

Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

Effects of thyme essential oil and A, C, and E vitamin combinations to diets on performance, egg quality, MDA, and 8-OHdG of laying hens under heat stress

Selma Büyükkılıç Beyzi, Yusuf Konca, Mahmut Kaliber, Savaş Sarıözkan, Berrin Kocaoğlu Güçlü, Eray Aktuğ & Meryem Şentürk

To cite this article: Selma Büyükkılıç Beyzi, Yusuf Konca, Mahmut Kaliber, Savaş Sarıözkan, Berrin Kocaoğlu Güçlü, Eray Aktuğ & Meryem Şentürk (2020) Effects of thyme essential oil and A, C, and E vitamin combinations to diets on performance, egg quality, MDA, and 8-OHdG of laying hens under heat stress, Journal of Applied Animal Research, 48:1, 126-132, DOI: 10.1080/09712119.2020.1746662

To link to this article: https://doi.org/10.1080/09712119.2020.1746662

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

Published online: 03 Apr 2020.

Submit your article to this journal

Article views: 443

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=taar20

JOURNAL OF APPLIED ANIMAL RESEARCH
2020, VOL. 48, NO. 1, 126–132
https://doi.org/10.1080/09712119.2020.1746662

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Effects of thyme essential oil and A, C, and E vitamin combinations to diets on performance, egg quality, MDA, and 8-OHdG of laying hens under heat stress

Selma Büyükkılıç Beyzi[a], Yusuf Konca[a], Mahmut Kaliber[a], Savaş Sarıözkan[b], Berrin Kocaoğlu Güçlü[c], Eray Aktuğ[c] and Meryem Şentürk[d]

[a]Department of Animal Science, Faculty of Agriculture, Erciyes University, Kayseri, Turkiye; [b]Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkiye; [c]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkiye; [d]Department of Biochemistry, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkiye

ABSTRACT

This experiment was conducted to evaluate the effects of supplementary thyme (*Thymus vulgaris*) essential oil (TEO) and A, C, and E vitamins on performance, egg quality and some biochemical parameters of laying hens under heat stress (HS). A total of 120 White Leghorn laying hens were randomly distributed into 5 experimental groups with 6 replicates and each replicate had 4 laying hens. The HS group was housed at 34°C between 08:00 am and 05:00 pm and the rest of the day at 22°C. The experimental groups were; 1: HS group; 2: HS + TEO (300 mg/kg TEO in diet), 3: HS + TEO + Vit-C (300 mg/kg TEO + 250 mg/kg vitamin C in diet), 4: HS + TEO + Vit-E (300 mg/kg TEO + 250 mg/kg vitamin E in diet), and 5: HS + TEO + Vit-A (300 mg/kg TEO + 15,000 IU/kg vit-A in diet). In the study, body weight, egg weight, feed intake and egg quality of the hens were not affected with treatment groups. Serum glucose level was decreased in the TEO group, plasma MDA was decreased in the TEO and TEO + Vit-C groups and 8-OHdG was decreased in the TEO + Vit-E. It was concluded that it would be more beneficial to use TEO against HS in laying hens with Vit-E and Vit-C instead of using it alone.

ARTICLE HISTORY
Received 4 February 2020
Accepted 16 March 2020

KEYWORDS
Layer; heat stress; essential oil; vitamin addition

1. Introduction

Heat stress in poultry may cause serious economic losses by reducing performance and increasing mortality (Khan et al. 2011). It is estimated that the economic losses will increase gradually in the near future due to the effects of global warming in tropical and semitropical regions (Khan et al. 2014). The manipulation of environment or ration formulation is closely related to the applied cost and performance increments (Safiullah et al. 2019). It is well known that when there is environmental heat stress in poultry, vitamins need such as vitamin C also gradually increases (Khan et al. 2012a). It has been reported that the combination of vitamins and herbs may be more effective than the individual use (Rahman et al. 2017).

Heat stress causes poor performance in laying hens due to increased lipid peroxide level, total oxidant capacity, and oxidative stress index such as malondialdehyde (MDA) (Ahmad et al. 2020). Lipid peroxidation occurs because of increased body temperature in poultry exposed to heat stress (Alzawqari et al. 2016). MDA and 8-hydroxy-2′-deoxyguanosine (8-OHdG) have been used as indicators of lipid peroxidation. The dietary supplementation of selenium and vitamin E has the potential to decrease serum MDA levels in laying quail (Shakirullah et al. 2017). There are many studies investigating the effects of supplementation vitamin C to the diet to reduce or eliminate the negative effects of heat stress in chickens (Attia et al. 2009; Das et al. 2011; El-Habbak et al. 2011). Vitamin C has an effect of increasing feed intake, improving feed efficiency, ovulation day and egg weight in laying hens under heat stress (Seven 2008). Vitamin E can help reduce heat stress removes free radicals and prevents lipid peroxidation (Rahman et al. 2017) and decrease plasma MDA level (Sahin et al. 2003) in poultry. *Thymus vulgaris* is the most used medicinal herb in the Lamiaceae family for gastric, cosmetic and medicinal purposes worldwide. Thyme extracts have antimicrobial, antiseptic and antioxidant functions (Khan et al. 2012b). The effects of thyme originated from thymol and carvacrol, which are the most important bioactive compounds that in present study carvacrol is the major component.

In previous studies, the effects of some vitamins and essential oils on performance, carcass, and blood parameters were investigated (Ghazi et al. 2015). However, to the best of our knowledge, the combination of essential oil and vitamin A, C or E on the performance and economics of laying hens under heat stress have not been studied. Therefore, the objective of this study was to find the effect of thyme essential oil with or without vitamin A, E and C on the performance and blood antioxidant in laying hens.

2. Material and methods

2.1. Experimental design and diets

The experiment was carried out in accordance with the established standards for the use of animals. The protocol was

CONTACT Yusuf Konca yusufkonca@erciyes.edu.tr Faculty of Agriculture, Department of Animal Science, Erciyes University, Kayseri 38039, Turkey

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

approved by the Ethical Commission the Erciyes University Animal Care and Use Committee 15/86.

A total of 120–25-week-old Lohman White Leghorn hens were used in the study. Prior to the start of the experiment, the daily egg yields of the chickens were recorded for 14 days and body weight (BW) measured and then distributed to groups according to minimal differences in terms of egg yields and body weights. Laying hens were divided into 5 treatment groups with 6 replicates (cages) and 4 birds in each cage (dimension of cage, 45 × 42 × 45 cm). Heat stress (HS) was set at 34°C between 08:00 and 05:00 pm (9h) and 22°C between 05:00 and 09:00 pm (15h). A thermostat-controlled radiator and split air conditioner were used to ensure a homogeneous temperature in the houses. During the study, the temperature and humidity in the houses were kept stable according to the heat stress schedule. Thyme essential oil (TEO) was bought from a commercial company (Botanika Tarım Ltd. Antalya, Turkey). Vitamin A, C, and E supplements were bought from a commercial company (Kartal Kimya A.Ş. İstanbul, Turkey). The experimental groups were as follows; 1: heat stress (HS, control, basal diet, 34°C room temperature), 2: HS + TEO (300 mg/kg TEO addition to diet), 3: HS + TEO + Vit- C (300 mg/kg TEO + 250 mg/kg vitamin C addition to diet), 4: HS + TEO + Vit-E (300 mg/kg TEO + 250 mg/kg vitamin E addition to diet), and 5: HS + TEO + Vit-A (300 mg/kg TEO + 15,000 IU/kg vitamin A addition to diet). The amount of feedstuff used in the diet and the nutrient contents are given in Table 1. The composition of essential oil used in the diet is given in Table 2. In the poultry houses, 16 h of light and 8 h of dark period were applied. Feed and water were given ad-libitum. The study was continued for 10 weeks and feed and egg data were collected every 2 weeks.

Table 1. Composition of the basal diet used in the experiment.

Items	g/kg DM
Corn	400.0
Wheat	170.0
Sunflower meal	170.0
Limestone	82.6
Soybean meal	62.0
Vegetable oil	25.0
Corn bran	32.0
Corn gluten meal	27.0
Meat-bone meal	21.0
Mycotoxin-binder	1.0
Salt	2.5
Vitamin-Mineral premix[a]	2.5
L-lysine	2.3
DL-methionine	1.0
Phytase[b]	0.6
Multi-enzyme[c]	0.5
Calculated nutrient composition	
Dry matter, %	88.2
Crude protein, %	17.5
Crude fat, %	5.1
Crude fibre, %	5.5
Calcium, %	3.5
Available phosphorus, %	0.4
Lysine, %	0.8
Methionine, %	0.4
Metabolizable energy, kcal/kg	2820.0

[a]Vitamin-mineral premix per kilogram of the diet, vitamin A, 10,000 IU; vitamin D3, 1200 IU; vitamin E, 20 mg; vitamin K, 3 mg; vitamin B1 (thiamine), 2 mg; vitamin B2 (riboflavin), 6 mg; vitamin B6, 4 mg; vitamin B12, 0.015 mg; niacin, 25 mg; calcium D-pantothenate, 5 mg; folic acid, 0.35 mg; choline chloride, 300 mg; manganese, 80 mg; iron, 40 mg; zinc, 60 mg; copper, 5 mg; iodine, 0.4 mg; cobalt, 0.1 mg; selenium, 0.15 mg assures.
[b]Contains 5000 FTU/kg phytase enzyme.
[c]Contains 9000 BGU/g β-gluconase, 40,000 XU/g xylanase, 1000 U/g mannanase, 50 FPU/g cellulase, 50 PGLU/g pectinase.

2.2. Performance, egg quality and sample collection

The laying hens at the beginning and end of the experiment were weighed individually, and body weight change during experiment was calculated. Feed intake was determined every two weeks by weighing in all subgroups. Feed conversion rate (FCR) was calculated by total amount of feed consumed: per kg of egg mass.

Egg yield records and the number of damaged (cracked, unshelled) eggs were recorded daily. To determine egg weights, two eggs were collected every two weeks' last consecutive 3 days and weight recorded. The egg's internal quality characteristics were measured with an egg quality measuring device (egg analyzer, ORKA Food Technology Ltd., Ramat Hasharon, Israel) in 24 eggs, each 28 days. The eggs' albumen and yolk height and diameter were measured. The Haugh unit was calculated by using these values. The eggshell thickness was determined by micrometer from the pointed middle and blunt ends of the eggs and average values were calculated from this data.

2.3. Blood analysis

At the end of the experiment, blood samples were collected from wing veins (12 hens, 2 from each subgroup), were kept at room temperature for 2 h, then centrifuged at 3000 rpm for 10 min, serum and plasma samples were separated and stored at −80°C until analysis was performed. Serum glucose levels were determined with a commercial kit (Biolabo, France) with spectrophotometer (UV/VIS Shimadzu 1208, Japan). The plasma levels of MDA (TBARS ELISA Kit, Cayman, USA, Cat. No. 10009055), which are indicative of lipid peroxidation, and 8-OHdG (Northwest Life Science Specialist, LLC, Washington, Code: NWK 8-OHdG 02), which are indicative of DNA destruction, were used in plasma samples (μQuant Bio-Tek, Turkey).

2.4. Economical and statistical analysis

In the economic analysis, total egg production obtained on a group basis during the experiment (70 days) was calculated. Accordingly, net profit amounts were calculated by subtracting production cost from total sales revenue. In the calculations, the

Table 2. Composition of thyme essential oil.

Components	%	Components	%
α-pinene	0.41	Terpinen-4-ol	0.84
Camphene	0.29	β-caryophyllene	1.33
β-myrcene	0.59	α-terpineol	0.29
α-terpinene	0.52	Borneol	1.60
β-phellandrene	0.31	β-Bisabolene	1.54
γ-terpinene	1.59	Caryophyllene oxide	0.27
β-cymene	3.18	Spathulenol	0.19
Trans-sabinene hydrate	0.37	Thymol	1.81
Linalool	3.00	**Carvacrol**	**81.89**

egg price in 2019 figures is 10 TL/package (30 eggs); feed price was taken as 2 TL/kg., 70% of the total feed expenses are considered as the remaining 30% of the other expenses. Thyme oil 600 TL/kg; Vitamin A 700 TL/kg; Vitamin E was taken as 42 TL/kg and vitamin C as 38 TL/kg.

Partial budgeting (Hady et al. 1994) was used to examine the economic impact of options to be used. The net income increase obtained as a result of the partial budget analysis was calculated with the following formula;

$$\text{Net Income} = (\text{Additional Revenue Increase} + \text{Decreasing Costs}) - (\text{Decreasing Revenue} + \text{Additional Costs})$$

During the experiment, sampling periods determined as 2-week intervals (totally 10 weeks: 5 times). Performance parameter measurements (feed intake, egg production, egg weight and FCR) were also taken at these times. A repeated measurements analysis was used in the GLM-procedure IBM SPSS Statistics version 22.0 for Windows (SPSS, Chicago, IL) to examine treatment and time effects. Differences between treatments were examined using Tukey post-hoc comparisons. For the body weight, egg quality parameters and serum glucose and plasma antioxidant parameters data were not appropriate with repeated measures analysis.

Differences were considered statistically significant at $p < 0.05$. Data were expressed as mean and pooled standard error mean (SEM) in the tables.

3. Results

3.1. Laying performance

There was no significant effect of the addition of TEO and TEO + vitamin (C, A, and E) combinations on BW and BW change (Table 3). In the study, it was determined that the TEO and TEO combinations with one of the vitamins C, E, or A in the diet did not affect feed intake, egg weight, egg production and FCR of laying hens exposed to heat stress (Table 4).

3.2. Egg quality

There were no significant differences among the treatment groups in terms of egg specific gravity (SG), shell thickness (ST), albumen height (AH), yolk colour (YC), and Haugh unit (HU) at 0–5 weeks (Table 5). In the period of 6–10 weeks, the SG of the TEO + Vit E group was higher than those of the other treatment groups ($P < 0.01$) and ST, AH, YC, and HU were not statistically influenced by the treatment groups.

Table 3. The effects of TEO and vitamin C, E, and A supplementation on body weight (g) in laying hens.

Items	C	TEO	TEO+Vit C	TEO+Vit E	TEO+Vit A	SEM	*P*
Initial BW	1506.67	1491.28	1553.38	1496.00	1515.62	11.31	0.451
Final BW	1475.43	1575.14	1512.33	1521.43	1528.72	16.34	0.446
BW change	−31.24	83.86	−41.05	25.43	13.1	3.40	0.254

Note: BW: body weigh; C: control diet; TEO: control diet + 300 mg/kg thyme essential oil; TEO + Vit C: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin C; TEO + Vit E: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin E; SEM: standard error of means; *P*: probability.

Table 4. The effects of TEO and vitamin C, E, and A supplementation on feed intake, egg weight, feed conversion ratio and egg production in laying hens.

Parameter	C	TEO	TEO+Vit C	TEO+Vit E	TEO+Vit A	SEM	*P*
Feed intake, g/hen/day	107.25	113.05	109.30	109.89	109.96	2.597	0.379
Egg weight, g/egg	59.57	60.70	60.12	60.84	59.99	0.907	0.734
Egg production, %	97.69	96.94	97.14	98.56	97.49	1.165	0.568
Feed conversion ratio*	1.80	1.86	1.82	1.81	1.83	0.039	0.466

Note: C: control diet; TEO: control diet + 300 mg/kg thyme essential oil; TEO + Vit C: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin C; TEO + Vit E: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin E; SEM: standard error of means; *P*: probability; [a, b]: The differences between the averages in the same row are significant, $P < 0.05$.; *: kg feed consumed/kg egg produced.

Table 5. Effects of TEO and vitamin C, E, and A supplementation on the egg quality of fresh eggs in laying hens.

Egg quality parameters	C	TEO	TEO+Vit C	TEO+Vit E	TEO+Vit A	SEM	*P*
0–5 weeks							
SG, g/g	1.10	1.10	1.11	1.11	1.11	0.002	0.266
ST, mm	0.39	0.39	0.37	0.38	0.39	0.003	0.066
AH, mm	5.05	4.97	4.92	4.84	4.79	0.075	0.875
Yolk colour	9.61	9.62	9.37	9.54	9.64	0.067	0.724
HU	67.42	66.64	66.88	65.44	65.24	0.775	0.917
6–10 weeks							
SG, g/g	1.05[b]	1.05[b]	1.05[b]	1.08[a]	1.05[b]	0.003	0.005
ST, mm	0.42	0.43	0.42	0.45	0.42	0.003	0.063
AH, mm	5.18	5.27	5.27	4.97	4.94	0.087	0.609
Yolk colour	8.94	9.00	9.00	8.97	9.11	0.059	0.913
HU	70.21	69.99	68.89	66.87	66.87	0.978	0.703

Note: SG: specific gravity; ST: shell thickness; AH: albumen height; HU: haugh unit; [a, b]: Differences between the averages in the same row are important, $P < 0.05$. C: control diet; TEO: control diet + 300 mg/kg thyme essential oil; TEO + Vit C: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin C; TEO + Vit E: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin E; SEM: standard error of means; *P*: probability a, b: The differences between the averages in the same row are significant, $P < 0.05$.

3.3. Economic analysis

Considering the total egg production and sales income, the highest value was obtained in the TEO + Vit E group (Table 6). The lowest production cost and the highest profitability were in the control group.

3.4. Biochemical parameters

Plasma glucose levels ranged between 204.13–235.54 and the lowest was observed in the TEO group with the highest level observed in the control group ($P < 0.05$) (Table 7). Plasma MDA level was decreased in TEO and TEO + Vit C group. Plasma 8-OHdG was decreased in TEO + vit E group compared to control and TEO + Vit A groups.

4. Discussion

4.1. Performance

Heat stress adversely affects production parameters and livability of egg-laying hens (Harsini et al. 2012; Ghazi et al. 2015). Balnave (2004) reported that temperature stress occurs when the ambient temperature rises above 25°C. However, in many regions of the world, temperature below 32°C is not considered extremely oppressive and higher temperature should be considered in other conditions. Even in tropical and subtropical areas, chicks may have exposure to higher temperature and yet their strength is better than chickens grown in moderate climate condition. In the current experiment, the chickens showed stability against heat stress during the day. They breathed more during the day with exposure to heat stress and decreased their feed intake. However, they consumed more feed in the evening from 05:00 until 09:00 pm. In addition, in early morning until the start of heat stress, layers may have consumed enough feed to continue production. The findings of the current study showed that the supplementation of thyme essential oil alone or in combination with one of the vitamins E, A and C in laying hens exposed to heat stress did not affect the body weight, feed intake, egg production, FCR and egg weight. In some studies, it was noted that the supplementation of oregano essential oil to the diet in laying hens did not affect the FCR (Mohebbifar and Torki 2010; Amad et al. 2011; Akbari et al. 2016; Torki et al. 2018). Feed intake and body weight did not change in groups supplemented with oregano essential oil have been reported in some other studies (Mohebbifar and Torki 2010; Amad et al. 2011; Özek et al. 2011). Lin et al. (2004) showed that feed intake, feed efficiency and body weight changed parallel in heat stressed hens supplemented with vitamin A. Kirunda et al. (2001) reported that the supplementation of 20 IU/kg of vitamin E does not affect feed intake, but the supplementation of 60 and 120 IU/kg to diet increases the feed intake in the hens under heat stress. Ghazi et al. (2015) found that the addition of thyme oil alone or in combination with vitamin C in broilers exposed to heat stress improves LW, BW gain and FCR, but does not affect feed intake.

Table 6. Effects of TEO and vitamin C, E, and A supplementation on economical parameters of laying hens*.

Parameter	C	TEO	TEO+Vit C	TEO+Vit E	TEO+Vit A
Total egg production, package	1.641	1.629	1.632	1.656	1.638
Total Production Cost (TL)	468.5	528.0	512.3	515.2	545.9
Total Sales Revenue (TL)	547	543	544	552	546
Net Profit (TL/group)	78.5	15.0	31.7	36.8	0.1

*Calculations were done on a group basis; C: control diet; TEO: control diet + 300 mg/kg thyme essential oil; TEO + Vit C: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin C; TEO + Vit E: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin E.

4.2. Egg quality

In the present study, egg yield and egg weight were not affected by the treatments. In the published literature, there are conflicting results on the effect of aromatic plants and essential oils on egg quality in chickens (Cross et al. 2002, 2007; Bampidis et al. 2005; Florou-Paneri et al. 2005; Arpasoval et al. 2013; El-Hack et al. 2019). In the current study, there was no significant difference between thyme essential oil and control groups in terms of egg quality parameters. This finding was similar to some studies indicating that essential oils were not effective to reduce the negative effects of heat stress (Montazeri et al. 2014). In the studies, it was determined that the addition of essential oil (*Nigella sativa*, *Myrtus communis*) to the hen diets did not affect the egg yield and weight (Özek et al. 2011; Torki et al. 2018).

At the end of the second period of the study (second 4 weeks), a significant increase in egg specific weight was detected in the TEO + Vit E group but was not affected by treatments in terms of HU, ST, AH and yolk colour. Akbari et al. (2016) reported that egg ST was not affected by the supplementation of oregano essential oil to the diet, but HU increased in a study conducted in laying hens. Ghasemi et al. (2010) reported that thyme powder added to the diet in ST improved in laying hens. Çabuk et al. (2006) reported that essential oil mixture including myrtle leaf oil does not affect parameters such as white and yellow index, Haugh unit, shell thickness and shell weight in laying quails. Özek et al. (2011) reported that the essential oil mixture increased egg white height and Haugh

Table 7. Effects of TEO and vitamin C, E, and A supplementation on serum glucose, plasma MDA and 8-OHdG of laying hens.

Parameter	C	TEO	TEO+Vit C	TEO+Vit E	TEO+Vit A	SEM	*P*
Serum glucose, mg/dL	235.54^{a}	204.13^{c}	216.29^{abc}	208.08^{bc}	230.22^{ab}	3.37	0.045
Plasma MDA, µmol/L	12.49^{a}	8.38^{b}	8.516^{b}	9.31^{ab}	12.00^{a}	0.49	0.048
Plasma 8-OHdG, ng/mL	8.02^{ab}	5.64^{bc}	5.93^{bc}	4.32^{c}	8.51^{a}	0.44	0.004

Note: C: control diet; TEO: control diet + 300 mg/kg thyme essential oil; TEO + Vit C: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin C; TEO + Vit E: control diet + 300 mg/kg thyme essential oil + 250 mg/kg vitamin E; SEM: standard error of means; *P*: probability $^{a, b, c}$: The differences between the averages in the same row are significant, $P < 0.005$.

unit and did not affect eggshell thickness, shell weight, shell weight ratio and yellow colour.

In poultry performance, the differences between the results were attributed generally to the variety of essential oils and their components, the levels used in diets, the type of breed, and experimental conditions. In the present study, vitamins supplementation had no effect on egg quality. However, in one study, it was reported that the addition of vitamin C (200 mg/kg) to rations in egg-laying hens increased eggshell thickness (Demir et al. 1995). Kirunda et al. (2001) reported that the addition of vitamin E (20, 60, 120 IU/kg) to rations in egg-laying hens increased egg HU. In the same study, it was reported that the addition of high doses (60 and 120 IU/kg) increased the brightness of the egg yolk. Lin et al. (2004) reported that the addition of vitamin A (9000 IU/kg) to the ration in egg-laying hens increased egg weight.

4.3. Economic evaluation

The economics TEO or combinations of with different vitamins (A, C, E) must be evaluated in term of production cost and egg sale income. Some researchers reported that, supplementation of essential oil improves egg weight in laying hens under heat stress (Özek et al. 2011). Similarly, under high ambient temperature conditions, supplementation of Vitamin C to the diet of laying quail's increased egg weight (Karimi et al. 2015). Higher egg weight increases the sale and generates more income and profit. On the other hand, due to especially higher cost of Vitamin A, the production cost was higher compared to the other groups.

4.4. Biochemical parameters

Under comfortable range of temperature (10–22°C), no balance can be established between the heat produced by the body and the heat lost from the body. Heat stress resulting from the increased body temperature leads to deterioration of health and reduced efficiency (Abu-Dieyeh 2006). Similar to the results of the current study, an increase in serum glucose levels in stress-exposed animals has been reported (Chand et al. 2018). The increase in glucose level may be due to stress-suppressed insulin and increased glucagon level (Sands and Smith 2002) as well as increased glucocorticoid release (Nazifi et al. 2003; Garriga et al. 2006) and apical sodium in the jejunum dependent glucose transporter-1 expression (Garriga et al. 2006).

Studies have been reported that the herb thyme has good capacity to prevent lipid peroxidation (Capecka et al. 2005). In this case, it is thought that heat stress increases serum glucose levels by increasing lipid peroxidation in chickens and thyme essential oil decreases glucose level by preventing this condition. Akbari et al. (2016) reported that the addition of oregano essential oil to rations did not affect serum glucose levels in stressed hens. Malondialdehyde which is considered as an indicator of lipid peroxidation, has been reported to reach detectable levels in blood and urine under oxidative stress conditions (Fang et al. 2002; Moreno et al. 2003; Fujii et al. 2005). The increase in plasma MDA level was related to the decreased glutathione peroxidase, and superoxide dismutase (Şentürk and Uyanık 2016; El-Soud et al. 2006). In addition, the inhibition of the activity of antioxidant enzymes (El-Soud et al. 2006) and numerical increase in blood glucose level leading to the autoxidation of glucose (Anderson et al. 2001). The decrease in plasma MDA levels as a result of the addition of TEO and TEO + Vit C is thought to be caused by the antioxidant effect of oregano oil (Capecka et al. 2005), and vitamin C (El-Habbak et al. 2011). Mousavi et al. (2017) showed that the addition of 200 mg/kg essential oil mixture to a laying hen ration caused a decrease in plasma MDA concentration. Studies have been reported that the antioxidant bioactive compounds of aromatic plants such as thyme and rosemary can be used effectively in the MDA content by affecting lipid peroxidation in the blood (Lee et al. 2004; Florou Paneri et al. 2005). 8-hydroxy-2′-deoxyguanosine is the result of adding OH group to the 8th C atom of guanine of ROT in the organism (Cadet et al. 2003; Valko et al. 2004; Dinçer et al. 2007). Since 8-hydroxy-2′-deoxyguanosine is an important marker of ROT-induced DNA damage (Chen et al. 2007; Hamurcu et al. 2010), in this study, TEO + vit E may be useful in the prevention of damage. This is thought to be due to the combination of thyme oil (Capecka et al. 2005) and vitamin E (Sarı et al. 2008) which have antioxidant effects.

5. Conclusion

In conclusion, it was found that thyme essential oil added to the ration alone and in combinations with some vitamins (A, C, and E) resulted in no improvement in performance. It was also observed that the addition of TEO alone to the diet decreased plasma glucose levels. Economically, all supplements decreased the net profit TEO and TEO + Vit C were more effective in decreased plasma MDA level and TEO + Vit E were more effective in decreased plasma 8-OHdG in the laying hen under heat stress.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Erciyes Üniversitesi [grant number TOA-2013-4491].

References

Abu-Dieyeh ZHM. 2006. Effect of chronic heat stress and long-term feed restriction on broiler performance. Int J Poult Sci. 5:185–190.

Ahmad M, Chand N, Khan RU, Ahmad N, Khattak I, Naz S. 2020. Dietary supplementation of milk thistle (*Silybum marianum*): growth performance, oxidative stress and immune response in natural summer stressed broilers. Trop Anim Health Prod. 52:711–715.

Akbari M, Torki M, 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). Int J Biometeorol. 60:447–454.

Alzawqari MH, Al-Baddany AA, Al-Baadani HH, Alhidary IA, Khan RU, Aqil GM, Abdurab A. 2016. Effect of feeding dried sweet orange (*Citrus sinensis*) peel and lemon grass (*Cymbopogon citratus*) leaves on growth

performance, carcass traits, serum metabolites and antioxidant status in broiler during the finisher phase. Environ Sci Poll Res. 23:17077–17082.
Amad AA, Männer K, Wendler KR, Neumann K, Zentek J. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult Sci. 90:2811–2816.
Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. 2001. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J American Coll Nutr. 20:212–218.
Arpasoval H, Kacaniova M, Galik B. 2013. The effect of oregano essential oil and pollen on egg production and egg yolk qualitative parameters. Anim Sci Biotech. 46:12–16.
Attia YA, Hassan RA, Qota EMA. 2009. Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: effect of ascorbic acid and different levels of betaine. Trop Anim Health Prod. 41:807–818.
Balnave D. 2004. Challenges of accurately defining the nutrient requirements of heat-stressed poultry. Poult Sci. 83:5–14.
Bampidis VA, Christodoulou V, Florou-Paneri P, Christaki E, Chatzopoulou PS, Tsiligianni T, Spais AB. 2005. Effect of dietary dried oregano leaves on growth performance, carcass characteristics and serum cholesterol of female early maturing turkeys. Br Poult Sci. 46:595–601.
Çabuk M, Bozkurt M, Alçiçek A, Çatli AU, Başer KHC. 2006. Effect of a dietary essential oil mixture on performance of laying hens in the summer season. S Afr J Anim Sci. 36:215–221.
Cadet J, Douki T, Gasparutto D, Ravanat JL. 2003. Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res. 531:5–23.
Capecka E, Mareczek A, Leja M. 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. Food Chem. 93:223–226.
Chand N, Naz S, Rehman Zu, Khan RU. 2018. Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. J Kor Soc Appl Bio Chem. 61:273–279.
Chen HI, Liou SH, Ho SF, Wu KY, Sun CW, Chen MF, Loh CH. 2007. Oxidative DNA damage estimated by plasma 8-hydroxydeoxyguanosine (8-OHdG): influence of 4, 4′-methylenebis (2-chloroaniline) exposure and smoking. J Occup Health. 49:389–398.
Cross DE, Acamovic T, Deans SG, McDevitt RM. 2002. The effect of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. Br Poult Sci. 43:33–35.
Cross DE, McDevitt RM, Hillman K, Acamovic T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br Poult Sci. 48:496–506.
Das S, Palai TK, Mishra SR, Das D, Jena B. 2011. Nutrition in relation to diseases and heat stress in poultry. Vet World. 4:429.
Demir E, Ozturkcan O, Gorgulu M, Kutlu HR, Okan F. 1995. Sicak kosullarda yumurta tavugu rasyonlarina eklenen vitamin A ve C'nin yumurta özelliklerine etkileri. J Agri Faculty ÇÜ. 10:123–132.
Dinçer Y, Erzin Y, Himmetoğlu S, Gunes KN, Bal K, Akcay T. 2007. Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. Dig Dis Sci. 52:1636–1641.
El-Habbak MM, El-Ghamry AA, El-Mallah GM, Younis H, El-Komy EM. 2011. Influence of dietary vitamin E and C supplementation on performance and some metabolic response of broiler chicks subjected to heat stress. World Agr Sci. 7:258–269.
El-Hack MEA, Alagawany M, Mahrose KM, Arif M, Saeed M, Arain MA, Fowler J. 2019. Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season. Anim Nutr. 5:49–55.
El-Soud SBA, Ebeid TA, Eid YZ. 2006. Physiological and antioxidative effects of dietary acetyl salicylic acid in laying Japanese quail (*Coturnix japonica*) under high ambient temperature. J Poult Sci. 43:255–265.
Fang YZ, Yang S, Wu G. 2002. Free radicals, antioxidants, and nutrition. Nutrition. 18:872–879.
Florou-Paneri P, Nikolakakis I, Giannenas I, Koidis A, Botsoglou E, Dotas V. 2005. Hen performance and egg quality as affected by dietary oregano essential oil and α-tocopheryl acetate supplementation. Int J Poult Sci. 4:449–454.
Fujii J, Luchi Y, Okada F. 2005. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reprod Biol Endocrinol. 3:43.
Garriga C, Hunter RR, Amat C, Planas JM, Mitchell MA, Moretó M. 2006. Heat stress increases apical glucose transport in the chicken jejunum. Am J Physiol Regul Integr Comp Physiol. 290:195–201.
Ghasemi R, Zarei M, Torki M. 2010. Adding medicunal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. American J Anim Vet Sci. 5:151–154.
Ghazi S, Amjadian T, Norouzi S. 2015. Single and combined effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. Inter J Biomet. 59:1019–1024.
Hady PJ, Lyoyd JW, Kaneene JB, Skidmore AL. 1994. Partial budget model for reproductive programs of dairy farm businesses. J Dairy Sci. 77:482–491.
Hamurcu Z, Bayram F, Kahrıman G, Dönmez-Altuntas H, Baskol G. 2010. Micronucleus frequency in lymphocytes and 8-hydroxydeoxyguanosine level in plasma of women with polycystic ovary syndrome. Gynecol Endocrinol. 26:590–595.
Harsini SG, Habibiyan M, Moeini MM, Abdolmohammadi AR. 2012. Effects of dietary selenium, vitamin E, and their combination on growth, serum metabolites, and antioxidant defense system in skeletal muscle of broilers under heat stress. Bio Trace El Res. 148:322–330.
Karimi N, Ahangari YJ, Zerehdaran S, Akhlaghi A, Hashemi SR, Adabi NA. 2015. Effects of the dietary supplementation of chromium and vitamin C on egg quality traits in heat-stressed Japanese quails (*Coturnix cot. japonica*). Poult Sci. 79:1–8.
Khan RU, Naz S, Dhama K. 2014. Chromium: pharmacological applications in heat stressed poultry. Intern J Pharmacol. 10:213–317.
Khan RU, Naz S, Nikousefat Z, Selvaggi M, Laudadio V, Tufarelli V. 2012a. Effect of ascorbic acid in heat-stressed poultry. World's Poultry Sci J. 68:477–490.
Khan RU, Naz S, Nikousefat Z, Tufarelli V, Javadani M, Rana N, Laudadio V. 2011. Effect of vitamin E in heat-stressed poultry. World's Poultry Sci J. 67:469–478.
Khan RU, Naz S, Nikousefat Z, Tufarelli V, Laudadio V. 2012b. Thymus vlugaris: alternative to antibiotics in poultry feed. World's Poultry Sci J. 68:401–408.
Kirunda DFK, Scheideler SE, McKee SR. 2001. The efficacy of vitamin E (DL-α-tocopheryl acetate) supplementation in hen diets to alleviate egg quality deterioration associated with high temperature exposure. Poult Sci. 80:1378–1383.
Lee KW, Everts H, Kappert HJ, Beye AC. 2004. Growth performance of broiler chickens fed a carboxymethyl cellulose containing diet with supplemental carvacrol and/or cinnamaldehyde. Int J Poult Sci. 3:619–622.
Lin YF, Chang SJ, Hsu AL. 2004. Effects of supplemental vitamin E during the laying period on the reproductive performance of Taiwan native chickens. British Poult Sci. 45:807–814.
Mohebbifar A, Torki M. 2010. Effects of adding mixed powder of garlic and thyme to diets included graded levels of rice bran on productive performance of laying hens and egg quality characteristics. Advan Environ Biol. 4:469–476.
Montazeri S, Jafari M, Khojasteh S. 2014. The effect of powder and essential oil of savory medicinal plant (*Satureja hortensis*) on performance and antioxidant status of broiler chicks under heat stress. Iranian J Appl Anim Sci. 4:573–577.
Moreno IM, Mate A, Repetto G, Vázquez CM, Cameán AM. 2003. Influence of microcystin LR on the activity of membrane enzymes in rat intestinal mucosa. J Physiol Biochem. 59:293–300.
Mousavi A, Mahdavi AH, Riasi A, Soltani-Ghombavani M. 2017. Synergetic effects of essential oils mixture improved egg quality traits, oxidative stability and liver health indices in laying hens fed fish oil. Anim Feed Sci Tech. 234:162–172.
Nazifi S, Saeb M, Rowghani E, Kaveh K. 2003. The influences of thermal stress on serum biochemical parameters of Iranian fattailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. Comp Clin Path. 12:135–139.
Özek K, Wellmann KT, Ertekin B, Tarım B. 2011. Effects of dietary herbal essential oil mixture and organic acid preparation on laying traits, gastrointestinal tract characteristics, blood parameters and immune response of laying hens in a hot summer season. J Anim Feed Sci. 20:575–586.

Rahman ZU, Chand N, Khan RU. 2017. The effect of vitamin E, L-carnitine and ginger on production traits, immune response and antioxidant status in two broiler strains exposed to chronic heat stress. Environ Sci Poll Res. 24:26851–26857.

Safiullah Chand N, Khan RU, Naz S, Ahmad M, Gul S. 2019. Effect of ginger (*Zingiber officinale* Roscoe) and organic selenium on growth dynamics, blood melanodialdehyde and paraoxonase in broilers exposed to heat stress. J Appl Anim Res. 47:212–216.

Sahin K, Sahin N, Onderci M, Gursu MF, Issi M. 2003. Vitamin C and E can alleviate negative effects of heat stress in Japanese quails. Food Agri Environ. 1:244–249.

Sands JS, Smith MO. 2002. Effects of dietary manganese proteinate or chromium picolinate supplementation on plasma insulin, glucagon, glucose and serum lipids in broiler chickens reared under thermoneutral or heat stress conditions. Int J Poult Sci. 1:145–149.

Sarı M, Bolat D, Çerçi İH, Önol AG, Deniz S, Azman MA, Şahin K, Güler T, Tatlı Seven P, Karslı MA, et al. 2008. Hayvan besleme ve beslenme hastalıkları. Malatya: Medipress Matbaacılık Ltd Şti.

Şentürk MG, Uyanık F. 2016. Yumurtacı Bıldırcınlarda Oluşturulan Isı Stresinde Krom ve Çinkonun Bazı Kan Parametrelerine Etkileri. ERU Vet Fak Derg. 13:38–49.

Seven PT. 2008. The effects of dietary Turkish propolis and vitamin C on performance, digestibility, egg production and egg quality in laying hens under different environmental temperatures. Asian-Australasian J Anim Sci. 21:1164–1170.

Shakirullah Qureshi MS, Akhtar S, Khan RU. 2017. The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. Appl Biol Chem. 60:585–590.

Torki M, Sedgh-Gooya S, Mohammadi H. 2018. Effects of adding essential oils of rosemary, dill and chicory extract to diets on performance, egg quality and some blood parameters of laying hens subjected to heat stress. J Appl Anim Res. 46:1118–1126.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 266:37–56.