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RESEARCH ARTICLE

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Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants

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Abstract

The current study was performed to evaluate the effects of the dietary inclusion of extra virgin olive oil (EVOO), betaine (BET), lemongrass essential oil (LGEO), gallic acid (GA), vitamin C (VC) and vitamin E (VE) on different body temperature traits and stress hormone and glucose levels in heat-stressed growing rabbits. Rabbits were fed diets with no supplementation (control group) or supplemented with 15 g of EVOO, 400 mg of LGEO, 500 mg of GA, 1000 mg of BET, 500 mg of VC, or 200 mg of VE per kg of diet. All tested feed additives, especially EVOO, had a lowering effect on various rabbit temperature traits. Both triiodothyronine (T_3) and tetraiodothyronine/thyroxine (T_4) were increased ($p<0.05$) by the addition of BET, VC, EVOO, and VE. With the exception of the VC group, all dietary groups showed no significant changes in the insulin level compared to the control group level. In contrast, the cortisol and glucose levels were diminished ($p<0.05$) in all treated groups compared to the control levels. The results suggested that all tested supplementations had positive ameliorating effects on growing rabbits under a severe heat load in terms of lowered body temperatures and a favourable stress hormone balance, with the most favourable results found in the EVOO, VC, and BET supplementation groups.

Additional keywords: betaine; body temperature; gallic acid; lemongrass; extra virgin olive oil; stress hormones.

Abbreviations used: BET (betaine); EVOO (extra virgin olive oil); GA (gallic acid); LGEO (lemongrass essential oil); RH (relative humidity); RIA (radioimmunoassay); T_3 (triiodothyronine); T_4 (tetraiodothyronine/thyroxine); THI (temperature humidity index); VC (vitamin C); VE (vitamin E).

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Introduction

Under the high environmental temperatures in tropical and subtropical regions, farm animals, including rabbits, are subjected to heat stress, which results in poor production traits. Provision of natural and safe dietary supplements to mitigate the negative impacts of rising temperatures has become necessary. Recently, climate change, especially the expected global rise in surface temperature, has constituted a serious hazard for livestock production (Dangi *et al.*, 2016). In particular, high ambient temperatures are a major stress factor for rabbits due to their dense fur and few functional sweat glands, which greatly hinder heat loss (Marai *et al.*, 2002). In tropical and subtropical regions, heat stress is aggravated by high relative humidity, which can reach 85% during hot months (Marai *et al.*, 2007). Alterations in the rectal, skin and ear temperatures; respiration rate; and thyroid and stress hormone, albumin, globulin, total lipid, glucose, sodium, potassium, calcium, magnesium and phosphorus levels are the main physiological responses to heat load in rabbits (Marai *et al.*, 2008). Heat load also causes increased oxygen-derived free radicals, which create a condition of oxidative stress (Sahin & Kucuk, 2003).

Various genetic, managerial, nutritional, buffering, hormonal and physical mitigating strategies have been adopted to palliate the adverse effects of heat stress (Fayez *et al.*, 1994; Marai *et al.*, 1999). Vitamins, essential oils, fats and amino acids are the main dietary supplements with marked antioxidant properties. Recently, the use of natural antioxidants of plant origin has caught the attention of both livestock producers

and nutritionists, as these supplements can be added to the daily diet with ease at an affordable price and are expected to have very few side effects (Tawfeek *et al.*, 2014). In rabbits, vitamin C (ascorbic acid) and E are the most common and widely used antioxidants to alleviate heat stress (Yassein, 2010; Arafa Mervat *et al.*, 2012). Betaine (BET), which is a trimethyl form of glycine, has been found to alleviate heat stress in sheep and poultry (Zulkifli *et al.*, 2004; DiGiacomo *et al.*, 2012).

Lemongrass essential oil (LGEO) is a volatile oil that can be extracted directly from fresh lemongrass (*Cymbopogon citratus*) using steam extraction; the grass contains 0.035% essential oil (Malee *et al.*, 2000). LGEO is characterized by the presence of various phyto-constituents, including α-citral, β-citral, isoneral and α-myrcene. Al-Sagheer *et al.* (2018) found improvements in the productive performance, immunity and disease resistance of Nile tilapia fed a diet supplemented with LGEO. Extra virgin olive oil (EVOO) is obtained through mechanical pressing of the olive mesocarp. This oil has potentially beneficial effects, including antimicrobial, antioxidant and anti-inflammatory properties, due to its components, such as phenolic compounds, carotenoids and tocopherol (Cicerale *et al.*, 2012). Gallic acid (GA) is a natural polyphenolic that possesses several pharmacological activities, including antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and anti-allergic activities (Jo *et al.*, 2006). However, little evidence is available to date regarding potential beneficial roles of LGEO, EVOO, and GA against heat stress in rabbits.

We previously discussed the effects of EVOO, LGEO and GA supplementation on the growth, nutrient digestibility, antioxidant status, and lipid peroxidation of growing rabbits (Al-Sagheer *et al.*, 2017). The objective of this study was to evaluate the effects of EVOO, LGEO, GA, and BET as non-traditional antioxidants on different body temperature traits and on stress hormone and glucose levels in growing rabbits under severe heat stress compared to the effects of vitamin C (VC) and vitamin E (VE) as traditional antioxidants.

Material and methods

Test compounds

Lemongrass herbs (*Cymbopogon citratus*) were collected in May 2015 from different farms in Egypt. The plant materials were stored in a cool and dry location for oil extraction. According to Guenther (1972), the essential oil was isolated through hydrodistillation using a Clevenger type apparatus for 4 h, and the solvent was evaporated under reduced pressure at 40ºC using a rotary evaporator. The obtained essential oil was sterilized by filtration using a Millipore cellulose filter membrane (0.45-mm pore diameter) and stored at low temperature. Gallic acid (99.5%) was obtained from Alpha Chemica (Mumbai, India). The EVOO (ILIADA PDO Kalamata Extra virgin olive oil; AGRO. VI. M.S.A., Kalamata, Greece) was obtained from a local market. VC (Microvit® C Promix 1000), VE (Microvit® E Promix 50) and BET (betafine®) were obtained from Adisseo (France).

Experimental rabbits and management

The study was conducted at the Rabbit Research Farm, Faculty of Agriculture, Zagazig University, Egypt. The experiment was run beginning in June and continued for 8 weeks. In total, 84 (male, 5 weeks old) New Zealand White (NZW) rabbits were purchased from the Laboratory Animal Farm, Zagazig University. During the experimental period, the rabbits were housed individually in galvanized wire cages (35×60×35 cm) in an artificially illuminated room. The building was naturally ventilated and provided with electric fans. The rabbits were kept under the same hygienic and managerial conditions. Faeces and urine were removed from the rabbitry floor every morning. The rabbits were adapted to the basal diet over a 2-week period. Feed and water were offered *ad libitum* and refilled at 8:30 am and 2:30 pm daily. The Ethics of Animal Use in Research Committee of Zagazig University approved all protocols involving animals.

Experimental design and diets

The rabbits were distributed into seven groups with 12 rabbits per treatment. The groups were fed the basal diet with no supplementation (control group, C) or a diet supplemented with 500 mg of VC, 200 mg of VE, 1000 mg of BET, 400 mg of LGEO, 500 mg of GA, or 15 g of EVOO per kg of diet. The basal diet composition (g/kg) was as follows: alfalfa hay 330, barley grain 250, wheat bran 250, soybean meal 150, sodium chloride 5, limestone 10, mineral-vitamin premix 3 and dl-methionine 2. The basal diet was pelleted and formulated to meet the nutrient requirements of growing rabbits according to the recommendations of De Blas & Mateos (2010). The chemical analysis of the basal diet revealed that it contained 90.82% organic matter, 18.20% crude protein, 32.12% neutral detergent fibre, 16.63% acid detergent fibre, 3.26% ether extract, 57.19% nitrogen-free extract and 9.18% ash (on a dry matter basis).

Throughout the experimental period, the relative humidity and ambient temperature were recorded using an automatic thermo-hygrometer (°C -10:60, RH 10–99%; TFA Dostmann GmbH & Co. KG, Wertheim, Germany) twice daily. Inside the rabbitry, the ambient temperature, relative humidity and temperature humidity index (THI) averages were 32.44 ± 0.19°C, 68.55 ± 0.76% and 84.67 ± 0.35, respectively, which indicated severe heat stress. The THI was calculated according to LPHSI (1990) using the following equation: THI = db°F - [(0.55-0.55RH) (db°F -58)] where db°F is the dry bulb temperature in degrees Fahrenheit and RH is the relative humidity (%). The THI values obtained were categorized as follows: <82, absence of heat stress; 82-<84, moderate heat stress; 84-<86, severe heat stress and ≥86, very severe heat stress.

Measurement of rectal, ear and skin temperatures

Using a digital thermometer (Type K Thermocouple, ± 0.1ºC) (Adamsons, 1959), the rectal, skin, and ear temperatures were measured at midday for each rabbit (n = 12 per treatment). The skin temperature was measured at one location between the neck and loin on the body surface. The ear temperature was measured by placing the digital thermometer in direct contact with the central area of the auricle. The rectal temperature was measured by inserting the thermometer probe into the rectum at a depth of 2 cm. All body temperatures measurement was carried out at the midday (12.00-14.00 p.m., heat stress period) by three persons where each one is concerned with a type of body temperature. Each measurement consumed about 30-60 seconds/rabbit. The duration of measurement were minimized as possible to be all in the heat stress period, so no differences related to the time of measurement could affect the results.

Serum biochemical analysis

Upon termination of the experiment, blood samples (n = 6 per treatment) were collected from the ear vein into clean, sterile tubes. The samples were left for 20 min at room temperature to coagulate and then centrifuged at 1075 × g in a refrigerated centrifuge (BOECO centrifuge C-28 A, Hamburg, Germany) for 10 min. The generated sera were stored at −20°C prior to analysis of the thyroid hormone, cortisol, insulin and glucose levels. Serum total triiodothyronine (T_3), tetraiodothyronine/thyroxine (T_4) and cortisol were analysed using commercial radioimmunoassay (RIA) kits (Vidas, Biomérieux, Lyon, France, Catalogue No. 30403, 30404, and 30451, respectively) following the manufacturer's instructions. The detection ranges of the T_3, T_4 and cortisol assays were 0.26-5.84 ng/mL, 0.46-24.8 µg/dL, and 2-650 ng/mL, respectively. Serum insulin was quantified using an ELISA kit (BioCheck, Uppsala, Sweden. Catalogue No. 10-1113-01) with a detection limit of <1 mU/L. All samples were measured at appropriate dilutions to ensure that the hormone activities were in the linear ranges of the standard curves constructed with pure enzymes. The serum glucose level was determined colourimetrically according to Trinder (1969) using a commercial kit (Biodiagnostic, Cairo, Egypt) according to the manufacturer's instructions.

Statistical analysis

Differences among treatments were analysed with a one-way ANOVA test in a completely randomized design using the SPSS software statistical analysis program (SPSS®, 2001). Significant differences among the means were compared using Duncan's new multiple-range test.

Results

Temperature humidity index

In the current experiment, the calculated THI ranged from 80.87 to 81.99 during the 1st and 2nd weeks of the experiment, respectively, indicating no heat stress (Table 1). Conversely, the estimated THI values ranged from 84.08-86.95 from the beginning of the 3rd week until the 8th week of the experiment, demonstrating fluctuation of heat stress from severe to very severe. Moreover, throughout the experiments, the overall mean THI value was 84.67, which reflected a state of severe heat stress.

Effect on rabbit rectal temperature

The effects of 8 weeks of supplementation with VC, BET, EVOO, GA, VE or LGEO on the rectal temperatures of growing NZW rabbits are presented in Table 2. During the overall trial period, all tested feed additives had a lowering effect on the rabbit rectal temperatures compared to the control group temperatures. When comparing the tested additives, BET, VC, EVOO and VE evoked a larger (p<0.05) reduction in rectal temperature, followed by GA and LGEO.

Effect on rabbit skin temperature

The skin temperatures are shown in Table 3. Compared with the control group temperatures, the

Table 1. Ambient temperature (AT), relative humidity (RH) and the calculated temperature-humidity index (THI) during the experimental period. Data are expressed as the mean ± standard error.

	Date	AT (°C)	RH (%)	THI
1st week	1-7 June	31.34 ± 0.25	54.86 ± 1.44	80.87 ± 0.47
2nd week	8-14 June	31.03 ± 0.20	64.29 ± 1.54	81.99 ± 0.42
3rd week	15-21 June	32.31 ± 0.29	70.00 ± 0.00	84.86 ± 0.43
4th week	22-28 June	33.99 ± 0.57	70.00 ± 0.00	87.37 ± 0.86
5th week	29 June-5 July	32.10 ± 0.58	70.57 ± 0.30	84.63 ± 0.84
6th week	6-12 July	33.34 ± 0.60	71.29 ± 0.52	86.63 ± 0.86
7th week	13-19 July	31.71 ± 0.24	70.71 ± 0.42	84.08 ± 0.39
8th week	20-26 July	33.58 ± 0.18	71.00 ± 0.46	86.95 ± 0.31
Overall period		32.44 ± 0.19	67.84 ± 0.76	84.67 ± 0.35

Table 2. Effect of feed additives on the rectal temperatures of growing New Zealand White rabbits at 7-14 weeks of age.

	Control	VC	BET	EVOO	GA	VE	LGEO	SEM	*p* value
1st week	39.04	39.00	38.66	38.84	39.00	38.89	38.94	0.10	0.17
2nd week	39.13	38.83	38.82	39.13	39.02	39.07	39.06	0.10	0.17
3rd week	39.31	39.02	39.00	38.97	39.22	39.00	39.17	0.09	0.07
4th week	39.59	39.20	39.29	39.17	39.37	39.25	39.32	0.10	0.07
5th week	39.64^{a}	39.29^{b}	39.29^{b}	39.33^{b}	39.47^{ab}	39.29^{b}	39.59^{ab}	0.10	0.03
6th week	39.81	39.62	39.52	39.57	39.54	39.56	39.60	0.07	0.11
7th week	39.78	39.44	39.53	39.53	39.62	39.52	39.60	0.07	0.07
8th week	39.98^{a}	39.68^{b}	39.66^{b}	39.68^{b}	39.76^{b}	39.69^{b}	39.83^{ab}	0.06	0.003
Overall	39.53^{a}	39.26^{bc}	39.23^{c}	39.26^{bc}	39.37^{b}	39.28^{bc}	39.39^{b}	0.04	0.001

VC: vitamin C. BET: betaine. EVOO: extra virgin olive oil. GA: gallic acid. VE: vitamin E. LGEO: lemongrass essential oil. SEM: standard error of the mean and n=12 treatments per week. Means within the same row carrying different superscripts are significantly different at $p<0.05$.

Table 3. Effect of some feed additives on skin temperatures of growing New Zealand White rabbits under heat stress conditions at 7-14 weeks of age.

	Control	VC	BET	EVOO	GA	VE	LGEO	SEM	*p* value
1st week	38.68	38.71	38.62	38.50	38.53	38.52	38.55	0.12	0.82
2nd week	38.92	38.63	38.68	38.67	38.56	38.90	38.79	0.10	0.14
3rd week	38.85^{a}	38.73^{ab}	38.64^{ab}	38.54^{b}	38.91^{a}	38.52^{b}	38.89^{a}	0.09	0.02
4th week	39.15^{a}	38.79^{ab}	38.70^{b}	38.67^{b}	39.07^{a}	38.81^{ab}	38.88^{ab}	0.11	0.03
5th week	39.33^{a}	38.89^{bc}	38.90^{bc}	38.80^{c}	38.90^{bc}	38.99^{abc}	39.22^{ab}	0.11	0.02
6th week	39.38	39.17	39.25	39.32	39.13	39.32	39.35	0.09	0.47
7th week	39.48	39.27	39.21	39.32	39.28	39.31	39.28	0.08	0.39
8th week	39.66^{a}	39.36^{bc}	39.30^{bc}	39.26^{c}	39.54^{ab}	39.36^{bc}	39.48^{abc}	0.08	0.02
Overall	39.17^{a}	38.91^{c}	38.94^{bc}	38.89^{c}	39.00^{bc}	38.96^{bc}	39.07^{ab}	0.05	0.001

VC: vitamin C. BET: betaine. EVOO: extra virgin olive oil. GA: gallic acid. VE: vitamin E. LGEO: lemongrass essential oil. SEM: standard error of the mean and n=12 treatments per week. Means within the same row carrying different superscripts are significantly different at $p<0.05$.

skin temperatures were significantly lower in the rabbits in all supplemented groups except LGEO. When the treatments were ranked according to their ability to ameliorate (p<0.01) skin temperature during the experimental period, the addition of EVOO and VC showed the lowest values, followed by BET, VE and GA.

Effect on rabbit ear temperature

As presented in Table 4, the mean ear temperatures of growing rabbits under severe heat stress were significantly (p<0.05) lowered in response to the addition of VC, BET, EVOO, and GA when compared to the temperatures of the control growing rabbits. When comparing the tested additives, VC, BET, GA, and EVOO evoked a larger (p<0.05) reduction in ear temperature, followed by GA and LGEO.

Effect on serum hormones and glucose

The serum hormonal component data showed that the cortisol concentrations were markedly diminished by 50, 32, 31 and 28% (p<0.05) with the addition of VC, EVOO, VE and LGEO, respectively, compared to the control group concentration (Fig. 1 a). Relative to the level in the control group, a notable increase (p<0.05) was observed in the serum T3 and T4 concentrations with all tested dietary supplements except GA and LGEO, which did not cause significant differences in the T3 concentrations (Fig. 1 b and c). As shown in Fig. 1 d, obvious significant (p<0.05) reductions in the blood glucose concentrations were observed with all dietary supplement groups. The insulin levels were not significantly affected by any of the treatments except for the VC group, which evoked a one-fold increase in insulin levels compared to the control values (Fig. 1 e).

Discussion

Heat stress can evoke multiple biological and physiological responses that can become fatal if not appropriately controlled (Ducray *et al.*, 2016). In the rabbit industry, heat stress is an important stressor that affects productive performance, and the use of eco-friendly dietary additives to alleviate the negative impacts of heat stress remains a vital issue (Ayyat *et al.*, 2018). Therefore, the present *in vivo* study aimed to evaluate the beneficial roles of the non-traditional feed additives EVOO, LGEO, GA, and BET in the alleviation of the effects of heat stress in growing rabbits compared to the effects of VC and VE.

Throughout the experimental period, thermoregulatory parameters, including rectal, ear and skin temperatures, were estimated as indicators for the initial response of rabbits to air temperature fluctuations, as adopted by previous investigators (Marai *et al.*, 2007, 2008). In the control group, exposure to severe heat stress (a THI of 84.67) resulted in a significant increase in all body temperature traits compared to those of the other additive-supplemented groups. Failure of the physiological mechanisms of animals to balance the excessive heat loads caused by exposure to high ambient temperatures could be responsible for the increase in both the rectal and ear temperatures of the heat-stressed rabbits (Habeeb *et al.*, 1998). Moreover, the high skin temperatures in hot climates may be

Table 4. Effect of feed additives on ear temperatures of growing New Zealand White rabbits at 7-14 weeks of age.

	Control	VC	BET	EVOO	GA	VE	LGEO	SEM	*p* value
1st week	38.40	38.37	38.35	38.21	38.41	38.50	38.36	0.09	0.54
2nd week	38.65	38.42	38.35	38.61	38.29	38.56	38.65	0.11	0.16
3rd week	38.64	38.56	38.43	38.62	38.50	38.50	38.55	0.10	0.81
4th week	39.03^{a}	38.69^{ab}	38.77^{ab}	38.56^{b}	38.7^{ab}	38.78^{ab}	38.97^{a}	0.11	0.04
5th week	38.84	38.61	38.75	38.90	38.79	38.91	39.02	0.12	0.36
6th week	39.35^{a}	38.99^{b}	38.98^{b}	38.99^{b}	39.07^{ab}	39.12^{ab}	39.28^{a}	0.09	0.02
7th week	39.36	39.15	39.23	39.08	39.15	39.23	39.26	0.07	0.20
8th week	39.38	39.18	39.25	39.23	39.42	39.28	39.40	0.06	0.07
Overall	38.96^{a}	38.74^{b}	38.77^{b}	38.79^{b}	38.78^{b}	38.84^{ab}	38.94^{a}	0.05	0.01

VC: vitamin C. BET: betaine. EVOO: extra virgin olive oil. GA: gallic acid. VE: vitamin E. LGEO: lemongrass essential oil. SEM: standard error of the mean and n=12 treatments per week. Means within the same row carrying different superscripts are significantly different at p<0.05.

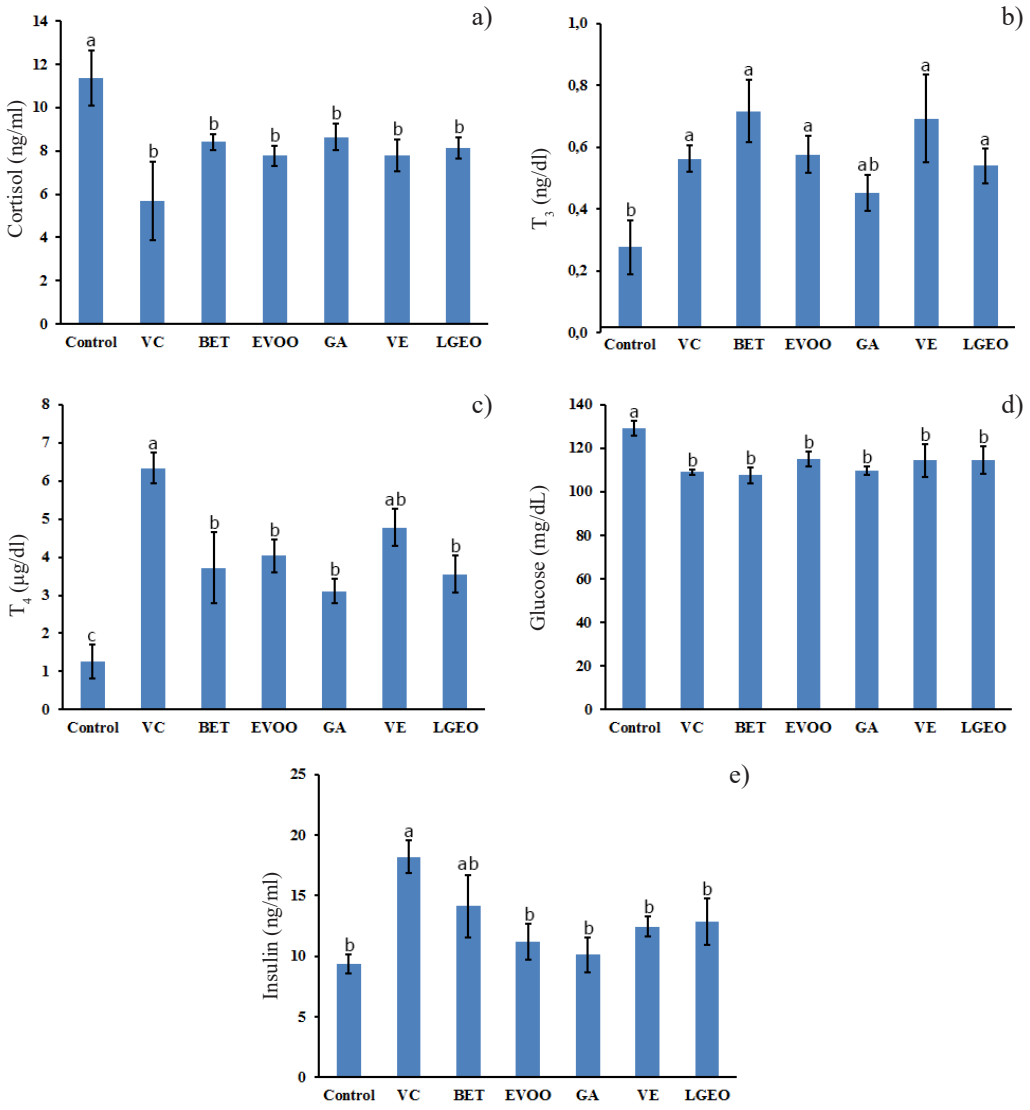

Figure 1. Effect of feed additives (VC: vitamin C, BET: betaine, EVOO: extra virgin olive oil, GA: gallic acid, VE: vitamin E, and LGEO: lemon grass essential oil) on the serum cortisol (a), triiodothyronine (T_3) (b), tetraiodothyronine/thyroxine (T_4) (c), glucose (d), and insulin (e) concentrations of growing New Zealand White rabbits. The values shown are the means ± SEs (n=6/treatment). Bars with different letters significantly differ from one another (p<0.05).

linked to the insulating effect of the hair coat (Marai *et al.*, 2008).

Among the treated groups, EVOO and BET showed significant improvements in different body temperature traits with potencies similar to those of VE, followed by GA and LGEO. Similarly, in slow-growing chicks, dietary supplementation with BET at 1 g/kg and VC at 250 mg/kg was equally potent for partial amelioration of the effects of heat stress on performance (Qota *et al.*, 2008). As an osmolyte and methyl group donor, betaine may maintain the thermo-neutral state of animals by reversing the heat-induced inhibition of the osmotic equilibrium and maintaining the tertiary structures of macromolecules in the kidney and other tissues (Lever *et al.*, 2004; Huang *et al.*, 2007).

High temperatures in mammals are known to cause physiological stress in organisms with enhanced generation of reactive oxygen species (ROS), leading to oxidative damage (Jena *et al.*, 2013). Hence, in the current study, the significant palliative effects of EVOO on various body temperature traits of growing rabbits may have been associated with the phenolic antioxidant components of EVOO, including hydroxytyrosol and oleuropein, and its monounsaturated fatty acids (MUFAs, especially oleic acid). In our earlier study using GC–MS analysis, EVOO was shown to contain 1-octadecene, octacosanol, delta-3-carene, docosane, 17-pentatriacontane, and n-hexadecanoic acid, whereas the major identified fractions of LGEO were citral, α-myrcene, and cis-geraniol (Al-Sagheer *et al.*, 2017).

The components of both EVOO and LGEO were reported to have antioxidant activity (Kumar *et al.*, 2010; Guimarães *et al.*, 2011; Gurnani *et al.*, 2016). Consequently, their antioxidant activity may be the key for their roles in improving rectal, ear and skin temperatures in heat-stressed growing rabbits (Nhu-Trang *et al.*, 2006). GA has also been reported to have potent free radical scavenging and antioxidant activities (Priscilla & Prince, 2009).

In the current experiment, hormonal and glucose assays were performed to further characterize the effects of the tested feed additives on the thermoregulation of heat-stressed growing rabbits. Primarily, a sharp rise in the cortisol levels was found in the control rabbits under heat stress. This outcome could be attributed to activation of the hypothalamic–pituitary–adrenal axis during heat stress conditions that subsequently caused an increase in the serum glucocorticoid concentrations (mainly cortisol). In line with these findings, Kowalska (2011) reported that the blood cortisol level was increased in NZW rabbits exposed to thermal stress. Nevertheless, all tested feed additives had a lowering effect on the rising cortisol levels compared with the control group levels during the overall trial period. This reduction could presumably be linked to their antioxidant activities, because the glucocorticoid response was shown to be associated with changes in redox physiology (Costantini *et al.*, 2011). In our previous study, dietary supplementation with EVOO, GA, or LGEO in heat-stressed growing rabbits enhanced catalase activity and reduced glutathione content, whereas EVOO-treated rabbits had the highest catalase activity and reduced glutathione content. Malondialdehyde activity was also reduced in response to all tested additives (Al-Sagheer *et al.*, 2017). The cortisol-reducing effect of BET may have been due to its positive effect on nitric oxide production, which in turn may inhibit the biosynthesis of glucocorticoids (Monau *et al.*, 2010; Messadek, 2012). GA has also been shown to inhibit the production of both prostaglandins and the enzymes involved in glucocorticoid production (Hsu *et al.*, 2007; Seo *et al.*, 2016).

In this study, thyroid hormone activities were significantly depressed in the non-supplemented heat-stressed growing rabbits. These results are in a harmony with those reported by Habeeb *et al.* (1993) in growing male rabbits, in which the thyroxine hormone decreased significantly by 17.86% when the rabbits were exposed to 35ºC for six hours daily. Gad (1996) also estimated declines in the T_3 levels of 21.7 and 20.7% in NZW and Californian rabbits, respectively. Thyroid hormones are the key hormones in the regulation of metabolism and adaptation of animals to stress (Brecchia *et al.*, 2010). At high ambient temperatures, the activity of thyroid hormones has been suggested to decline due to the decrease in endogenous heat production by the animals and thus depress its growth performance (Leung *et al.*, 1984). In contrast, both BET and EVOO supplementation elevated the serum T_3 and T_4 levels, which could be directly linked to their antioxidant activities; indeed, hypothyroidism has been shown to be related to oxidative stress and cellular damage (Cano-Europa *et al.*, 2012). Notably, VC and VE produced the highest increased in T_4. Similarly, serum concentrations of T_4 increased to a greater extent by increasing dietary VC or VE levels of Japanese quails reared under heat stress (Sahin *et al.*, 2002). Mechanism for increasing the levels of T_4 by VC or VE is not well known (Omidi *et al.*, 2015). However, it may occur in part because of their antioxidant properties (Al-Sagheer *et al.*, 2017).

The blood glucose levels showed significant elevation in the control heat-stressed growing rabbits in the present work. In the same line, Rashidi *et al.* (2010) reported that heat stress condition increased blood glucose levels. The increase in the serum glucose levels under hot climatic conditions could be akin to the decrease in glucose utilization, depression of both catabolic and anabolic enzyme secretions and subsequent reduction in metabolic rates and the preservation of energy (Hassan *et al.*, 2016). Also, the increase in glucose concentration is directly responsive to an increase in glucocorticoids resulting from heat stress (Borges *et al.*, 2007). In contrast, a significant decline in the blood glucose concentration was recorded in all dietary supplement groups. Similarly, in Japanese quails reared under heat stress, blood glucose level was observed to move down constantly with increasing dietary levels of VC or VE (Sahin *et al.*, 2002). Also, Kutlu & Forbes (1993) reported that VC supplementation in heat-stressed broilers significantly decreased blood glucose concentration. Because oxidative stress in the cell blocks normal glucose metabolism, the antioxidant properties of the tested additives could be the key to their beneficial roles in the manipulation of glucose levels (Shah *et al.*, 2007). Additionally, the lowering of glucose level could be related to the reduced secretion of glucocorticoids following antioxidants dietary supplementation (Sarıca *et al.*, 2017). Also, the decline in the glucose levels could be correlated to lesser extent with the effects of the insulin hormone, because most of the dietary groups showed a trend toward increase in insulin levels which was significant in VC treated rabbits compared with the control group. The observed increase in the insulin level is linked to the decrease in glucose due to an improvement of non-oxidative glucose metabolism (Paolisso *et al.*, 1994).

These results suggest that in the warm, subtropical environmental conditions of Egypt, the adverse impacts

of the exposure of growing rabbits to severe heat stress can be alleviated using natural feed additives of plant origin, including extra virgin olive oil, lemongrass essential oil, gallic acid and betaine. The former outcome was obvious in terms of thermoregulatory parameters and glucose and stress hormone levels. Notably, extra virgin olive oil and betaine showed high potency in heat-stress amelioration that was similar to that of the traditional antioxidants vitamin C and vitamin E.

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