kg
Corn	492.00	Metabolizable energy (kcal/kg)	2,904
Soybean meal	244.00	Crude protein	170.20
Barley	93.30	Crude fat	84.78
Limestone	53.50	Crude fiber	39.93
Cottonseed meal	50.00	Moisture	97.93
Soybean oil	40.00	Calcium	25.20
Dicalcium phosphate	18.50	Available phosphorus	4.50
Salt	5.00	Lysine	8.10
Premix [1]	2.50	Methionine	4.00
DL-Methionine	1.20	Cystine	3.00
Total	1,000.00	Methionine + cystine	7.00
		Treonin	6.50

[1]Premix provided/kilogram of diet: manganese, 60 mg; iron, 30 mg; zinc, 50 mg; copper, 5, 1, and 1.1 mg; selenium, 0.1 mg, vitamin A, 8.800 IU; vitamin D_3, 2.200 IU; vitamin E, 11 mg; nicotine acid, 44 mg; Cal-D-Pan, 8.8 mg; riboflavin, 4.4 mg; thiamin, 2.5 mg; vitamin B_{12}, 6.6 mg; folic acid, 1 mg; D-biotin, 0.11 mg; coline, 220 mg.

Evaluation of performance indicators and egg production

To assess performance parameters, each of the 90 experimental chukar partridges underwent initial and final individual weighing using a precision balance with an accuracy of ± 0.01 g. Changes in body weight (in grams) were calculated based on these measurements. Feed intake, measured in grams per chukar per day, was monitored by recording the total feed dispensed and the remaining feed daily, following the methodology described by Gül et al. (2023). Egg production was assessed by calculating the daily egg count per 100 chukar. Eggs collected during the last three days of the study were weighed using a high-precision balance (± 0.01 g). Egg mass (grams per chukar per day) and feed conversion ratio (FCR) were determined using the methods outlined by Sarmiento-García et al. (2023).

Egg quality assessment

All eggs acquired during the final three days (n=240) were analysed at the Partridge Breeding Unit laboratory (Bahri Dağdaş International Agricultural Research Institute, Türkiye) to assess eggshell quality. Eggshell breaking strength was quantified utilizing the Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel), applied to the blunt part of the egg. Eggshell thickness was measured at three points (equator, blunt, and pointed ends) using a micrometer (Mitutoyo, 0.01 mm, Japan) and averaged.

After assessing the external quality characteristics of the eggs, they were carefully cracked open on a sanitized glass surface, and any residue on the eggshell was meticulously removed. Yolk colour was assessed using a yolk colour fan (F. Hoffmann-La Roche Ltd., Basel, Switzerland). All subsequent procedures followed the methodologies outlined by Sarmiento-García et al. (2024). The relative eggshell weight was determined by precisely weighing the cleaned and dried shells and dividing this weight by the corresponding egg weights. Following this, the albumen was separated from the yolk. The heights of the albumen and yolk were measured using a height gauge, and their length and width were recorded using a digital caliper with a precision of 0.01 mm (Mitutoyo, Japan).

The albumin index was then calculated using the formula (1):

$$Albumen\ index = \frac{albumen\ height\ (mm)}{\frac{albumen\ width + albumen\ length}{2}\left(mm\right)}\ x\ 100 \quad (1)$$

For determining yolk index, the following formula (2) was applied:

$$Yolk\ index = \frac{height\ of\ yolk\ (mm)}{diameter\ of\ yolk\ (mm)}\ x\ 100 \quad (2)$$

The Haugh unit was then calculated using the egg weight and albumen height data, following the formula (3) outlined by Stadelman and Cotterill (1995).

$$Haugh\ unit = 100\ x\log\left(albumen\ height + 7.57 - 1.7\ x\ egg\ weight^{0.37}\right) \quad (3)$$

Mineral content analysis of the manure and serum samples

To determine mineral content in the excreta, two birds per subgroup (n=60) were temporarily housed in cages measuring 70 x 70 x 40 cm for one day, specifically between days 35 and 40 of the experiment. Each bird was individually placed in these cages to facilitate the collection of droppings samples. Once sufficient droppings had been gathered, the birds were transferred back to their original cages. The collected manure samples were stored at -20°C until analysis. The mineral content was determined using a wet digestion method as described by Olgun et al. (2024). The analysis was performed using ICP-OES equipped with a Thermo Scientific 7200 analyser (Thermofisher Scientific, Waltham, United States).

On day 40, blood samples were taken from the jugular vein of each chukar in the treatment groups. Samples were drawn into coagulation tubes and processed immediately. The samples were centrifuged at 4,000 × g for 10 minutes to separate the serum. The separated serum was stored at -20°C until mineral content analysis. The analysis utilized the same ICP-OES equipment and method as previously described.

Formal analysis

Data from the study were subjected to statistical analysis using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). The cage of animals served as the experimental unit for performance parameters, while individual animals were used as the experimental unit for all other assessed parameters. Shapiro Wilk's test was used to assess the normality of errors in this study. The effects of TEO supplementation on performance parameters, egg quality, and mineral content were analysed using a one-way analysis of variance (ANOVA). When significant differences were found ($p<0.05$), means were compared using the Duncan Multiple Comparison Test. The results were expressed as mean values with their standard errors of the mean (mean ± SEM). Statistical significance was defined as a probability value of $p <0.05$, while a value of $p < 0.10$ indicated a trend

Results

Performance and egg production

All animals survived until the conclusion of the study. The results obtained from incorporating TEO into the diet of chukar partridges are presented in Table 2. Thyme essential oil supplementation had no significant effect on performance parameters ($p>0.05$), including final body weight, weight gain, feed intake, and FCR. However, notable differences were observed in egg parameters based on the level of TEO supplementation. In comparison to both the control group and the group supplemented with 100 mg/kg TEO, the inclusion of the lowest TEO dosage (50 mg/kg) in the chukar diet led to increased egg production ($p<0.01$), with similar results observed at higher TEO doses (150 and 200 mg/kg). Similarly, chukar receiving the lowest TEO dosage (50 mg/kg) exhibited higher egg mass compared to the control group, while intermediate values were observed for the remaining TEO dosage levels assessed.

Table 2. The effect of different dietary thyme essential oil levels on the performance of chukar partridge (n=90).

Parameters	Dietary thyme essential oil supplementation (mg/kg)					
	0	**50**	**100**	**150**	**200**	***p-value***
Initial body weight	540.83±8.30	540.83±6.70	538.75±9.20	542.50±11.30	530.25±7.60	0.872
Final body weight	452.50±15.90	457.20±16.50	474.90±14.80	446.00±28.90	436.20±23.30	0.854
Body Weight Gain	-88.30 ±15.30	-83.60±12.11	-63.90±14.81	-96.50±31.50	-61.10±24.51	0.703
Feed intake	36.57±1.60	37.00±2.60	37.58±0.80	35.82±1.60	37.23±1.30	0.960
Feed conversion ratio	6.52±0.20	5.89±0.40	6.52±0.10	5.97±0.30	6.05±0.30	0.528
Egg production.	29.07^{c} ±0.50	32.22^{a} ±0.80	29.25^{bc} ±0.30	31.66^{abc} ±0.60	31.85^{ab} ±0.70	0.003
Egg mass	5.60^{b} ±0.10	6.29^{a} ±0.10	5.76^{ab} ±0.10	6.02^{ab} ±0.20	6.18^{ab} ±0.10	0.001

Initial body weight, final body weight, and body weight gain are expressed as g. Feed intake is expressed as g/ day/chukar. The feed conversion ratio is expressed as g feed/g egg. Egg production is expressed as a percentage. The egg mass is expressed as g/day/chukar.
^{a,b,c}: Means within a row lacking a common superscript differ ($p < 0.05$).

Egg quality parameters

As can be observed in Table 3, no differences in egg weight were observed among the experimental groups, although a higher eggshell ratio was detected in the group receiving 150 g/kg TEO compared to the control and the group supplemented with 200 g/kg TEO. Interestingly, chukar eggshell thickness was significantly reduced ($p<0.05$) in groups receiving 200 mg/kg TEO and 100 mg/kg TEO compared to the other experimental groups and the control. However, egg-breaking strength was not affected ($p>0.05$).

Table 3. The effect of different dietary thyme essential oil levels on egg quality parameters (n=240) of Chukar partridge.

Parameters	Dietary thyme essential oil supplementation (mg/kg)					
	0	**50**	**100**	**150**	**200**	***p-value***
Egg weight	19.28±0.20	19.55±0.20	19.70±0.10	18.99±0.30	19.39±0.10	0.295
Eggshell ratio	9.58^{b} ±0.10	10.04^{ab} ±0.10	9.79^{ab} ±0.10	10.35^{a} ±0.10	9.63^{b} ±0.10	0.007
Eggshell thickness	0.257^{a} ±0.002	0.266^{a} ±0.002	0.254^{b} ±0.006	0.266^{a} ±0.003	0.255^{b} ±0.003	0.046
Eggshell breaking strength	2.44±0.08	2.38±0.12	2.81±0.11	2.49±0.11	2.56±0.09	0.474
Albumin İndex	5.23±0.20	5.14±0.20	4.58±0.10	5.19±0.20	5.49±0.30	0.209
Yolk İndex	40.65^{ab} ±2.30	49.53^{a} ±2.10	38.85^{b} ±1.60	41.31^{ab} ±1.80	43.84^{ab} ±2.90	0.023
Haugh Unit	68.82±1.10	68.59±1.00	67.50±0.50	69.42±0.90	70.78±1.20	0.277
Egg Shape index	75.35±0.90	74.03±0.80	74.58±0.40	75.54±0.70	74.89±1.00	0.352
Yolk colour score	6.60^{ab} ±0.10	6.40^{b} ±0.20	6.90^{a} ±0.20	6.50^{ab} ±0.10	6.50^{ab} ±0.10	0.008

The parameters above the line correspond to the external parameters of the egg. Parameters below the line correspond to internal egg parameters. Egg weight is expressed as g. Eggshell ratio is expressed as a percentage. Eggshell thickness is expressed as µm. Egg-breaking strength is expressed as kg/cm^2.
^{a,b}: Means within a row lacking a common superscript differ ($p < 0.05$).

Regarding egg internal parameters, neither the Haugh unit, albumin index, nor egg shape index were influenced ($p>0.05$) by TEO supplementation. Differences were observed in the yolk index, which increased ($p<0.05$) in chukars supplemented with the lowest doses of TEO compared to those supplemented with 100 mg/kg, while the other experimental groups (including the control) showed similar values.

Egg yolk colour received a significantly higher score ($p<0.01$) when chukars received the lowest TEO dosage (50 mg/kg) compared to those receiving 100 mg/kg of TEO. Intermediate values were observed for the remaining doses (150 and 200 mg/kg) and the control group.

Faecal mineral content

The faecal analysis of six minerals (Table 4) revealed comparable values ($p>0.05$) between the control group and those supplemented with TEO, irrespective of the dosage. Zinc consistently emerged as the predominant mineral excreted, followed by calcium and copper, across all experimental conditions.

Table 4. The effect of different dietary thyme essential oil levels on faecal mineral contents (n=60) of chukar partridge.

Parameters (mg/kg)	Dietary thyme essential oil supplementation (mg/kg)					
	0	50	100	150	200	*p-value*
Calcium	72.10±4.04	85.99±3.27	85.23±4.02	82.78±4.54	74.65±6.71	0.149
Phosphorus	2.68±0.26	2.94±0.38	3.34±0.25	2.96±0.09	3.35±0.28	0.339
Copper	16.29±0.33	16.34±0.99	14.78±0.50	15.14±1.14	16.70±0.68	0.368
Magnesium	5.23±0.26	5.68±0.17	5.65±0.18	4.97±0.24	5.08±0.12	0.056
Manganese	34.15±4.54	37.33±6.61	29.64±3.41	34.28±6.01	33.93±3.93	0.879
Zinc	125.50±15.95	122.90±16.81	122.60±20.48	134.40±17.11	149.30±18.30	0.803

Serum mineral content

Table 5 shows that the most abundant mineral in blood serum regardless of the experimental group were iron, followed by copper and zinc. No significant differences ($p>0.05$) were observed in the levels of the nine minerals analysed in serum between the control group and the groups supplemented with TEO. However, a trend ($p<0.1$) was observed for copper, which decreased numerically from the control group to the group receiving 150 mg/kg of TEO. However, a numerical increase was detected for 200 mg/kg dose.

Table 5. The effect of different dietary thyme essential oil levels on blood serum mineral contents of Chukar partridge (n=60)

Parameters (mg/L)	Dietary thyme essential oil supplementation (mg/kg)					
	0	50	100	150	200	*p-value*
Calcium	1.63±0.10	1.55±0.09	1.65±0.10	1.72±0.11	1.46±0.09	0.430
Phosphorus	0.29±0.04	0.29±0.06	0.30±0.06	0.23±0.04	0.30±0.05	0.891
Cooper	9.01±0.55	8.31±0.25	7.70±0.58	6.77±0.46	8.15±0.56	0.051
Zinc	4.26±0.36	3.95±0.28	4.50±0.50	5.12±0.48	4.64±0.25	0.320
Iron	28.75±1.82	23.58±1.87	24.89±1.53	25.63±1.73	27.43±3.18	0.457
Magnesium	0.14±0.007	0.13±0.006	0.13±0.006	0.14±0.007	0.14±0.008	0.857
Manganese	0.53±0.16	0.40±0.17	0.64±0.13	0.77±0.05	0.49±0.22	0.557
Sodium	3.30±0.05	3.20±0.08	3.27±0.06	3.52±0.32	3.31±0.13	0.687
Potassium	0.31±0.02	0.29±0.02	0.27±0.01	0.28±0.02	0.33±0.06	0.594

Discussion

Performance parameters and egg production

Essential oils derived from herbs are generally believed to positively influence feed consumption due to their aromatic properties, which positively affect performance parameters (Gholami-Ahangaran et al., 2022; Puvača et al., 2022; Yesilbag et al., 2022). Despite this, our current research demonstrated that TEO did not impair developmental parameters, including feed intake. No statistically significant differences were observed between the experimental and control groups in final body weight, weight gain, or FCR. These results are consistent with the findings of Bala et al. (2021), who reported similar outcomes in laying hens fed with TEO. Wade et al. (2021) also observed no significant differences in performance parameters of broilers fed with TEO compared to control groups, which aligns with the results described by Ding et al. (2017) for laying hens when a mixture of essential oils was added to the diet. These results may be attributed to the fact that healthy, unstressed poultry kept in optimal conditions often do not show a response to performance-enhancing supplements (Torki et al., 2021). Akbari et al. (2016) hypothesise that the antioxidant properties of TEO are likely more effective under stress conditions, such as extreme temperatures. Thyme essential oil protects pancreatic tissue against oxidative damage, potentially aiding proper pancreatic function, including the secretion of digestive enzymes and improved nutrient digestibility. The effects of TEO could be more pronounced in compromised health conditions or stressful situations. In contrast, Zaazaa et al. (2022) found that while TEO did not impair feed intake in broiler production, it did modify FCR and body weight gain. Authors suggested that improvements in production parameters might be more evident when increased TEO doses, ranging from 300 to 1000 mg/kg, are supplied to the animals.

While no significant differences in body weight gain were observed between the groups, the results indicate a trend of decreased weight gain across all treatments. This reduction, however, is a well-known trade-off in high-performing laying birds. During peak egg production, nutrients and energy are often redirected from somatic growth towards reproductive functions (Olgun et al., 2014). This physiological shift is particularly evident in the 50 mg/kg TEO group, where egg production was significantly improved. Thus, although body weight gain was lower, the prioritisation of reproductive output aligns with the expected physiological adaptations in laying partridges, where maintaining optimal egg production takes precedence over growth.

These findings suggest that TEO supplementation at 50 mg/kg enhances production efficiency without compromising overall health. The improvements in egg production can be attributed to key components found in TEO, such as thymol and carvacrol, known for their ability to enhance nutrient absorption and thereby improve egg production (Gholami-Ahangaran et al., 2022). Since the chukars were at their peak production, a period of maximum nutritional demand, it is plausible that the benefits of TEO supplementation were more evident during this time.

However, it has been described that increased concentrations of carvacrol or thymol have negative effects on intestinal epithelial cells and beneficial gut bacteria, potentially compromising feed utilization in laying birds (Feng et al., 2021) It is suggested that dietary supplementation of TEO may offer beneficial effects at optimal levels (e.g., 50 mg/kg). However, adverse effects at higher concentrations (>50 mg/kg) could potentially overshadow these benefits, as indicated by the current findings.

Previous reports on the effects of including EO in laying diets on egg production parameters are unclear. For instance, Bozkurt et al. (2009) found that EO supplementation significantly increased the egg production rate and egg weight in laying hens, while egg mass, feed intake, and FCR were unaffected. Similarly, Akbari et al. (2016) described that laying hens fed a diet supplemented with peppermint and thyme oils exhibited a lower FCR and higher egg production and mass than the control diet. However, in the same research, those authors found no differences in egg production parameters when only TEO was added to the diet. Similar results were observed by Feng et al. (2021) when oregano EO was added to the laying hens' diet, aligning with findings by Ding et al. (2017) and Büyükkılıç Beyzi et al. (2020) who added a mixture of EO to the layers diet. Variations among studies may arise from several factors, such as the type and concentration of essential oil used, the composition of the oil itself, differences in feed formulations, the genetic lineage of the birds, and the age of the animals involved (Ding et al., 2017).

Egg quality parameters

Eggshell quality remains a critical concern in avian production, often leading to significant economic losses in the industry (Akbari et al., 2016; Olgun et al., 2024). In this study, egg weight was not affected by TEO supplementation. In contrast, the eggshell ratio reached the maximum value at 150 and 50 mg/kg levels compared to the control and 200 mg/kg groups. Conversely, the eggshell thickness was reduced in the 100 mg/kg and 200 mg/kg groups compared to the control and the rest of the experimental groups, although this did not impair egg-breaking strength. The differential effects of TEO on egg quality parameters may be due to dose-dependent variations in nutrient absorption, calcium metabolism, and hormonal regulation. The findings of the current research suggest that moderate doses of TEO might optimize egg metabolism processes, enhancing egg weight and shell weight, while higher doses could disrupt calcium utilisation, reducing shell thickness without affecting egg-breaking strength. However, researchers have not reached a consensus on the effects of EO supplementation on egg quality in layer diets. For example, contrary to our results Akbari et al. (2016) described an increase in eggshell thickness from laying hens fed a diet containing a combination of EOs under cold conditions compared to those fed the control diet. These authors suggest that while the mechanism underlying this association remains incompletely understood, one plausible reason could be the impact of essential oils on alkaline phosphatase. This effect could prevent calcium storage in bones, providing more calcium for eggshell formation, and subsequently increasing shell thickness. However, the same authors showed that when TEO was individually assessed at 100 mg/kg, no differences were observed in eggshell thickness or eggshell weight compared to the control which is in line with those described by previous authors (Büyükkılıç Beyzi et al., 2020; Torki et al., 2021). According to Büyükkılıç Beyzi et al. (2020), variations in results were mainly attributed to the diversity of essential oils and their components, the levels employed in diets, the breed types, and the experimental conditions.

The albumin index, Haugh unit, and egg shape index remained unaffected by TEO supplementation. However, the yolk index peaked at the 50 mg/kg TEO level compared to the 100 mg/kg TEO group. Essential oils enhance uterine health, increase calcium storage, and stimulate pancreatic secretions, improving nutrient digestion (Abdel-Wareth & Lohakare, 2020). This leads to better eggshell and egg quality. These findings reaffirm previous results, identifying 50 mg/kg as the optimal dose of TEO for chukar, likely due to enhanced nutrient absorption at this specific concentration. Nevertheless, the effect on egg internal quality parameters of including EO in the layers diet is unclear. For example, Akbari et al. (2016) observed effects on the Haugh unit when TEO was combined with peppermint, but no differences were recorded when both essential oils were provided individually. On the opposite, Abdel-Wareth & Lohakare (2020) demonstrated a linear decrease in yolk percentage as a result of including peppermint oil. However, these authors observed that eggshell percentage and thickness, albumin height, and Haugh unit exhibited a consistent increase in laying hens aged 38 and 44 weeks. As described above, the age range, the birds used, and, above all, the essential oil included in the diet and the dose are responsible for the variations between studies (Büyükkılıç Beyzi et al., 2020).

Mineral content

The poultry industry has prioritized nutritional strategies aimed at minimizing mineral residues in faeces, which pose environmental risks (Olgun et al., 2024). The findings of this study indicate that there were no notable differences in faecal mineral concentrations among the experimental groups. The lack of significant variation in faecal mineral content across different TEO dosages suggests that TEO could be a viable dietary supplement that does not exacerbate environmental mineral pollution. To our knowledge, only one study has evaluated the effect of including EOs on mineral excretion (Olgun & Yıldız, 2014). These authors proposed that higher levels of EOs (400 and 600 mg/kg) might lead to reduced ash and mineral excretion compared to lower dosage groups. The discrepancy between these studies could be attributed to several factors. Differences in the type and concentration of essential oils used, the duration of the studies, the specific diets and conditions under which the birds were raised, and the inherent variability in how different species metabolise and excrete minerals could all contribute to the observed variations. Nevertheless, further research is needed to understand these differences fully and to determine the most effective and environmentally friendly levels of EO supplementation for avian diets.

In our investigation, the levels of minerals in the blood were observed to fall within the optimal physiological range for avian species (Olgun et al., 2024) and no differences were reported based on the TEO dosage, suggesting an optimal physiological state for all animals. These findings align with those described by Adewole et al. (2021) who included different TEO concentrations in broiler nutrition, and with the findings of Çiftçi et al. (2018) for chukar partridge. Conversely, Cengiz et al. (2015) reported that serum calcium levels were significantly higher in groups receiving 100 mg/kg fennel essential oil and a 400 mg/kg thyme, rosemary, and fennel essential oil mixture compared to the control and vitamin E groups. The discrepancies between studies could be attributed to several factors, including variations in EO types and concentrations, differences in study duration, the specific diets and conditions under which the birds were raised, and species-specific metabolic and excretory differences. Further research should explore the long-term effects of TEO on mineral metabolism and its potential interactions with other dietary components to elucidate the mechanisms involved fully.

Essential oils are promising candidates in avian nutrition. This study has demonstrated that TEO at a concentration of 50 mg/kg significantly improved egg production, and egg mass without impairing performance parameters or serum and faecal mineral composition. These beneficial effects are likely due to the active components of TEO, particularly thymol, and carvacrol, which could enhance nutrient utilisation. However, higher concentrations of TEO did not yield further improvements and, in some cases, led to a decrease in some of them including eggshell thickness, and yolk index suggesting a potential negative impact on nutrient absorption at elevated doses. Hence, these findings suggest that 50 mg/kg TEO is an optimal dosage for enhancing certain parameters of chukar partridges. Nevertheless, further research is recommended to understand the underlying mechanisms of the TEO effects.

Ethical approval: The Ethics Committee of Bahri Dağdaş International Agricultural Research Institute approved all processes adopted in the current experiment. The animal ethics approval Decision was NO: 77. Moreover, for the entirety of the trial, strict adherence to the guidelines established in the European Animal Protection Policy (EPCEU, 2010) was maintained.

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