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RESEARCH ARTICLE

Effects of Thyme-supplemented Diet on Laying Hens Eggs' Shape, Volume and Surface Area

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ABSTRACT

In the study, a mathematical model with bare minimum variables that may easily be interpreted with least-squares calculations was presented for the egg size assessments to investigate the effects of thyme supplementation on the geometrical properties of laying hens' eggs. A total of 120 24-week-old Lohmann-LSL (initial live body weight = 1350 g ± 80 g) hens were used in the study. The experimental groups received different concentrations (0.1 %, 0.5 %, and 1 %) of thyme supplementation, while the control group was fed with the standard laying hens ration for 30 days. Two eggs from each sub-group (10 eggs per the main groups) were collected weekly to evaluate the eggs' geometrical properties. All collected eggs were digitally photographed, and the measurements concerning egg volume, length, width, and surface area were performed on the digital images at the end of week 4. Overall, data revealed a significant decrease (p =0.009) in the egg length with the highest thyme concentration. The study was designed as a method modeling in an attempt to provide contributions to the further studies to be carried out with higher concentrations of thyme or other phytogenic additives to monitor the potential effects of the relevant supplements on the eggs' metrics.

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Introduction

Geometrical properties of eggs are utilized in various fields, ranging from ecological, physiological, and morphological studies concerning birds to assessing prospective chick yield in the poultry industry (Troscianko, 2014). Egg size is estimated due to egg mass and volume, and linear measurements, which are generally converted to mass and volume values through various geometrical and regression equations to compare egg size. All these methods have their inconveniences (Hoyt 1979; Boersma and Rebstock, 2010). Egg mass measurement is a reliable tool for assessing egg size; however, an extended shelf life alters the egg mass due to gradual water loss, which renders the measurements reliable only for the freshly harvested products (Troscianko, 2014). Field-fabricated egg volume measurements are not eligible and thus quite rarely applied.

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Photographic measurement methods to assess egg volume are evaluated based on the distance between the camera and the egg and the shooting angle by processing the images captured in a comparative approach on the software program (Boersma and Rebstock, 2010). The photography techniques utilized for egg measurements offer several advantages. Digital cameras are easily accessible and affordable devices that enable a rapid, relatively laborless in situ imaging of eggs with the least casualty (Bridge et al., 2007; Troscianko, 2014).

Measurement of egg surface area, which plays a significant role in eggshell permeability, is rather complicated due to the variety in eggs' sizes of different species. For instance, owl eggs are spherical, while seagull eggs are pointy at one end. The eggshell permeability is a constituent of utmost importance that enables heat, metabolic gasses, water vapor exchange, and nutrient transfer for the eggs' developing embryos (Paganelli et al., 1974; Bridge et al., 2007). Egg shape fitting methods to calculate egg size are considered advantageous; however such methods require further and laborious egg breadth measurements at different distances, although they offer reliable matching for the eggs commonly found in nature (Monus and Barta, 2005).

Thyme, as an ancient plant belonging to the family Lamiaceae (Farsani et al., 2016). ranks among the healing herbs widely used in the food and drug industry. (Yalcin el.,2020). Thymus vulgaris contains 1% to 2.5 % essential oils, a monoterpene mixture, including thymol and its isomer carvacrol (Hosseinzadeh et al., 2015). It has long been utilized for medical purposes due to the outdrawn antimicrobial effects associated with its thymol and carvacrol content (Helander et al., 1998; Gholijani and Amirghofran 2016). Moreover, thyme and thyme based compounds were shown to elicit antifungal, anti-inflammatory, and antioxidant properties (Bukovska et al. 2007; Gholijani and Amirghofran 2016, Yalçin et al., 2020).

To the best of our knowledge, there is no study available concerning the geometrical properties of eggs in chicken fed on thyme supplemented diet despite a large number of studies that investigated the effects of thyme supplementation on egg quality parameters (Bolukbasi and Erhan, 2007; Ghasemi et al., 2010; Abd El-Hack and Alagawany, 2015; Yalcin et al., 2020). Intrinsic and extrinsic egg quality parameters were evaluated in such studies. Bolukbasi et al. (2008) indicated that supplementation of the basal diet with essential oils of phytobiotic properties, including thyme oil, significantly increased eggshell quality when compared with the control. Furthermore, the thyme oil supplemented group revealed the highest ratios. In another study (Abd El-Hack and Alagawany, 2015), thyme supplementation was shown to have affected all egg quality parameters investigated. Yalçın et al. (2000) indicated the statistical insignificancy of the effects of thyme-supplemented diet on the egg quality parameters. On the other hand, Manafi et al. (2016) and Vakili and Majidzadeh Heravi (2016) showed that thyme supplementation improved the eggshell quality.

In the study, a mathematical model with as minimum variables as possible (Troscianko, 2014) was presented to painlessly calculate the egg size by the least-squares method to investigate the effects of thyme supplementation on eggs' metrics.

Materials and Methods

Laying Hens and Experimental Design

A total of one hundred and twenty 24-week-old, Lohmann brown-classic laying hens obtained from a commercial company, were used in the study. All hens were allowed four weeks to adapt before the experiment. Afterward, they were randomly divided into four groups, each containing 30 hens. Each group was replicated five times, with six birds per pen. Thyme leaves provided from a commercial company (MBD Yem San. Tic. Ltd. Şti.) were initially powdered and then added to the ration at concentrations of 0.1%, 0.5%, 1% by the chicken feed manufacturing company. The experimental groups received thyme supplemented (at different concentrations) basal diet (Bolukbasi and Erhan, 2007), while the control group was fed on the standard commercial laying hens feed for 30 days. The amount of feed to be consumed by a hen per day was calculated according to Lohmann Company's instructions (Lohmann Tierzucht GmbH, 2008). Water was provided *ad libitum*.

Content of the Rations

The composition of the laying hens' diet was shown in Table 1. Thyme powder was supplied from a commercial feed manufacturer (MBD Yem San. Tic. Ltd. Şti.). The trial feeds' chemical analyses were performed according to the instructions established by the AOAC (1984), and the results were given in Table 2.

Table 1. Composition of the ration given to the hens

Feedstuff	%
Corn	28.00
Soybean meal (SBM)	15.091
Sunflower seed meal (SFM)	12.959
Wheat	38.00
Marble dust	5.00
L-Lysine	0.12
DL-Methionine	0.136
Salt	0.30
Vitamin-Mineral Premix*	0.20
Vegetable oil	0.194

Vitamin and trace mineral complexes provided the followings (per kg of the diet): Vitamin B1, 3 mg; vitamin B2, 7.5 mg; D-pantothenic acid, 25 mg; vitamin B6, 5 mg; vitamin B12, 0.002 mg; biotin, 0.5 mg; niacin, 25 mg; vitamin K3, 1.25 mg; folic acid, 1.5 mg; choline chloride, 750 mg; cobalt, 1.2 mg; copper, 8.8 mg; zinc, 84 mg; manganese, 106 mg; iron, 44 mg; iodine, 1.2 mg; and selenium, 0.15 mg.vitamin A (retinyl palmitate), 15.000 IU; vitamin D3, 2.500 IU; vitamin E (DL-α-tocopheryl acetate), 20 mg;

Table 2. Nutrient content (%) and energy levels (ME, MJ/kg feed) of the rations

Experimental Groups	Moisture %	Dry matter %	Crude protein %	Crude fat %	Crude fibre %	Ash %	Nitrogen-free core matter* %	Starch	Sugar	Calcium %	Utilizable phosphorus %	ME, MJ/kg feed**
Control	12.6	87.4	16.18	2.03	4.8	7.7	56.69	42.17	42.17	41.09	41.52	42.5
0.1 % thyme	11.92	88.08	16.55	2.17	4.83	8.18	56.35	41.09	41.09	41.09	41.52	42.5
0.5 % thyme	12.4	87.6	16.06	2.1	5.13	7.75	56.56	41.52	41.52	41.09	41.52	42.5
1 % thyme	11.97	88.03	16.17	2.08	4.99	7.81	56.98	42.5	42.5	41.09	41.52	42.5

* Nitrogen-free core matter*, % = Dry matter, % - (crude protein, % + crude fat, % + crude fiber, % + ash, %)

** ME, MJ/kg feed = (0.03431 x crude fat, g/kg) + (0.01551 x crude protein, g/kg) + (0.01669 x starch, g/kg) + (0.01301 x sugar, g/kg).

The Composition of the Standard Basal Diet given to all Hens in the Study

The rations were formulated to be isocaloric and isonitrogenous by feed optimization software, assuring that it contained 16 % crude protein and 10.65 MJ/kg metabolizable energy. The common ingredients in all rations included corn, sunflower seed meal (SFSM), wheat, soybean meal (SBM).

Assessment of Nutrient and Energy Contents of the Rations

Chemical analyses of experimental rations were carried out following the instructions proposed by the AOAC (1984). Nitrogen free core matter contents and metabolizable energy levels (ME, MJ/kg feed) of the rations were calculated based on the data derived by chemical analyses, as follows (Kırchgessner, 1997):

ME, MJ/kg feed = (0.03431 x crude fat, g/kg) + (0.01551 x crude protein, g/kg) + (0.01669 x starch, g/kg) + (0.01301 x sugar, g/kg).

CAGES and Housing Conditions of Hens

All hens were housed in commercial stainless steel batteries, consisting of wire mesh cages (width = 100 cm, length = 70 cm, height = 75 cm), equipped with a feeder and nipple drinkers. A total of 1166 cm^2 space was provided per chicken, including the nest box and the perch. The housing conditions (e.g., light, temperature, and ventilation) were maintained according to the laying hens (Lohmann brown) breeding standards of the Lohmann Company (Lohmann Tierzucht GmbH, 2008).

Measurement of Egg Shape

Two eggs were collected from each sub-group (10 eggs from each group) per week. The eggs were digitally photographed with a specific shooting angle using a ruler, and the weekly digital images were stored (iPhone 7, 750*1334px, Apple) (Figure 1). At the end of week 4, egg volume, size, and surface area were calculated on the open-source digital imaging software, "Image J" (Schneider et al., 2012; Schindelin et al., 2015) with the "Egg shape tool" (Troscianko, 2014). A mathematical model with as minimum variables as possible, which could easily be interpreted by the least-squares method, was applied in the study to measure eggs' metrics (Figure 2).

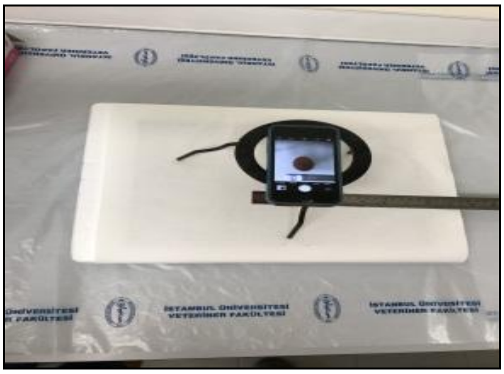
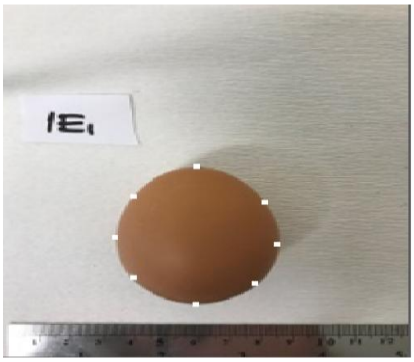
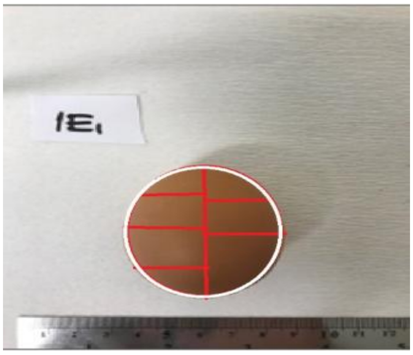


Figure 1. Digital imaging of the eggs





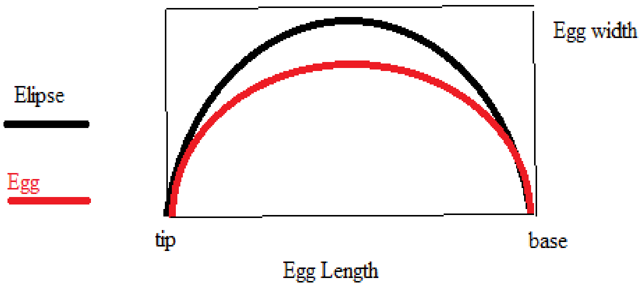


Figure 2. Elliptical egg shape

It is considered that egg shape formation mostly depends on the pressure it is exposed to along the oviduct, which tends to be spherical other than elliptical. The model applied in the study to calculate the eggs' geometrical properties was based on the assumption that pressure was applied across the egg's surfaces along its length in a normal elliptical distribution.

The egg's total width, pressure distribution, and the vertex values of pressure distribution along the egg's length were obtained. The least-squares method was applied to determine the egg's metrics by designating at least three additional measurement points located at the basis, apex, and the egg's two side margins. After the egg's curvatures were determined, egg size, volume, and surface area were presumptively calculated on a circular cross-section basis (Troscianko, 2014). The egg volumes were measured using the least-squares approach on Image J software (Schneider et al., 2012; Schindelin et al., 2015) in a comparative fashion with the species-specific egg size coefficients proposed by Hoyt (1979).

Statistical Analyses

Data collected from all groups were analyzed via Shapiro-Wilk tests to check for normal distribution. Normally distributed parameters were compared by one-way ANOVA and non-normal distributions were subjected to Kruskal Wallis non-parametric tests. Bonferroni correction was applied for non-normally distributed parameters, and overall data of the groups were compared via Mann Whitney U tests. The statistical significance level was established as P = 0.05. SPSS software program (SPSS for Windows, version 11.5.2.1) was used for the statistical analyses (SPSS, 2003).

Results and Discussion

Egg length was reduced (p =0.009) in the group that received 1 % thyme than those of the other groups. On the other hand, thyme supplementation failed to affect egg width, egg surface area, and volume (p > 0.05). In the study, egg length was significantly reduced with the highest thyme supplementation (p =0.009). Similarly, other parameters, such as egg width, volume, and surface area, are inclined to have reduced with the increasing thyme concentrations despite the nominal statistical values. The relevant significant differences (Table 3) in the egg length were considered to be associated with the escalated mineral and protein absorption due to the organic acid content of the thyme included in the laying hens' rations (Soltan, 2008). A few and mostly contradictory studies are available concerning the effects of phytogenic additives on the egg quality of laying hens. Organic acid combinations used in the quails' diet were demonstrated to have induced more elongated and widened egg formations and thickened eggshell with more enlarged surface area rendering them resistant to breakage (Manafi et al., 2016). The egg size index assessed by measuring eggs' metric values is calculated as the egg width's proportional value to the egg length (Abd El-Hack and Alagawany, 2015). Egg length, width, and the egg size index determined based on these two parameters are considered external quality parameters (Sandikci Altunatmaz et al., 2020). In a study (Abd El-Hack and Alagawany, 2015), the egg size index was shown to have increased with 6 g · kg–1 thyme supplementation in the laying hens. On the other hand, Mohebbifar and Torki (2010) observed no significant differences in the egg quality parameters with the thyme-supplemented basal diet. In a recent study, different concentrations (1 % and 2%) of thyme supplementation in the diet were shown to have no effect on the egg quality parameters (Yalcın et al., 2020). Essential oils contained in thyme were reported to have altered eggshell composition. Likewise, Akbari et al. (2016) demonstrated in a study carried out with laying hens that a thyme oil and mint oil-supplemented diet provided calcium for eggshell formation due to the chemical interaction of essential oils with the alkaline phosphatase enzyme. Trace elements and essential oils, in particular, were noted to have played a crucial role in increasing the eggshell thickness (Akbari et al., 2016).

Table 3. Effect of thyme supplementation on the geometrical properties of eggs

Group	Egg length (mm)		Egg width (mm)		Egg volume mm^3		Egg surface area mm^2	
	Means	SE	Means	SE	Means	SE	Means	SE
Control	63.01^a	0.25	49.19	0.31	79265.65	1261.18	9009.25	94.1
0.1 % Thyme	63.07^a	0.45	49.19	0.27	79222.76	1366.66	9005.41	103.72
0.5 % Thyme	63.02^a	0.43	49.55	0.36	80445.65	1652.69	9095.53	123.38
1 % Thyme	61.44^b	0.32	48.82	0.27	76257.03	1035.47	8767.8	81.42
P -value	0.009		0.613		0.173		0.273	

[a, b]: There is a significant difference between the means indicated with different letters on the same column ($p<0.05$).

Conclusion

Based on comprehensive data, it can be deduced that a thyme-supplemented diet in the laying hens solely impacted the egg length among the other geometrical properties of eggs. The study was designed as a modeling method for egg size assessments using digital photographic images, which is deduced to have elicited substantial data for future research with the potential effects of higher concentrations of thyme or other phytogenic additives on the geometrical properties of laying hens' eggs.

Compliance with Ethical Standards

a) Authors' Contributions

Initials of Author 1: Designed the study, performed the laboratory work, interpreted data, and drafted the paper.

b) Acknowledgments

This work was supported by the Research Fund of Istanbul University (Project number: BAP 23747).

c) Conflict of Interest

The authors declare that there is no conflict of interest.

d) Statement on the Welfare of Animals

Ethical approval was obtained from the Animal Care and Ethics Committee of the Institute of Istanbul University (28/11/2016-35980450-050.01.04).

e) Statement of Human Rights

This study does not involve human participants.

References

Abd El-Hack, M.E., and Alagawany, M., 2015. Performance, egg quality, blood profile, immune function, and antioxidant enzyme activities in laying hens fed diets with thyme powder. *Journal of Animal and Feed Sciences,* 24(2):127-133. https://doi.org/10.22358/jafs/65638/2015

Akbari, M., Torki, M., and Kaviani, K., 2016. Single and combined effects of peppermint and thyme essential oils on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition (6.8±3 °C). *International Journal of Biometeorology,* 60(3): 447-454. https://doi.org/10.1007/s00484-015-1042-6

AOAC., 1984. *Official Methods of Analysis,* 14th ed. Association of Official Agricultural Chemist. Washington, DC.

Boersma, P.D., and Rebstock, G., 2010. Calculating egg volume when shape differs: when are equations appropriate? *Journal of Field Ornithology,* 81(4): 442–448.

https://doi.org/10.1111/j.1557-9263.2010.00300.x

Bolukbasi, Ş.C., Erhan, M.K., and Kaynar, O., 2008. The effect of feeding thyme, sage and rosemary oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces. *Archive for poultry science,* 72(5): 231- 237.

Bolukbasi, S.C., and Erhan, M.K., 2007. Effect of dietary thyme (*Thymus vulgaris*) on laying hens performance and Escherichia coli (E. coli) concentration in feces. *International Journal of Natural and Engineering Sciences,* 1(2): 55-58.

Bridge, E.S., Boughton, R.K., Aldridge, R.A., Harrison, T.J.E., Bowman, R., and Schoech, S.J., 2007. Measuring egg size using digital photography: testing Hoyt's method using Florida Scrub Jay eggs. *Journal of Field Ornithology,* 78(1): 109–116.

https://doi.org/10.1111/j.1557-9263.2006.00092.x

Bukovska, A., Cikos, S., Juhas, S., Ilkova, G., Rehak, P., and Kop-pel, J., 2007. Effects of a combination of Thyme and Oregano essential oils on TNBS-induced colitis in mice. *Mediators of Inflammation,* 2007: 1-9. https://doi.org/1-9. 10.1155/2007/23296

Farsani M.S., Behbahani, M., and H.Z. Isfahani (2016). The effect of root, shoot and seed extracts of the Iranian Thymus L (family: lamiaceae) species on HIV-1 replication and CD4 expression. *Cell Journal (Yakhteh),* 18(2): 255-261.

Ghasemi R., Zarei, M., and Torki, M., 2010. Adding medicinal herbs including garlic (allium sativum) and thyme (Thymus vulgaris) to diet of laying hens and evaluating productive performance and egg quality characteristics. *American Journal of Animal and Veterinary Sciences,* 5(2): 151-154.

Gholijani, N., and Amirghofran, Z., 2016. Effects of thymol and carvacrol on T-helper cell subset cytokines and their main transcription factors in ovalbumin-immunized mice. *Journal of Immunotoxicology,* 13(5):729–737. https://doi.org/10.3109/1547691X.2016.1173134

Helander, I.M., Alakomi, H.L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M., and Von Wright, A., 1998. Characterization of the action of selected essential oil compo- nents on Gram-negative bacteria. *Journal of Agricultural and Food Chemistry,* 46(9), 3590–3595. https://doi.org/10.1021/jf980154m

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., and Armand, R., 2015. The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine,* 6(09), 635–642. https://doi.org/10.4236 / ijcm.2015.69084

Hoyt, D.F., 1979. Practical methods of estimating volume and fresh weight of bird eggs. *The Auk,* 96(1): 73-77. https://doi.org/10.1093/auk/96.1.73

Kırchgessner, M., 1997. *Animal nutrition,* 10th revised edition, Guide to study, advice and practice. Publishing Union Agrar, ISBN 3-7690-0549-X, Seite. 140.

Lohmann Tierzucht GmbH, 2008. *Layer Management Guide Lohmann Brown Classic,*Cuxhaven, Germany

Manafi, M., Hedayati, M., and Khalaji, S., 2016. Effectiveness of phytogenic feed additive as alternative to bacitracin methylene disalicylate on hematological parameters, intestinal histomorphology and microbial population and production performance of Japanese Quails. *Asian-Australasian Journal of Animal Sciences,* 29(9): 1300-1308. https://doi.org/10.5713/ajas.16.0108

Mohebbifar, A., and Torki, M., 2010. Effects of adding mixed powder of gar- lic and thyme to diets included graded levels of rice bran on productive performance of laying hens and egg quality characteristics. *Advances in Environmental Biology,* 4(3): 469-476.

Mơnus, F., and Barta, Z., 2005). Repeatability analysis of egg shape in a wild Tree Sparrow (*Passer montanus*) population: a sensitive method for egg shape description. *Acta Zoologica Academiae Scientiarum Hungaricae,* 51(2): 151–162.

Paganelli, C.V., Olszowka, A., and Ali, A., 1974. The avian egg: surface area, volume, and density. *The Auk,* 91: 132–138. https://doi.org/10.2307/1366345

Sandikci Altunatmaz, S., Aksu, F., Aktaran Bala, D., Akyazi, I., and Celik, C., 2020. Evaluation of quality parameters of chicken eggs stored at different temperatures. *Kafkas University Journal of Veterinary Faculty,* 26(2):247-254.

https://doi.org/10.9775/kvfd.2019.22856

Schindelin, J., Rueden, C.T., Hiner, M.C., and Eliceiri, K.W., 2015. The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and Development,* 82(7-8): 518–529.

https://doi.org/ 10.1002/mrd.22489

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* 9(7): 671-675.

https://doi.org/ 10.1038/nmeth.2089

Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. *International Journal of Poultry Science,* 7(6): 613-621. https://doi.org/ 10.3923/ijps.2008.613.621

SPSS (2003). *SPSS Version 11.5.2.1 for Windows,* SPSS Inc

Troscianko, J., 2014. A simple tool for calculating egg shape, volume and surface area from digital images. *International Journal of Avian Science,* 156(4): 874-878. https://doi.org/10.1111/ibi.12177

Vakili, R., and Majidzadeh Heravi, R., 2016. Performance and egg quality of laying hens fed diets supplemented with herbal extracts and flaxseed. *Poultry Science,* 4(2), 107–116. https://doi.org/10.22069/PSJ.2016.9833.1156

Yalcin, S., Eser, H., Onbasilar, I., and Yalcin, S., 2020. Effects of dried thyme (Thymus vulgaris L.) leaves on performance, some egg quality traits and immunity in laying hens. *Veterinary Journal of Ankara University,* 67(3): 303-311. https://doi.org/10.33988/auvfd.677150