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## Influence of dietary protein levels and some cold pressed oil supplementations on productive and reproductive performance and egg quality of laying Japanese quail

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**ABSTRACT:** The present study was carried out to examine the effect of dietary protein levels (18, 20, and 22%) and addition of cold-pressed oils (no addition, 1 g thyme, 1 g oregano, and 0.5 g thyme + 0.5 g oregano /kg diet) on the productive and reproductive performance and egg quality of Japanese quail at the laying period. 324 mature ten-week-old Japanese quails (216 females and 108 males) were used. Quails were randomly distributed to 12 treatment groups, 27 in each group, with 3 replicates of 9 quails (6 females and 3 males) per group. Feed conversion ratio (FCR) significantly improved in the layers fed with the diets containing 20 or 22% CP in all experimental periods, except in 3- to 4-month-old birds. The diets supplemented with thyme only or interaction with oregano improved FCR significantly compared to the control or oregano -administered group in all experimental periods, except in 2- to 3-month-old birds. Dietary supplementation with thyme oil caused the egg number and egg mass to increase significantly in all experimental periods compared to the other dietary groups. Fertility percentages were significantly higher in the groups which had oregano than in the control group. Dietary supplementation with thyme oil significantly increased the egg number ( $P<0.05$ ) and egg mass ( $P<0.01$ ) in all experimental periods compared to other dietary groups. Fertility and hatchability percentages were not significantly affected due to varying dietary protein levels throughout the experiment. In conclusion, the quails fed with a diet containing 20 to 22% CP or supplemented with oregano or thyme oil exhibited an improved productive and reproductive performance without any detrimental impacts on the other parameters studied.

**Keywords:** Cold pressed oil, Protein, Performance, Production, Reproduction, Quails

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## INTRODUCTION

Nutritional costs account for about 65-70% of the total costs of poultry production (FAO, 2006; Alagawany et al., 2016). Protein is one of the most important and expensive nutrients in the rations of poultry - so much so that its quality and quantity are considered as the main limiting factors to the productivity and efficiency of poultry. Shrivastav et al. (1994) concluded that laying quail performed well when given 19% dietary protein and 2750 kcal ME/kg and when early diets contained 24 and 20% CP in the starter and finisher periods, respectively. The protein and energy requirements of grower and layer Japanese quail have been reported under different conditions (Alagawany et al., 2014a,b; Reda et al., 2015; Reda et al., 2020).

The effects of some aromatic medicinal plants and their extracts and cold pressed oils on broiler activity have been studied, and the addition of feeds or water has been found to improve the feed intake (FI), and feed conversion ratio (FCR) (Alagawany and Abd El-Hack, 2015; Alagawany et al., 2017). Thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare*) and their effective components improve the activity of digestive enzymes such as protease, amylase and lipase, which results in a reduced nutrient digestibility (Abd El-Hack and Alagawany, 2015; Badiri and Saber, 2016). Additionally, these grasses of the Labiatae family are used to improve digestion and absorption. These effects are able to increase the length of intestine and the depth and width of villi, which creates better good conditions for dietary absorption. Nonetheless, while there are abundant studies on thyme essential oils, there is limited knowledge about the effects of this plant alone.

Phytogenic feed additives such as thyme played an active role in forming chelates with metal ions and preventing or decreasing the oxygen formation (Dharma et al., 2015; Mohamed et al., 2017). Regarding the combination of protein and herbal oils, the quails fed with a diet containing 24% or 26% CP or the diets enriched with oregano or thyme oils or both exhibited an improvement in performance, antioxidant capacity, and immunity (Mohamed et al., 2019). But, in the literature, there is a limited number of studies in this regard. So, this study aimed to examine the effects of supplementation with dietary protein levels and cold-pressed oils (thyme and oregano) on the productive and reproductive performance and egg quality of Japanese quail in the production stage.

## MATERIALS AND METHODS

### *Experimental Design and Animal Husbandry*

completely random design with factorial arrangement (3×4) was performed to reveal the effect of three levels of CP (18, 20, and 22%, Table 1) with the addition of cold-pressed oils (no addition, 1 g/kg thyme, 1 g/kg oregano and 0.5 g/kg thyme + 0.5 g/kg oregano) on the productive and reproductive indices and egg quality criteria of laying Japanese quail in the production stage. The oil types were purchased from the company named "El Hawag for Natural Oils", Cairo, Egypt. A total of 324 mature ten-week-old Japanese quails (216 females and 108 males) were used. Quails were randomly distributed into 12 treatment groups, 27 in each group, with 3 replicates of 9 quails (6 females and 3 males) per group. Birds were housed in a naturally ventilated house measuring 5×3m<sup>2</sup> equipped with laying cages measuring 144×70×30cm<sup>3</sup>. All birds were reared under similar managerial and hygienic conditions at a temperature of 35-38°C and a humidity of about 60-80%. Feed and water were provided *ad libitum* throughout the experiment.

The diets were formulated according to NRC (1994) and the required amount of cold pressed oil supplement for each diet was initially blended with vegetable oil of the diet, then mixed with small amount of the diet, and finally mixed with the remaining of the diet. The cumulative and per-period average daily feed intake (FI), body weight gain (BWG), and FCR were calculated using these data. Feed lost was recorded every day, and the information was used to estimate the feed utilization. Animal care and maintenance were performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the Guide for the Care and Use of Laboratory Animals (2011).

### *Data Collection*

FI was recorded weekly, calculated as grams of diet consumed per 7 days, and divided by the number of birds in each replicate group. On the other hand, the feed conversion of feed (g feed/ g egg) was calculated as the rate of the feed intake to the egg mass. The eggs were weighed and the egg number was counted every day to measure the egg mass (egg number × egg weight) (Alagawany et al., 2014). The egg characteristics parameters were recorded monthly using three eggs from each replicate. The external and internal egg quality characteristics (yolk percentages, albumen, and shell; shape index of egg (ESI); thickness of shell; unit surface shell weight (USSW), and Haugh unit) were indicated according to Romanoff and Romanoff (1949).

**Table 1.** Ingredient and nutrient contents of experimental diets of laying Japanese quail.

| Items                    | Crude protein levels (%) |       |       |
|--------------------------|--------------------------|-------|-------|
|                          | 18                       | 20    | 22    |
| Ingredient (%)           |                          |       |       |
| Yellow corn              | 65.20                    | 58.70 | 53.00 |
| Soybean meal 44%         | 22.80                    | 27.50 | 31.00 |
| Corn gluten meal 62 %    | 3.50                     | 4.60  | 6.22  |
| Vegetable oil            | 0.8                      | 1.8   | 2.46  |
| Limestone                | 5.5                      | 5.54  | 5.52  |
| Di-calcium phosphate     | 1.27                     | 1.20  | 1.20  |
| Salt                     | 0.30                     | 0.30  | 0.30  |
| Premix*                  | 0.30                     | 0.30  | 0.30  |
| L-Lysine%                | 0.18                     | 0.06  | 0.00  |
| DL-Methionine %          | 0.07                     | 0.00  | 0.00  |
| Total                    | 100                      | 100   | 100   |
| Calculated composition** |                          |       |       |
| ME, Kcal /Kg             | 2902                     | 2901  | 2900  |
| Crude protein %          | 18.00                    | 20.00 | 22.00 |
| Calcium %                | 2.50                     | 2.50  | 2.50  |
| Nonphytate P %           | 0.35                     | 0.35  | 0.35  |
| Lysine %                 | 1.00                     | 1.00  | 1.03  |
| TSAA %***                | 0.70                     | 0.70  | 0.77  |

\*Layer Vitamin-mineral Premix, Each 1Kg consists of vit. A, 8000 IU ;vit. D3, 1300 ICU, vit. E 5 mg ; vit. K, 2 mg ; vit B1 ,0.7 mg; vit. B2, 3 mg; vit. B6, 1.5 mg; vit. B12, 7 mg; Biotin 0.1 mg; Pantothenic acid , 6 g; Niacine, 20 g ; Folic acid, 1 mg , manganese , 60 mg ; Zinc, 50 mg, Copper , 6 mg ; Iodine, 1 mg , Selenium, 0.5 mg ; Cobalt, 1mg

\*\*Calculated according to NRC {30}.

\*\*\*TSAA: Total sulfur amino acids

### Fertility and hatchability

At the end of first, second, and third month of each experimental period, 20 eggs were collected from each replicate. The eggs were then incubated at 37.6°C and 65% RH in an automatic incubator and turned 45° every 1 h. Beginning on the 14<sup>th</sup> day of incubation, the eggs were maintained at 37.5°C and 70% RH without turning until hatching. After hatching, chicks were counted and unhatched eggs were broken to determine the percentages of fertility and hatchability. The hatchability was expressed as hatched chicks from the total egg set. Fertility and hatchability percentages were calculated as follows: fertility percentage = (number of fertile eggs / total eggs set) × 100; hatchability percentage = (number of hatched chicks / total number of fertile eggs) × 100; hatchability percentage = (number of hatched chicks / total eggs set) × 100 (Alagawany and Attia, 2015).

### Statistical analysis

The data of productive and reproductive performance as well as egg quality criteria were analyzed with a generalized linear model using the normal distribution and the identity link function (SAS Institute Inc., 2001). The model used included the protein lev-

els and cold pressed oils, as well as the interaction effects:

$$Y_{ijk} = \mu + A_i + S_j + AS_{ij} + e_{ijk},$$

Where  $Y_{ijk}$  = an observation,  $\mu$  = the overall mean,  $A_i$  = effect of protein level ( $j = 18, 20$  and  $22\%$ ),  $S_j$  = effect of cold pressed oils (0, 1 g/kg thyme, 1 g/kg oregano and 0.5 g/kg thyme + 0.5 g/kg oregano),  $AS_{ij}$  = interaction effect between CP levels and cold pressed oils supplementation ( $j = 1, 2 \dots$  and  $12$ ) and  $e_{ijk}$  = random error. The differences between means were calculated using the *post hoc* Tukey's test. The statistical significance was set at  $P < 0.05$  unless otherwise stated.

## RESULTS AND DISCUSSION

### Feed intake and Feed Conversion Ratio

The average FI and FCR of Japanese quails were affected by dietary protein values, cold pressed oils and their mixture in the laying period, which is presented in Table 2. The different protein levels did not have a significant effect on FI in all experimental periods, except in 3- to 4-month-old birds; while feeding the laying quails with a diet containing 22% CP

increased FI significantly compared to the diets with lower CP content. The FCR significantly improved in the layers fed with the diets containing 20 or 22% CP in all experimental periods, except in 3- to 4-month-old birds in which the dietary protein levels did not significantly affect the FCR. These results were in line with the results of Abd-Elsamee et al. (2001) who showed that the average FI values did not differ significantly depending on the increasing dietary CP. On the other hand, the lowest feed intake values were observed in the groups that took low levels of CP. The protein consumption and protein efficiency ratio differ significantly depending on strain, protein level, and age (Hammouda et al., 2001). Alagawany et al. (2011) asserted that the FI in 20- to 30-week-old laying hens improved and the feed conversion ratio in 22- to 34-week-old hens at the laying period also improved when the birds were fed with a diet containing 20% CP compared to the hens fed with the diets containing 16% and 18% CP. Protein efficiency rates were significantly higher for the 16% and 18% CP diets than that for the 20% CP diet in almost all the experimental periods. These results were in line with the results of Dean et al. (2006) who reported a reduced gain/feed ratio when CP levels were below 22%.

The results presented in Table 2 show that FI showed no significant response to cold pressed oils throughout the entire experimental period. The diets supplemented with thyme only or interaction with oregano improved the FCR significantly compared to the control or oregano-administered group in all experimental periods, except in 2- to 3-month-old birds

in which the herbal oil supplementation had no significant effect on FCR. Sengul et al. (2008) found that supplementation with thyme oil significantly lowered the feed intake compared to the antibiotic-supplemented and control diets. These results may have stemmed from the good taste of the phenolic components in thyme oil. Parlat et al. (2005) reported that the thyme oil supplementation improved FCR. Oils of plant origin can stimulate the activity of enzymes and improve the absorption of feed, and thyme oil has a toxin binding effect and effectively diminishes the detrimental effects of aflatoxin on performance (Parlat et al., 2005). Also, Osman et al. (2010) reported that chicks fed with the basal diets containing 0.5 g/kg rosemary, oregano or sweet basil supplementation consumed less food and had an improved FCR ( $P < 0.05$ ) compared to the control. This may be due to the ability of oregano to increase the efficiency of digestion by increasing saliva, the amount of digestive enzymes; quieting the stomach and digestive system; improving craving; curing or preventing basic intestinal infections; and relieving diarrhea and constipation, thereby maximizing the benefit of feed without increasing FI (Badiri and Saber, 2016). The interaction between dietary protein levels and herbal oil supplementation had no significant effects on FI and FCR in any experimental period, except for the effects on FCR during the whole laying period (2- to 5-month-old birds), when the layers fed with a diet containing 22% CP supplemented with thyme oil. In this combination, FCR improved significantly compared to the other combinations.

**Table 2.** Feed intake and average feed conversion ratio ( $n=3$ ) of laying Japanese quails as affected by dietary protein levels, cold pressed oils and their interaction during the laying periods (2-5 months of age).

| Items  | Feed intake (g) |                         |            |            | Feed conversion ratio (g feed/ g egg) |                        |                         |                         |
|--|-----------------|-------------------------|------------|------------|---------------------------------------|------------------------|-------------------------|-------------------------|
|  | 2-3 months      | 3-4 months              | 4-5 months | 2-5 months | 2-3 months                            | 3-4 months             | 4-5 months              | 2-5 months              |
| <b>Dietary protein level</b>                     |                 |                         |            |            |                                       |                        |                         |                         |
| 18   | 30.09±0.28      | 32.16±0.35 <sup>b</sup> | 30.83±0.34 | 31.03±0.25 | 3.22±0.03 <sup>a</sup>                | 3.12±0.06              | 3.19±0.05 <sup>a</sup>  | 3.17±0.03 <sup>a</sup>  |
| 20   | 30.11±0.25      | 32.02±0.27 <sup>b</sup> | 30.97±0.33 | 31.03±0.21 | 3.02±0.05 <sup>b</sup>                | 2.97±0.06              | 2.90±0.06 <sup>b</sup>  | 2.96±0.02 <sup>b</sup>  |
| 22   | 30.27±0.31      | 33.36±0.38 <sup>a</sup> | 31.56±0.22 | 31.37±0.21 | 3.05±0.05 <sup>b</sup>                | 2.96±0.10              | 2.89±0.08 <sup>b</sup>  | 2.96±0.07 <sup>b</sup>  |
| <b>Cold pressed oils (g/kg diet)<sup>1</sup></b> |                 |                         |            |            |                                       |                        |                         |                         |
| 0.0  | 30.01±0.26      | 32.65±0.33              | 31.41±0.31 | 31.36±0.26 | 3.14±0.043                            | 3.27±0.06 <sup>a</sup> | 3.16±0.08 <sup>a</sup>  | 3.19±0.05 <sup>a</sup>  |
| 1 Org  | 30.33±0.32      | 32.73±0.48              | 30.88±0.35 | 31.32±0.30 | 3.09±0.07                             | 3.07±0.08 <sup>b</sup> | 3.04±0.07 <sup>ab</sup> | 3.06±0.05 <sup>b</sup>  |
| 1 Thy  | 30.01±0.30      | 32.73±0.43              | 31.14±0.37 | 31.29±0.23 | 3.03±0.06                             | 2.88±0.06 <sup>c</sup> | 2.83±0.08 <sup>c</sup>  | 2.91±0.06 <sup>c</sup>  |
| 0.5 Org + 0.5 Thy                                | 30.28±0.41      | 31.95±0.47              | 31.05±0.42 | 31.09±0.35 | 3.14±0.06                             | 2.85±0.08 <sup>c</sup> | 2.95±0.09 <sup>bc</sup> | 2.97±0.05 <sup>bc</sup> |
| <b>Probabilities</b>                             |                 |                         |            |            |                                       |                        |                         |                         |
| Dietary protein                                  | N.S             | *                       | N.S        | N.S        | **                                    | N.S                    | **                      | **                      |
| Cold pressed oils                                | N.S             | N.S                     | N.S        | N.S        | N.S                                   | **                     | **                      | **                      |
| Interaction                                      | N.S             | N.S                     | N.S        | N.S        | N.S                                   | N.S                    | N.S                     | *                       |

Means in the same column within each classification bearing different letters are significantly different. \*\*( $P \leq 0.01$ ), \*( $P \leq 0.05$ ) and NS = not significant.

<sup>1</sup>Org = oregano; Thy = Thyme



**Table 3.** Average egg number and egg weight (n=3) of laying Japanese quails as affected by dietary protein levels, cold pressed oils and their interaction during the laying periods (2-5 months of age).

| Items                                      | Average egg number (h/month) |                         |                         |                         | Average egg weight (g) |                         |                         |                         |
|--|------------------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|
|  | 2-3 months                   | 3-4 months              | 4-5 months              | 2-5 months              | 2-3 months             | 3-4 months              | 4-5 months              | 2-5 months              |
| Dietary protein level                      |                              |                         |                         |                         |                        |                         |                         |                         |
| 18   | 21.04±0.14 <sup>b</sup>      | 22.83±0.27 <sup>b</sup> | 21.90±0.13 <sup>b</sup> | 65.78±0.30 <sup>c</sup> | 12.42±0.06             | 12.68±0.09 <sup>b</sup> | 12.37±0.13 <sup>b</sup> | 12.49±0.04 <sup>b</sup> |
| 20   | 22.19±0.27 <sup>a</sup>      | 23.15±0.24 <sup>b</sup> | 23.00±0.27 <sup>a</sup> | 68.35±0.46 <sup>b</sup> | 12.66±0.05             | 13.07±0.12 <sup>a</sup> | 13.04±0.18 <sup>a</sup> | 12.90±0.07 <sup>a</sup> |
| 22   | 22.36±0.28 <sup>a</sup>      | 24.14±0.62 <sup>a</sup> | 23.07±0.54 <sup>a</sup> | 69.57±1.37 <sup>a</sup> | 12.46±0.07             | 13.16±0.11 <sup>a</sup> | 13.34±0.12 <sup>a</sup> | 12.99±0.06 <sup>a</sup> |
| Cold pressed oils (g/kg diet) <sup>1</sup> |                              |                         |                         |                         |                        |                         |                         |                         |
| 0.0  | 21.52±0.20 <sup>b</sup>      | 21.78±0.23 <sup>c</sup> | 21.70±0.38 <sup>c</sup> | 64.10±0.63 <sup>d</sup> | 12.45±0.03             | 12.87±0.16              | 12.86±0.22              | 12.73±0.12              |
| 1 Org                                      | 21.44±0.35 <sup>ab</sup>     | 23.15±0.37 <sup>b</sup> | 22.22±0.18 <sup>c</sup> | 67.31±0.63 <sup>c</sup> | 12.57±0.79             | 12.92±0.13              | 12.85±0.22              | 12.80±0.10              |
| 1 Thy                                      | 22.39±0.39 <sup>a</sup>      | 24.48±0.50 <sup>a</sup> | 23.67±0.46 <sup>a</sup> | 70.54±1.28 <sup>a</sup> | 12.42±0.78             | 13.04±0.14              | 13.05±0.21              | 12.84±0.90              |
| 0.5 Org + 0.5 Thy                          | 21.61±0.32 <sup>b</sup>      | 24.09±0.33 <sup>a</sup> | 23.04±0.42 <sup>b</sup> | 68.74±0.86 <sup>b</sup> | 12.53±0.09             | 13.06±0.12              | 12.88±0.22              | 12.82±0.10              |
| Probabilities                              |                              |                         |                         |                         |                        |                         |                         |                         |
| Dietary protein                            | **                           | **                      | **                      | **                      | NS                     | **                      | **                      | **                      |
| Cold pressed oils                          | **                           | **                      | **                      | **                      | NS                     | NS                      | NS                      | NS                      |
| Interaction                                | **                           | **                      | **                      | **                      | NS                     | NS                      | NS                      | NS                      |

Means in the same column within each classification bearing different letters are significantly different. \*\*( $P \leq 0.01$ ), \*( $P \leq 0.05$ ) and NS = not significant.

<sup>1</sup>Org = oregano; Thy = Thyme

### Egg Production

The results in the Tables 3 and 4 show an increase in egg number and egg mass for the 2- to 3-month-old and 4- to 5-month old birds fed with the diets containing 20 and 22% CP compared to the birds fed with 18% CP. Moreover, the egg number and egg mass significantly and gradually increased as the dietary CP levels increased, up to 22%. The egg weight significantly improved in the birds fed with the diets containing 20 and 22% CP in all experimental periods ( $P < 0.01$ ), except in the 2- to 3-month-old birds, compared to those fed with the diets supplemented with 18% CP (Tables 3 and 4). Alagawany et al. (2011) found that the egg number in 26- to 30-week-old birds and the egg mass in 22- to 34-week-old laying hens increased significantly ( $P < 0.01$ ) in response to the diets containing 20 and 18% CP compared to the birds fed with diets containing 16% CP. These results were in line with Novak et al. (2006) who found that the egg production decreased by 2% in response to feeding with a low-protein diet, whereas the same values were recorded for the same characteristics in the hens fed with high and medium levels of protein diets. According to Bunchasak et al. (2005), the dietary protein levels did not affect egg production, but the diets with higher protein levels (16 and 18% CP) tended to yield a good egg production percentage than the diets with 14% CP. In the study in question, the hens fed with 16 and 18% CP diets yielded a significantly higher egg mass than those fed with a 14% CP diet due to heavier egg weight.

The results in the Tables 3 and 4 reveal that the

dietary supplementation with thyme oil significantly increased the egg number and egg mass in all experimental periods compared to the other dietary groups. On the other hand, the egg weight did not differ significantly in response to the cold pressed oils supplementation in all experimental periods. According to Botsoglou et al. (2005), the volatile components in the extracts may cause the hens fed a diet with thyme or its extracts to give heavier eggs. It was reported that carvacrol and thymol in thyme oil increased the nutrition metabolism in hepatocytes and could also improve the activity of antioxidants (Liu, 2011). Radwan et al. (2008) reported that adding 0.5% oregano to the feed of laying hens increased both egg weight and egg mass. Furthermore, the power of this effect increased significantly as the level of oregano was increased to 1.0%. The pure components of essential oils were found to reduce the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CO A) reductase, which is a regulatory enzyme in the synthesis of cholesterol (Crowell, 1999). Ali et al. (2007) reported that adding thyme to hens' feed increased their egg number. However, Cetingul et al. (2009) indicated that egg weight was not significantly affected when oregano was added to the diets of quails. Regarding the combination between dietary protein values and herbal oil, it could be concluded that a diet with 22% CP and supplemented with thyme would produce the highest egg number and egg mass. In contrast, egg weight showed no significant response to the interaction between dietary protein levels and herbal oil supplementation.

**Table 4.** Average egg mass and percentages of fertility (n=60) of laying Japanese quails as affected by dietary protein levels, cold pressed oils and their interaction during the laying periods (2-5 months of age).

| Items                                      | Average egg mass          |                          |                           |                           | Percentage of fertility  |                         |                          |                          |
|--|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
|  | 2-3 months                | 3-4 months               | 4-5 months                | 2-5 months                | 2-3 months               | 3-4 months              | 4-5 months               | 2-5 months               |
| Dietary protein level                      |                           |                          |                           |                           |                          |                         |                          |                          |
| 18   | 261.30±1.47 <sup>b</sup>  | 289.57±4.10 <sup>c</sup> | 271.00±3.53 <sup>b</sup>  | 821.63±5.24 <sup>c</sup>  | 81.94±1.73 <sup>b</sup>  | 84.03±1.61              | 81.25±1.49               | 82.40±1.29               |
| 20   | 279.67±3.67 <sup>a</sup>  | 302.73±4.64 <sup>b</sup> | 299.86±5.92 <sup>a</sup>  | 881.69±6.19 <sup>b</sup>  | 88.19±1.91 <sup>a</sup>  | 84.72±2.01              | 78.47±2.04               | 83.79±1.77               |
| 22   | 278.68±3.50 <sup>a</sup>  | 318.10±9.74 <sup>a</sup> | 307.93±8.21 <sup>a</sup>  | 904.13±19.63 <sup>a</sup> | 87.50±1.92 <sup>a</sup>  | 85.41±1.81              | 79.86±2.16               | 84.26±1.50               |
| Cold pressed oils (g/kg diet) <sup>1</sup> |                           |                          |                           |                           |                          |                         |                          |                          |
| 0.0  | 268.06±3.03 <sup>b</sup>  | 280.13±3.80 <sup>c</sup> | 279.23±6.92 <sup>c</sup>  | 827.46±11.40 <sup>d</sup> | 81.48±2.31 <sup>b</sup>  | 80.55±1.96 <sup>b</sup> | 75.00±1.96 <sup>b</sup>  | 79.01±1.47 <sup>c</sup>  |
| 1 Org                                      | 275.10±5.40 <sup>a</sup>  | 299.30±6.65 <sup>b</sup> | 285.70±6.05 <sup>bc</sup> | 860.86±13.67 <sup>c</sup> | 83.33±1.96 <sup>b</sup>  | 82.40±1.67 <sup>b</sup> | 80.55±2.78 <sup>ab</sup> | 82.01±1.80 <sup>bc</sup> |
| 1 Thy                                      | 278.16±5.30 <sup>a</sup>  | 319.30±9.40 <sup>a</sup> | 309.46±9.77 <sup>a</sup>  | 906.35±21.53 <sup>a</sup> | 91.67±1.96 <sup>a</sup>  | 89.81±1.85 <sup>a</sup> | 85.18±1.85 <sup>a</sup>  | 88.89±0.92 <sup>a</sup>  |
| 0.5 Org + 0.5 Thy                          | 270.67±3.92 <sup>ab</sup> | 314.85±6.17 <sup>a</sup> | 297.30±9.47 <sup>ab</sup> | 881.92±15.30 <sup>b</sup> | 87.04±1.46 <sup>ab</sup> | 86.11±1.39 <sup>a</sup> | 78.70±1.46 <sup>ab</sup> | 83.95±0.77 <sup>b</sup>  |
| Probabilities                              |                           |                          |                           |                           |                          |                         |                          |                          |
| Dietary protein                            | **                        | **                       | **                        | **                        | *                        | N.S                     | N.S                      | N.S                      |
| Cold pressed oils                          | *                         | **                       | **                        | **                        | **                       | *                       | *                        | **                       |
| Interaction                                | **                        | *                        | NS                        | **                        | N.S                      | N.S                     | N.S                      | N.S                      |

Means in the same column within each classification bearing different letters are significantly different. \*\*( $P \leq 0.01$ ), \*( $P \leq 0.05$ ) and NS = not significant.

<sup>1</sup>Org = oregano; Thy = Thyme

### Fertility and Hatchability Percentage

The results shown in the Tables 4 and 5 reveal that the fertility percentage, hatchability percentage from the total egg set and the hatchability percentage from fertile eggs did not vary significantly in response to varying dietary protein levels in all periods. However, increasing the dietary protein level to 20 or 22% CP significantly increased the fertility percentage and hatchability percentage from the total egg set in 2- to 3-month-old birds. Alagawany et al. (2014a) found that the high fertility percentages were observed in the quails fed with a diet having a reduced level of CP, followed by those fed with the same diet supplemented with valine. In contrast, a diet containing 16% CP supplemented with an amino acid mixture resulted in the lowest fertility value in the first trial period, but no differences were observed in fertility percentage in 4- to 5-month-old birds or during the overall feeding period. The same results were also found by Zeweil et al. (2011). In contrast, no differences ( $P > 0.05$ ) were detected in the hatchability percentage of total eggs or that of fertile eggs in the other periods.

The results presented in the Tables 4 and 5 show that the dietary supplementation of thyme alone or in combination with oregano improved the fertility percentage significantly in all experimental periods, except in the whole period (2- to 5-month-old birds),

when the thyme supplementation produced the highest fertility percentage, followed by the supplementation with oregano alone or in combination with thyme, compared to the control group. The hatchability percentage from the total eggs set significantly improved in all experimental periods, except in 4- to 5-month-old birds, when dietary thyme oil produced the highest hatchability percentage from total eggs set, compared to the other groups. In contrast, hatchability percentage from fertile eggs did not vary significantly in response to dietary cold pressed oils supplementation (Tables 5 and 6).

Fertility percentages were significantly higher in the groups which had oregano than in the control group. The increase in fertile eggs in the oregano-treated groups could be due to a significant increase in sperm motility, live sperm percentage, ejaculate volume, sperm concentration, total sperm/ejaculate, and total live sperm/ejaculate compared to those in the control group (Daghighi et al., 2016; Alagawany et al., 2018, 2020a,b; Mbaye et al., 2019). Additionally, hatchability percentages (hatchability of total and fertile eggs) were significantly higher in oregano groups than in the control group. This increase may be due to the increased egg shell thickness in most treated groups compared to the control group (Soliman et al. 2016).

**Table 5.** Average percentages of hatchability from total eggs set and hatchability from fertile eggs (n=60) of laying Japanese quails as affected by dietary protein levels, cold pressed oils and their interaction during the laying periods (2-5 months of age).

| Items  | Percentage of hatchability from total eggs set |                          |                         |                          | Percentage of hatchability from fertile eggs |            |            |            |
|--|--|--------------------------|-------------------------|--------------------------|--|------------|------------|------------|
|  | 2-3 months                                     | 3-4 months               | 4-5 months              | 2-5 months               | 2-3 months                                   | 3-4 months | 4-5 months | 2-5 months |
| <b>Dietary protein level</b>                     |  |                          |                         |                          |  |            |            |            |
| 18   | 68.75±1.49 <sup>b</sup>                        | 71.53±2.16               | 68.75±2.32              | 69.68±1.47               | 84.08±1.74                                   | 85.19±2.31 | 84.43±1.69 | 84.57±1.33 |
| 20   | 76.39±2.01 <sup>a</sup>                        | 72.92±2.08               | 67.36±1.91              | 72.22±1.64               | 86.71±1.68                                   | 86.41±2.63 | 86.41±2.81 | 86.51±1.99 |
| 22   | 75.00±2.29 <sup>a</sup>                        | 71.53±2.40               | 66.67±2.05              | 71.06±1.98               | 85.90±2.41                                   | 83.90±2.72 | 83.99±3.00 | 84.60±2.07 |
| <b>Cold pressed oils (g/kg diet)<sup>1</sup></b> |  |                          |                         |                          |  |            |            |            |
| 0.0  | 68.52±1.85 <sup>b</sup>                        | 66.67±1.96 <sup>c</sup>  | 62.04±2.02 <sup>b</sup> | 65.74±1.22 <sup>c</sup>  | 84.46±2.74                                   | 83.14±3.18 | 83.46±4.19 | 83.68±2.61 |
| 1 Org  | 69.45±1.39 <sup>b</sup>                        | 68.52±1.85 <sup>bc</sup> | 67.59±2.17 <sup>b</sup> | 68.52±1.30 <sup>bc</sup> | 83.69±2.74                                   | 83.64±3.60 | 84.33±2.83 | 83.89±2.64 |
| 1 Thy  | 79.63±2.82 <sup>a</sup>                        | 77.78±1.96 <sup>a</sup>  | 74.07±2.17 <sup>a</sup> | 77.16±1.29 <sup>a</sup>  | 86.84±2.20                                   | 86.82±2.55 | 87.17±2.71 | 86.94±1.34 |
| 0.5 Org + 0.5 Thy                                | 75.92±1.67 <sup>a</sup>                        | 75.00±2.40 <sup>ab</sup> | 66.67±1.39 <sup>b</sup> | 72.53±1.57 <sup>a</sup>  | 87.27±1.53                                   | 87.07±2.33 | 84.81±1.72 | 86.39±1.44 |
| <b>Probabilities</b>                             |  |                          |                         |                          |  |            |            |            |
| Dietary protein                                  | **   | N.S                      | N.S                     | N.S                      | N.S  | N.S        | N.S        | N.S        |
| Cold pressed oils                                | *  | **                       | **                      | **                       | N.S  | N.S        | N.S        | N.S        |
| Interaction                                      | N.S  | N.S                      | N.S                     | N.S                      | N.S  | N.S        | N.S        | N.S        |

Means in the same column within each classification bearing different letters are significantly different. \*\*( $P \leq 0.01$ ), \*( $P \leq 0.05$ ) and NS = not significant.

<sup>1</sup>Org = oregano; Thy = Thyme

**Table 6.** External and internal egg quality characteristics (n=9) of laying Japanese quails as affected by dietary protein levels, cold pressed oils and their interaction during the whole laying period (2-5 months of age).

| Items  | External egg quality |                      |                  |   |              | Internal egg quality |                          |              |                           |
|--|----------------------|----------------------|------------------|---|--------------|----------------------|--------------------------|--------------|---------------------------|
|  | Shell percent        | Shell thickness (mm) | ESI <sup>1</sup> | USSW (mg/cm <sup>2</sup> ) <sup>2</sup> | Yolk percent | Albumen percent      | Albumen height (mm)      | Yolk index   | Haugh Unit                |
| <b>Dietary protein level:</b>                    |                      |                      |                  |   |              |                      |                          |              |                           |
| 18   | 11.46 ± 0.09         | 0.237 ± 0.002        | 78.08 ± 0.51     | 47.59 ± 0.05                            | 30.88 ± 0.39 | 57.70 ± 0.40         | 4.43 ± 0.10 <sup>c</sup> | 46.64 ± 0.41 | 88.16 ± 0.56 <sup>b</sup> |
| 20   | 11.45 ± 0.10         | 0.240 ± 0.003        | 77.11 ± 0.79     | 47.90 ± 0.08                            | 31.79 ± 0.67 | 56.80 ± 0.68         | 4.67 ± 0.06 <sup>b</sup> | 46.65 ± 0.36 | 89.27 ± 0.36 <sup>b</sup> |
| 22   | 11.78 ± 0.21         | 0.242 ± 0.001        | 77.19 ± 0.35     | 48.09 ± 0.08                            | 31.70 ± 0.47 | 56.56 ± 0.56         | 4.94 ± 0.09 <sup>a</sup> | 47.45 ± 0.57 | 90.60 ± 0.48 <sup>a</sup> |
| <b>Cold pressed oils (g/kg diet)<sup>3</sup></b> |                      |                      |                  |   |              |                      |                          |              |                           |
| 0.0  | 11.96 ± 0.23         | 0.239 ± 0.003        | 78.23 ± 0.35     | 47.65 ± 0.09 <sup>b</sup>               | 31.17 ± 0.49 | 56.91 ± 0.55         | 4.66 ± 0.11              | 46.98 ± 0.52 | 89.43 ± 0.57              |
| 1 Org  | 11.56 ± 0.10         | 0.242 ± 0.002        | 76.64 ± 0.48     | 48.02 ± 0.11 <sup>a</sup>               | 31.20 ± 0.70 | 57.27 ± 0.76         | 4.84 ± 0.10              | 46.88 ± 0.64 | 90.14 ± 0.51              |
| 1 Thy  | 11.35 ± 0.12         | 0.236 ± 0.003        | 77.46 ± 0.92     | 47.86 ± 0.07 <sup>a</sup>               | 31.49 ± 0.77 | 57.19 ± 0.82         | 4.45 ± 0.12              | 46.31 ± 0.49 | 88.04 ± 0.66              |
| 0.5 Org + 0.5 Thy                                | 11.39 ± 0.13         | 0.239 ± 0.003        | 77.51 ± 0.77     | 47.91 ± 0.12 <sup>a</sup>               | 31.95 ± 0.45 | 56.71 ± 0.52         | 4.76 ± 0.12              | 47.49 ± 0.45 | 89.77 ± 0.63              |
| <b>Probabilities</b>                             |                      |                      |                  |   |              |                      |                          |              |                           |
| Dietary protein                                  | NS                   | NS                   | NS               | NS                                      | NS           | NS                   | **                       | NS           | **                        |
| Cold pressed oils                                | NS                   | NS                   | NS               | *                                       | NS           | NS                   | NS                       | NS           | NS                        |
| Interaction                                      | NS                   | NS                   | NS               | NS                                      | NS           | NS                   | NS                       | NS           | NS                        |

Means in the same column within each classification bearing different letters are significantly different. \*\*( $P \leq 0.01$ ), \*( $P \leq 0.05$ ) and NS = not significant.

<sup>1</sup>ESI = egg shape index; <sup>2</sup>USSW = unit surface shell weight

<sup>3</sup>Org = oregano; Thy = Thyme

Radwan et al. (2008) who showed that the use of 1% oregano to hens' feed significantly increased (by 8.23%) the percentages of fertility in comparison to those in the hens fed with control diets. This improvement can be explained by the fact that oregano, which has antioxidant activities, decreased malondialdehyde (MDA) formation in egg yolk and, consequently, improved the semen characteristics. The addition of 1% thyme to hens' diets significantly increased the percentages of hatchability in comparison to those of

the hens fed with control diets for fertile fresh eggs. The addition of thyme to hens' diets significantly increased the percentages of fertility and hatchability of eggs compared to those of the hens fed with control diets. This improvement in hatchability in response to thyme supplementation can be explained by the effect of thyme on decreasing plasma total lipid (TL), and consequently, the lipid and oxidized compounds pass to egg (Ali et al., 2007). The results presented in the Tables 4 and 5 reveal that the dietary herbal



oil supplementation had no significant effects on the fertility percentage or hatchability percentage from total egg set or the hatchability percentage from fertile eggs due to the interaction between protein levels and herbal oils.

### Egg Quality

The results presented in the Table 6 revealed that no egg quality parameters were significantly affected by dietary protein levels, except Haugh unit and albumen height. Where, increasing the dietary protein level to 22% increased the Haugh unit and albumen height in comparison to the low CP level. In this respect, the increases in CP did not improve the Haugh unit score, egg shell percentage or shell thickness (Junqueira et al., 2006; Alagawany, 2012). No significant effect was observed on all egg quality characteristics due to herbal oil supplementation, except USSW. Ali et al. (2007) found that the addition of thyme insignificantly increased the percentage of ESI, shell thickness, and shell weight compared to those in the birds fed with a free diet. The addition of 0.5% oregano in the diets of laying hen insignificantly decreased the albumen weight percentage and increased the yolk weight percentage. The addition of 1.0% thyme had a significant effect additionally; the yolk index percentage was significantly higher in the birds fed with the diet having 0.5-1.0% oregano (Radwan et al., 2008).

Additionally, Ali et al. (2007) found that the use of thyme increased the percentage of shell weight and

the thickness of shell compared to those of the hens fed with a control diet. Because thyme is known as an antioxidant, it may improve the environment of the uterus (site of calcium deposition) and also increase the shell weight and shell thickness. On the other hand, Arpasova et al. (2013) suggested that no egg yolk quality parameters (egg yolk weight (g) and egg yolk index) were significantly influenced by oregano oil. In the present study, none of the studied external egg quality characteristics were significantly influenced by the combination between dietary protein traits and herbal oil supplementation in any experimental laying period.

### CONCLUSION

In the present study, it was found that the quails fed with a diet containing 20 to 22% CP or supplemented with oregano or thyme oil had improved productive and reproductive performances without any detrimental impacts on the other parameters studied. It could be concluded that a diet with 22% CP and supplemented with thyme would produce the highest egg number and egg mass. Dietary supplementation of thyme alone or in combination with oregano improved the fertility percentage significantly in all experimental periods, except in the whole period (2- to 5-month-old birds).

### CONFLICT OF INTEREST STATEMENT

The author has declared that no competing interest exists.

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