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RESEARCH ARTICLE

Palliative effects of extra virgin olive oil, gallic acid, and lemongrass oil dietary supplementation on growth performance, digestibility, carcass traits, and antioxidant status of heat-stressed growing New Zealand White rabbits

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Abstract This study explored the effects of supplemental dietary extra virgin olive oil (EVOO), gallic acid (GA), or lemongrass essential oil (LGEO) on growth performance, nutrient digestibility, carcass traits, lipid peroxidation, hematological, and antioxidative status in growing rabbits under heat stress conditions. A total of 48 male growing New Zealand White rabbits were randomly divided into four equal groups, which received a basal diet without any supplementation or supplemented with 15 g EVOO, 500 mg GA, or 400 mg LGEO/kg of diet, for eight consecutive weeks. Results revealed that the overall mean of temperature humidity index was 84.67 ± 0.35, reflecting a state of severe heat stress. Moreover, dietary supplementation with EVOO, GA, or LGEO significantly increased live body weight and daily body weight gain but decreased both feed conversion ratio and daily water consumption. Additionally, a significant increase in both organic matter and crude protein digestibility besides a remarkable elevation in the nutritive values of digestible crude protein, total digestible nutrients, and digestible energy, as well as an increase in the numbers of WBCs, lymphocytes, and heterophils was significant in EVOO-supplemented rabbits. Supplementation with EVOO, GA, or LGEO in the heat-stressed growing rabbit's diet enhanced catalase activity and reduced glutathione content, whereas EVOO-treated rabbits had the highest values. Also, malondialdehyde activity was reduced in response to all tested additives. In conclusion, these findings suggested that addition of EVOO, GA, or LGEO in growing rabbit's diet could be used effectively to alleviate negative impacts of heat stress load on performance, nutrient digestibility, oxidative status, and hemato-biochemical features. Furthermore, among these additives, EVOO achieved the best effects.

Keywords Extra virgin olive oil · Gallic acid · Lemongrass essential oil · Nutrients digestibility · Heat stress · Antioxidant status · Rabbits

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Introduction

In European and North African countries, rabbit industry plays a vital role in the national economies of these countries (Ebeid et al. 2013). Nevertheless, in recent years, a strong correlation between the progress of this industry and the global rise in surface temperature has been an issue of great interest. In Egypt, during the hot months, the heat stress is aggravated with the high relative humidity which is normally over 85% during the day and can reach 100% during the night (Marai et al. 2007). Additionally, summer and early autumn seasons are not within the thermal neutral zone (Attia et al. 2011).

High ambient temperature causes significantly economic losses in the rabbit industry due to its adverse impacts on feed intake, live body weight gain, feed efficiency, reproductive performance, and health of rabbits (Marai et al. 2002; Finzi et al. 2010). Additionally, heat stress has been reported to suppress diverse components of the immune system and, in that way, enhances vulnerability of an animal to various diseases (Aggarwal and Upadhyay 2013).

Several studies confirmed that the drastic impacts of heat stress are mainly linked to an extreme generation of free

radicals and reactive oxygen species (ROS) and a dwindle in antioxidant resistance (Ganaie et al. 2013; Nisar et al. 2013). Hence, several in vivo and in vitro trials confirmed the importance of antioxidant supplementation in ameliorating heat stress impacts (Alhidary et al. 2012; McKee and Harrison 2013). Vitamins, essential oils, fats, and amino acids are the main dietary supplements with marked antioxidant properties. But, for the time being, the use of natural antioxidants of plant origin has paid great interest from both livestock producers and nutritionists, as such supplements can be added with ease and affordable at low price to the daily diet and are expected to have very few undesirable effects (Tawfeek et al. 2014).

The extra virgin olive oil (EVOO) is obtained by mechanical cold pressing of mesocarps of olives. EVOO has been shown to possess anti-microbial, antioxidant, and anti-inflammatory properties because of its components such as phenolic compounds, tocopherol, and carotenoids (Cicerale et al. 2012). Gallic acid (GA), a metabolite of propyl gallate, is natural polyphenolics known to have various pharmacological actions including antioxidant, anti-carcinogenic, anti-mutagenic, anti-allergic, and anti-inflammatory (Jo et al. 2006).

Lemongrass essential oil (LGEO) is a volatile oil which can be extracted directly from fresh lemongrass (*Cymbopogon citratus*) using steam extraction and the grass contains 0.035% essential oil (Malee et al. 2000). LGEO was characterized by the presence of various phytoconstituent like delta-3-carene, geranial, *trans*-caryophyllene, and menthone. Malee et al. (2000) found a marked enhancement in the productive performance of weanling pigs fed diet supplemented with lemongrass oil.

But, so far, little evidence is yet available with regard the potential beneficial role of EVOO, GA, and LGEO against heat stress in growing rabbit. Hence, the current work was undertaken to investigate potential effects of these additives on growth performance, nutrients digestibility, carcass traits, lipid peroxidation, hematological, and antioxidative status in growing rabbits reared under hot climatic conditions.

Materials and methods

Experimental animals and management

The study was conducted in Rabbit Research Farm, Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The study was initiated in June 2015 and continued for 8 weeks. The Ethics of Animal Use in Research Committee (EAURC) of Zagazig University approved all protocols involving animals here. All experimental procedures were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

A total of 48 growing New Zealand White (NZW) rabbits (male, 5 weeks of age, 640 ± 11.7 g) were used in this study. Rabbits were purchased from the Laboratory Animal Farm at Zagazig University. Throughout the experimental period, rabbits were housed individually in galvanized wire cages (35 × 35 × 60 cm). All animals were kept under the same managerial and hygienic conditions. Throughout the experimental period, each cage contained a feeder and potteries to provide free access to feed and fresh water, respectively. Urine and feces on the rabbitry floor were removed every morning. The feed and water was offered ad libitum and refilled at 8:30 a.m. and 14.30 p.m. daily.

Feed additives and diets preparation

The extra virgin olive oil (Iliada PDO Kalamata Extra virgin olive oil) was obtained from AGRO. VI. M.S.A., (Kalamata, Greece). Gallic acid (99.5% purity) was purchased from Alpha Chemica (Mumbai, India). Lemongrass herbs (*C. citratus*) were collected at May 2015 from different farms in Egypt. Plant materials were stored in cool and dry place for extraction of oil. According to Guenther (1972), the essential oil was isolated by hydrodistillation using a Clevenger type apparatus for 4 h and evaporates the solvent under reduced pressure at 40 °C using rotary evaporator. The essential oil obtained was sterilized by filtration using Millipore cellulose filter membrane (0.45 mm pore diameter) and stored at low temperature.

Gas chromatography/mass spectrometry analysis of EVOO and LGEO

The GC–MS analysis of EVOO and LGEO was carried out at the Molecular and Atomic Physics Unit, Experimental Nuclear Physics Dept., Nuclear Research Centre, Egyptian Atomic Energy Authority, Cairo, Egypt. A 1310 TRACE GC Ultra Gas Chromatographs (Thermo Fisher Scientific Inc., Waltham, MA, USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC–MS system was equipped with a DB5 MS column (30 m × 0.25 mm × 0.25 μm film thickness) (JandW Scientific, Folsom, CA, USA). Analyses were carried out using helium as carrier gas at a flow rate of 1.5 ml/min at a split ratio of 1:10 and the following temperature programs: 50 °C for 1 min; rising at 10 °C/min to 150 °C and held for 2 min; and rising at 5 °C/min to 250 °C and held for 1 min. The injector and detector were held at 250 and 300 °C, respectively. Diluted samples (1:10 hexane, *v*/*v*) of 0.2 μl of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m*/*z* 40–450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and National Institute of Standards and Technology

(NIST) library). The separated components of the essential oil were identified by matching their mass spectra with the NIST published data (Adams 2007).

Experimental design and temperature humidity index calculation

The rabbits were randomly divided into four equal groups (12 rabbits per group): (1) control group, rabbits fed the basal diet without any supplementation; (2) EVOO group, rabbits fed the basal diet supplemented with 15 g EVOO/kg of diet; (3) GA group, rabbits fed the basal diet supplemented with 500 mg GA/kg of diet; and (4) LGEO group, rabbits fed the basal diet supplemented with 400 mg LGEO/kg of diet. All additives were pre-mixed with 1 kg of each diet and successively mixed into the remaining diet to obtain the homogenous inclusion level. The basal diet was formulated to cover the recommended nutrient requirements of growing rabbits according to (NRC 1977). The formulation and chemical analysis of the basal diet are shown in Table 1.

Table 1 Formulation and chemical analysis of the basal diet fed to rabbits

Item	Amount (g/kg)
Ingredients	
Alfalfa hay	330
Barley grain	250
Wheat bran	250
Soybean meal	150
Sodium chloride	5
limestone	10
Mineral[a]	1.5
Vitamins[b]	1.5
DL-Methionine	2
Total	1000
Chemical analysis (on DM basis)	
Organic matter	908.2
Crude protein	182.0
Crude fiber	121.7
Ether extract	32.6
Nitrogen free extract	57.19
Ash	91.8
Neutral detergent fiber (NDF)	321.2
Acid detergent fiber (ADF)	166.3
Hemicellulose	154.9

[a] Each 1.5 kg contains the following: manganese 80 g, zinc 60 g, iron 30 g, copper 4 g, iodine 0.5 g, selenium 0.1 g, and cobalt 0.1 g

[b] Each 1.5 kg contains the following: vitamin A 12000000 IU, vitamin D_3 3,000,000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, biotein 75 mg, folic acid 1000 mg, nicotinic 30,000 mg, and pantothenic acid 10,000 mg

Throughout the experimental period, ambient temperatures and relative humidity were measured in the rabbitry using an automatic thermo-hygrometer (OF 14:140, H 10–99%; TFA Dostmann GmbH + Co. KG, Wertheim, Germany) twice a day at 8:30 a.m. and 14:30 p.m. The temperature humidity index (THI) was calculated according to LPHSI (1990) as the following equation: THI = db°F − [(0.55–0.55RH) (db°F − 58)], where db°F is dry bulb temperature in Fahrenheit degrees, and RH is the relative humidity as a percentage. The THI values obtained were then categorized as follows: <82 = absence of heat stress, 82–<84 = moderate heat stress, 84–<86 = severe heat stress and 86 and more = very severe heat stress.

Growth performance

Feed intake and water consumption were recorded weekly by weighing and measuring the residual amounts of feed and water then subtracting them from the offered before putting the new ones. All rabbits were individually weighed at the beginning of the experiment and at weekly intervals throughout the experimental period. Live body weight (LBW) was recorded in the morning before having access to feed and water at 08:30 a.m. Daily body weight gain (DBWG) and feed conversion ratio (FCR) were calculated according to Berger and Halver (1987). Mortality rate was recorded as a number of dead animals in each group during the whole experimental period.

Digestibility trial

At the last week of the experimental period, four rabbits from each group were randomly chosen and individually housed in metabolic cages (i.e., designed for complete separation of feces) for 7 days digestibility trial. The daily collected feces from each animal was weighed, then all the feces were bulked, 50% subsample was taken, oven dried at 60 °C for 24 h, and stored for laboratory analysis. Feed and feces samples were chemically analyzed according to AOAC (2006). Total digestible nutrients (TDN) were calculated according to Cheeke et al. (1982) as follows

$$\mathrm{TDN}\ (\%) = \mathrm{DCP}\ (\%) + \mathrm{DCF}\ (\%) + \mathrm{DNFE}\ (\%) + (\mathrm{DEE}\ (\%) \times 2.25)$$

The digestible energy (DE) values (Kcal/Kg diet (of the experimental diets were calculated according to Schieman et al. (1972) as follows

$$\mathrm{DE}\ (\mathrm{Kcal/Kg\ diet}) = 5.28\ \mathrm{DCP}\ (\mathrm{g}) + 9.51\ \mathrm{DEE} + 4.2\ (\mathrm{DCF} + \mathrm{DNFE})$$

where DCP = digestible crude protein, DEE = digestible ether extract, DCF = digestible crude fiber and DNFE = digestible nitrogen free extract.

Collection of biologics from the rabbits

Two separate blood samples were collected from the ear vein of each rabbit: 2.5 ml was collected into an EDTA tube for use in hematological evaluations. Another 2.5 ml second sample was taken into a glass tube (without EDTA) and left for 20 min at room temperature to coagulate; after centrifugation at 3000 rpm for 10 min, the generated serum was isolated and placed at −20 °C until used (within 2 weeks) in the biochemical assays outlined below.

Hematological study and biochemical assay

In the whole blood samples, determinations of total red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, lymphocytes, granulocytes, and monocytes were carried out according to the method of (Grindem 2011) using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy). Oxidative status was assessed by evaluation of the enzymatic antioxidant biomarker, catalase (CAT), according to Aebi (1984). Reduced glutathione (GSH) determinations were made using the protocol described by Beutler et al. (1963). Lipid peroxidation was evaluated through measurement of malondialdehyde (MDA) according to Uchiyama and Mihara (1978).

Carcass traits

At the end of the experimental period (13 weeks of age), 16 rabbits from the different experimental groups were randomly kept off feed for 12 h, individually weighed and slaughtered for carcass traits. After bleeding, rabbits were weighed and skinned. After skinning, the carcasses were weighed before and after viscera removal. According to Blasco et al. (1993), the weight of the liver, kidneys, heart, lungs, testes, and caecum were weighed and expressed as grams per kilogram of slaughter weight (SW). Also, caecum length was measured. Carcass part weight was measured as whole carcass and each part (fore, mid, and hind part) as relatives to carcass weight. Dressing percentage was calculated by dividing the carcass weight on pre-slaughter weight and expressed as a percentage.

Statistical analysis

The differences among treatments were statistically analyzed with a one-way ANOVA test in a completely randomized design as the following model

$$Y_{ij} = \mu + T_i + e_{ij}$$

where μ = the overall mean, T_i = the fixed effect of treatment, and e_{ij} = residual error. The significant differences among means were compared using Duncan's new multiple-range test (Duncan 1955). Statistics 21 software (SPSS, Chicago, IL) was used for analysis of the data.

Results

EVOO and LGEO constituents by GC–MS analysis

The GC–MS analysis of EVOO and LGEO revealed the main constituents, their relative percentage of the total peak area, and retention times as presented in Tables 2 and 3 and Figs. 1 and 2, respectively. 1-Octadecene (24.27%), octacosanol (11.97%), delta-3-carene (7.44%), docosane (7.31%), 17-pentatriacontane (5.31%), and *n*-hexadecanoic acid (3.32%) were the main components identified in EVOO. Citral (63.07%) (both E and Z isomers) was the major constituent identified in LGEO followed by α-myrcene (14.17%) and *cis*-geraniol (3.63%).

Temperature humidity index

As shown in Fig. 3, during the first and second week of the experiment, the calculated THI were 80.87 and 81.99, respectively, eluciditaing no heat stress. However, from the the beginig of the third till the eight week of the experiment, the estimated THI values ranged from 84.08 to 86.95, indicating a flactuation of heat stress from severe to very sevare degree. Morever, the overall mean of THI throuhgout the experiments was 84.67, reflecting a state of severe heat stress.

Effects on growth performance, water consumption, and carcass traits

The effects of dietary EVOO, GA, or LGEO supplementation on growth performance and water consumption of heat-stressed growing rabbits are presented in Tables 4 and 5, respectively. By the end of the eight week of the trial, LBW was significantly higher in all supplemented groups than that of the control group ($p < 0.05$). Growing rabbits supplemented with EVOO, GA, or LGEO had significantly ($p < 0.05$) higher values of DBWG by 14.57, 11.29, and 14.90% through out the experimental period compared with the non-supplemented ones. On the other hand, along the experimental period, there was no marked effects on DFI attributable to the supplementation of diet with any of the tested additives. Furthermore, a significant ($p < 0.05$) decrease in FCR was evident in growing rabbits supplemented with EVOO or LGEO by 13.55 and 12.44% with respect to the control ones. Generally, mortality rate was low and there was no difference among the groups. The results of water consumption as milliliters per head, milliliters per kilogram body weight, and milliliters per gram feed

Table 2 Chemical composition of extra virgin olive oil (EVOO) detected by gas chromatography/mass spectrometry

Peak	Compounds	MW	Formula	R_t	Area%
1.	1-Octadecene	252	$C_{18}H_{36}$	17.51	24.27
2.	Octacosanol	410	$C_{28}H_{58}O$	21.66	11.97
3.	Delta-3-Carene	136	$C_{10}H_{16}$	3.89	7.44
4.	Docosane	310	$C_{22}H_{46}$	5.88	7.31
5.	17-Pentatriacontane	490	$C_{35}H_{70}$	9.40	5.31
6.	*n*-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	22.86	3.32
7.	8-Octadecanone	268	$C_{18}H_{36}O$	20.24	3.15
8.	1-Decanol, 2-hexyl-	242	$C_{16}H_{34}O$	7.50	2.81
9.	Eicosyl nonyl ether	424	$C_{29}H_{60}O$	17.39	2.56
10.	Nonacosane	408	$C_{29}H_{60}$	15.48	2.54
11.	Dodecane, 5,8-diethyl-	226	$C_{16}H_{34}$	7.39	1.89
12.	2,5-Di-tert-butyl-1,4-benzoquinone	220	$C_{14}H_{20}O_2$	11.91	1.63
13.	Clocortolone pivalate	494	$C_{27}H_{36}ClFO_5$	18.49	1.46
14.	Cyclohexane,1,1′-(1,4-butanediyl)bis-	222	$C_{16}H_{30}$	14.72	1.97
15.	Cyclohexane, 1,1′-dodecylidenebis[4-methyl-	362	$C_{26}H_{50}$	5.27	1.81
16.	8-Pentadecanone	226	$C_{15}H_{30}O$	16.12	1.27
17.	Lucenin 2	610	$C_{27}H_{30}O_{16}$	20.65	1.28
18.	Undecane (alkanes)	156	$C_{11}H_{24}$	3.52	0.79
19.	Glycyl-L-histidyl-L-lysine acetate			6.23	0.71
20.	25-Norisopropyl-9,19-cyclolanostan-22-en-24-one,3-acetoxy-24-phenyl-4,4,14-trimethyl-	516	$C_{35}H_{48}O_3$	4.73	0.56
21.	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	378	$C_{27}H_{54}$	10.75	0.54
22.	2(3H)-Furanone, 5-dodecyldihydro- (hexadecanoic acid)	254	$C_{16}H_{30}O_2$	17.17	0.49
23.	16-Octadecenal	266	$C_{18}H_{34}O$	19.51	0.46
24.	2-Acetyl-3-(2-cinnamido) ethyl-7-methoxyindole	362	$C_{22}H_{22}N_2O_3$	10.49	0.44
25.	Cyclohexane, 1,4-dimethyl-2-octadecyl-	364	$C_{26}H_{52}$	8.50	0.40
26.	7-Methyl-1,3,5-cycloheptatriene	106	C_8H_{10}	3.13	0.33
27.	Cyclohexane,1,1′-dodecylidenebis[4-methyl- (cyclohexane)	362	$C_{26}H_{50}$	7.84	0.33
28.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	366	$C_{26}H_{54}$	16.76	0.32
29.	Quercetin	344	$C_{18}H_{16}O_7$	5.81	0.17

R_t retention time (min); MW molecular weight

intake (Table 5) showed that the different estimated values were significantly ($p < 0.01$) decreased as a result of EVOO,GA, or LGEO addition than that of the basal ration. In particular, daily water consumption (ml/g feed intake) in of heat-stressed growing rabbits was significantly ($p < 0.001$) reduced by 28.35, 25.52, and 28.35% in response to supplementation of EVOO, GA, or LGEO, respectively, compared to control rabbits.

As presented in Table 6, no significant difference was recorded in dressing percentage and the proportions of various carcass organs of the heat-stressed growing rabbits fed diet supplemented with EVOO, GA, or LGEO compared to the control group. Concerning the carcass parts, only the mid part showed significant increase in the group fed diet supplemented with the feed additives, while the other carcass parts showed no significant changes compared with control.

Effect on nutrient digestibility

The influences of dietary supplementation with EVOO, GA, or LGEO on dry matter, organic matter (OM), ether extract, crude protein (CP), and nitrogen free extract digestibilities and DCP,TDN and DE values of heat-stressed growing rabbits are shown in Table 7. Both OM and CP digestibility were significantly ($p < 0.05$) higher in growing rabbits fed diets supplemented with EVOO by 5.39 and 6.93%, respectively, compared to control group. Also, a significant ($p < 0.05$) elevation in the nutritive values of DCP, TDN, and DE was recorded in the EVOO-supplemented rabbits by 6.62, 5.41, and 5.47%, respectively, compared to the non-supplemented ones. LGEO supplementation to the heat-stressed growing rabbits evoked a significant ($p < 0.05$) increase in the digestibility of CP (3.66%) and the nutritive values of DCP (3.65%) compared to control group. Despite the increasing trend in nutrient

Table 3 Chemical composition of lemongrass essential oil (LGEO) detected by gas chromatography/mass spectrometry

Peak	Compounds	MW	Formula	R_t	Area%
1-	2,6-Octadienal, 3,7-dimethyl-, (E)- (α-citral)	152	$C_{10}H_{16}O$	7.33	32.65
2-	2,6-Octadienal, 3,7-dimethyl-, (Z)- (β-citral)	152	$C_{10}H_{16}O$	6.76	30.42
3-	α-Myrcene	136	$C_{10}H_{16}$	3.49	14.17
4-	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (*cis*-geraniol)	154	$C_{10}H_{18}O$	6.54	3.63
5-	Linalool	154	$C_{10}H_{18}O$	4.47	2.70
6-	2-(1-Hydroxyethyl)-2-cyclopenten-1-one	126	$C_7H_{10}O_2$	3.75	2.41
7-	Isoneral	152	$C_{10}H_{16}O$	5.60	2.41
8-	(R)-Lavandulyl acetate	196	$C_{12}H_{20}O_2$	9.03	1.67
9-	Limonene oxide	152	$C_{10}H_{16}O$	5.34	1.25
10-	177-Trimethylbicyclo[2.2.1]heptan-2-ol (borneol)	154	$C_{10}H_{18}O$	5.15	0.67
12-	2,2-Dideuterooctadecanal	268	$C_{18}H_{34}D_2O$	18.89	0.44
13-	1-Benzoxepin-2(3H)-one, octahydro-	168	$C_{10}H_{16}O_2$	6.19	0.38
14-	*trans*-Ocimene	136	$C_{10}H_{16}$	3.90	0.30
15-	1-Cyclohexene-1-acetaldehy de, α,2-dimethyl-	152	$C_{10}H_{16}O$	5.03	0.25
16-	*trans*-α-Bergamotene	204	$C_{15}H_{24}$	12.24	0.21
17-	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (−)- (β-chamigrene)	204	$C_{15}H_{24}$	12.80	0.21
18-	16-Octadecenal	66	$C_{18}H_{34}O$	17.91	0.21
19-	*trans*-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	21.01	0.21
20-	Caryophyllene oxide	220	$C_{15}H_{24}O$	16.28	0.20
21-	Benzene, 1-(1,5-dimethylhexyl)-4-methyl- (dihydrocurcumene)	204	$C_{15}H_{24}$	9.83	0.19
22-	*trans*-Caryophyllene	204	$C_{15}H_{24}$	10.58	0.18
23-	1-Eicosanol	298	$C_{20}H_{42}O$	13.42	0.17
24-	2,3-Epoxy-gerianial	168	$C_{10}H_{16}O_2$	10.02	0.16
25-	Carveol, dihydro-, *cis*-	154	$C_{10}H_{18}O$	3.26	0.16
26-	2,6-Octadienoic acid, 3,7-dimethyl-, (E)- (geranic acid)	168	$C_{10}H_{16}O_2$	8.57	0.14
27-	Oleic acid	282	$C_{18}H_{34}O_2$	20.88	0.14
28-	17-Pentatriacontane	490	$C_{35}H_70$	21.94	0.13
29-	Isobutyric acid	224	$C_{14}H_{24}O_2$	14.42	0.11
30-	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, oxime (camphor)	167	$C_{10}H_{17}NO$	18.60	0.11
31-	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	114	$C_6H_{10}O_2$	4.21	0.10

R_t retention time (min); MW molecular weight

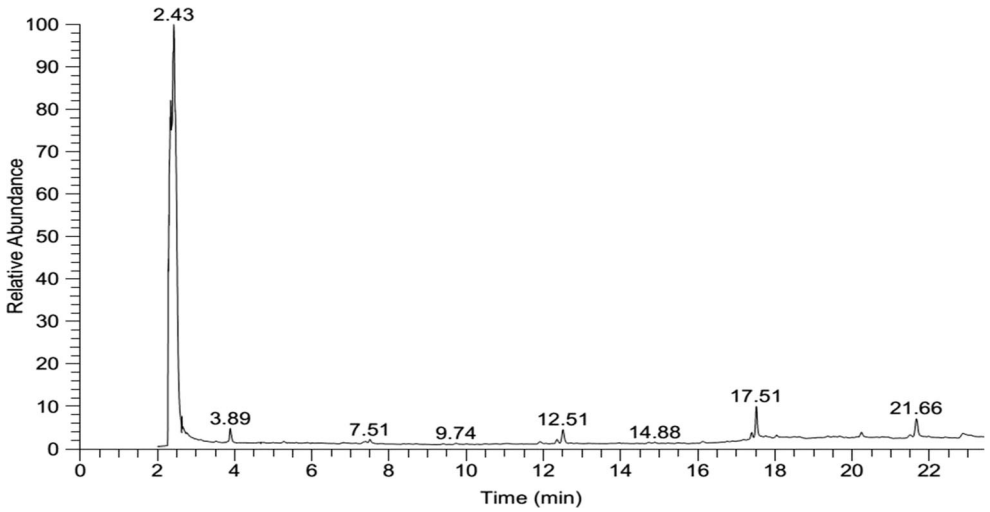

Fig. 1 Gas chromatographic chromatogram of extra virgin olive oil (EVOO)

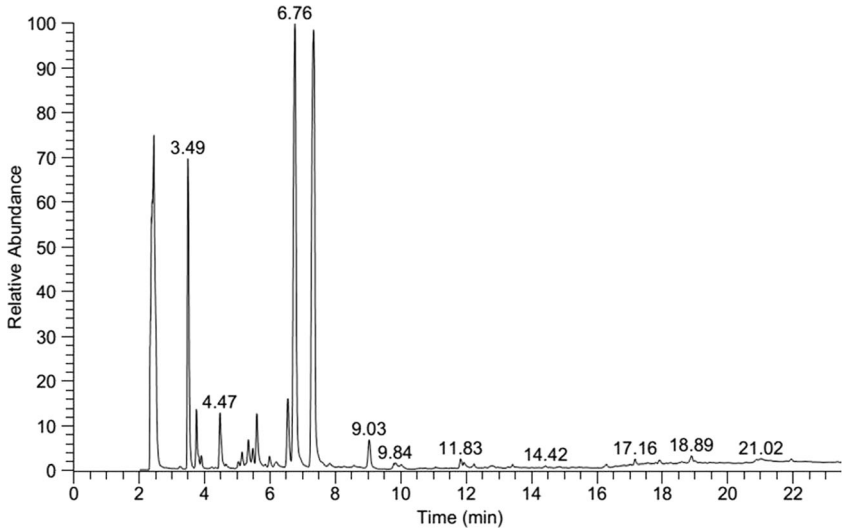

Fig. 2 Gas chromatographic chromatogram of lemongrass essential oil (LGEO)

digestibility and DCP, TDN, and DE values of heat-stressed growing rabbits fed GA-supplemented diets, these values were still not significant from control.

Effect on hematological parameters

The effects of 8-weeks supplementation of heat-stressed growing rabbits with EVOO, GA, or LGEO on hematological variables (erythrograms and leukograms) are shown in Table 8. With regard to the erythrograms, the mean values of RBCs, Hb, MCV, MCHC, and platelet count were not significantly different in all supplemented growing rabbits relative to those in the control one. On the other hand, relative to control rabbits levels, the numbers of WBCs, lymphocytes, and heterophils were significantly increased only in EVOO-supplemented rabbits by 96.19, 89.18, and 400%, respectively, compared to those in the control one. The later values showed a trend toward increase in GA, or LGEO groups, but these values still not significant from control.

Effect on antioxidants and oxidative stress indices

The effects of dietary supplementation with EVOO, GA, or LGEO on enzymatic (CAT) and non-enzymatic (GSH) antioxidant of growing rabbits are graphically presented in Fig. 4. The inclusion of EVOO, GA, or LGEO in the heat-stressed growing rabbits diet significantly ($p < 0.05$) increased the serum catalase activity by 55, 44, and 27% relative to the control group, while the pool of GSH was significantly ($p < 0.05$) maximized in response to EVOO or LGEO by more than two-fold, respectively, compared to the corresponding value of the controls. Also, a trend toward the increase in GSH content was observed GA-supplemented group, but without a significant difference with the control group. As shown in Fig. 5, using MDA as an indicator of lipid oxidation, it could be noted that EVOO, GA, or LGEO supplementation significantly ($p < 0.05$) suppressed MDA values in heat-stressed growing rabbits by 52, 43, and 40%, respectively, compared to the control group.

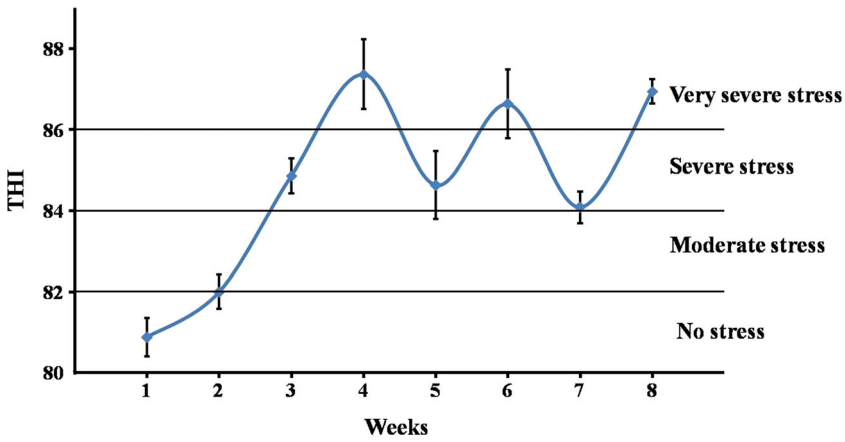

Fig. 3 Calculated temperature-humidity index (THI) throughout the experimental period in Egypt, June–July, 2015

Table 4 Effect of feed additives (EVOO, extra virgin olive oil; *GA*, gallic acid; LGEO, lemongrass essential oil) on growth performance in growing New Zealand White rabbits ($n = 12$)

	Experimental diets				SEM	*p* value
	Control	EVOO	GA	LGEO		
Live body weight (g) at						
5 weeks	646	642	637	639	15.24	0.998
9 weeks	1194	1319	1308	1302	21.78	0.133
Final (13 weeks)	1822^{b}	1990^{a}	1946^{a}	1990^{a}	23.17	0.025
Daily body weight gain (g)						
5–9 weeks	19.59	24.17	23.95	23.64	0.57	0.057
9–13 weeks	22.41	23.98	22.79	24.60	0.45	0.081
Overall (5–13 weeks)	21.00^{b}	24.06^{a}	23.37^{a}	24.13^{a}	0.36	0.001
Daily feed intake (g)						
5–9 weeks	72.46	72.90	78.05	79.81	1.40	0.146
9–13 weeks	122.21	120.08	125.19	119.37	1.14	0.286
Overall (5–13 weeks)	94.53	93.55	101.27	94.98	1.86	0.499
Feed conversion ratio (g feed/g gain)						
5–9 weeks	3.70^{a}	3.02^{c}	3.26^{bc}	3.38^{ab}	0.07	<0.001
9–13 weeks	5.45^{a}	5.01^{b}	5.49^{a}	4.85^{b}	0.07	<0.001
Overall (5–13 weeks)	4.50^{a}	3.89^{b}	4.33^{a}	3.94^{b}	0.07	0.014
No. of dead rabbits	2	1	1	1	–	–

Means in the same row bearing different letters differ significantly ($p < 0.05$)

SEM standard error of the means

Discussion

In rabbit industry, heat stress is a major cause of production loss whereas usage of safe dietary additives to maintain the animal within the range of its thermo-neutral state remains a challenge (El Saidy et al. 2016). Therefore, the present study sought to assess the beneficial role of phytogenic feed additives EVOO, GA, and LGEO in alleviating heat stress impacts in growing rabbits.

Herein, throughout the experimental period, growing rabbits suffer from severe heat stress as reflected by the high values of THI (84.67 on average). Nonetheless, distinct betterment in growth performance and nutrient digestibility were observed as a result of diet supplementation with EVOO, GA, and LGEO. To our knowledge, no previous reports of EVOO

Table 5 Effect of feed additives (EVOO, extra virgin olive oil; GA, gallic acid; LGEO, lemongrass essential oil) on daily water consumption in growing New Zealand White rabbits ($n = 12$)

	Experimental diets				SEM	*p* value
	Control	EVOO	GA	LGEO		
Daily water consumption as: ml/head						
5–9 weeks	246^{a}	212^{b}	183^{b}	186^{b}	6.03	<0.001
9–13 weeks	387^{a}	314^{b}	331^{b}	330^{b}	8.72	0.011
Overall (5–13 weeks)	315^{a}	263^{b}	254^{b}	242^{b}	7.72	0.002
ml/kg body weight						
5–9 weeks	253^{a}	206^{b}	182^{b}	186^{b}	7.03	<0.001
9–13 weeks	252^{a}	188^{b}	206^{b}	190^{b}	6.10	<0.001
Overall (5–13 weeks)	251^{a}	201^{b}	195^{b}	185^{b}	6.22	<0.001
ml/g feed intake						
5–9 weeks	3.92^{a}	2.98^{b}	2.80^{b}	2.72^{b}	0.10	<0.001
9–13 weeks	3.85^{a}	2.58^{b}	3.07^{b}	2.85^{b}	0.11	<0.001
Overall (5–13 weeks)	3.88^{a}	2.78^{b}	2.89^{b}	2.78^{b}	0.09	<0.001

Means in the same row bearing different letters differ significantly ($p < 0.05$)

SEM standard error of the means

Table 6 Effect of feed additives (EVOO, extra virgin olive oil; GA, gallic acid; LGEO, lemongrass essential oil) on dressing (%), parts relative to carcass weight (CW), and organs relative to slaughter weight (SW) in growing New Zealand White rabbits ($n = 4$)

	Experimental diets				SEM	p value
	Control	EVOO	GA	LGEO		
Dressing, %	60.27	60.94	60.49	61.36	0.29	0.641
Fore part, %CW	34.29	33.21	32.85	34.61	0.39	0.343
Mid part, %CW	18.90^{b}	24.41^{a}	24.36^{a}	24.31^{a}	0.76	0.004
Hind part, %CW	30.08	32.59	31.51	31.22	0.42	0.211
Liver, g/kg SW	26.16	27.13	28.17	24.26	0.98	0.581
Kidney, g/kg SW	5.83	6.08	5.87	5.27	0.13	0.150
Heart, g/kg SW	2.61	2.50	2.71	2.38	0.08	0.560
Lunges, g/kg SW	6.50	7.03	6.57	6.52	0.20	0.798
Testes, g/kg SW	3.15	3.05	2.93	3.31	0.07	0.318
Spleen, g/kg SW	0.43	0.49	0.43	0.52	0.04	0.877
Caecum, g/kg SW	4.01	3.64	3.11	3.07	0.17	0.135
Caecum length, cm	10.68	11.65	11.20	11.03	0.19	0.348

Means in the same row bearing different letters differ significantly ($p < 0.05$)

SEM standard error of the means

effects on growth performance and nutrient digestibility in heat-stressed growing rabbits have been published. However, regarding LGEO, comparable results in weanling pigs were observed by Malee et al. (2000) who demonstrated that LGEO addition to their diets resulted in higher productive performance. Likewise, supplementation with chestnut tannins, derivatives of GA, enhanced the growth performance of rabbits reared under high ambient temperature (35 °C) (Liu et al. 2011).

High ambient temperature has been reported to decrease growth performance, probably because of excessive reactive oxygen species (ROS) that oxidize and destroy cellular biological molecules, inhibit some ATPases activities ($Na^{+}K^{+}$-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase), and lastly cause many impairments to intestinal tissues and impaired growth and feed utilization (Hayashi et al. 1994; Payne and Southern 2005; Zhao and Shen 2005; Josephine et al. 2008). In this context, Kermauner and Laurenčič (2008) reported that the natural antioxidants can guard intestinal mucosa against oxidative damage and pathogens and limit peristaltic movement in digestive disorders preventing diarrhea and enhancing animal performance. Consequently, it could postulate that such improvements in growth performance in response to EVOO and LGEO could be linked to the antioxidant activity of their components detected by GC–MS analysis. 1-Octadecene, octacosanol, delta-3-carene, docosane, 17-pentatriacontane, and *n*-hexadecanoic acid represent the main fractions of EVOO and have already been reported to have antioxidant and anti-microbial activity (Gurnani et al. 2016; Kumar et al. 2010). Also, the immunomodulating activity of EVOO could partially explain its favorable effects in rabbits' growth (Khalil et al. 2013). Whereas major identified fractions of LGEO were citral, α-myrcene, and *cis*-geraniol which have been known by their antioxidant potential (Guimarães et al. 2011), this has been confirmed by the GC–MS characterization of EVOO and LGEO in the current study.

In the same way, GA has been known with its significant biological activities such as antioxidant and anti-inflammatory (Ow and Stupans 2003). Additionally, Liu et al. (2011) demonstrated that the dietary inclusion of GA derivatives in heat-stressed rabbit significantly solve troubles related to heat stress-induced intestinal impairment through maintaining the

Table 7 Effect of feed additives (EVOO, extra virgin olive oil; GA, gallic acid; LGEO, lemongrass essential oil) on digestibility and nutritive values in growing New Zealand White rabbits ($n = 4$)

	Experimental diets				SEM	p value
	Control	EVOO	GA	LGEO		
Nutrient digestibility (%)						
Dry matter	64.12	67.69	65.10	65.66	0.52	0.065
Organic matter	64.99^{b}	68.49^{a}	65.77^{b}	66.22^{ab}	0.51	0.049
Ether extract	66.91	71.00	70.17	69.35	1.14	0.646
Crude protein	73.81^{c}	78.75^{a}	75.67^{bc}	76.51^{ab}	0.60	0.007
Crude fiber	34.32	39.93	35.70	35.88	1.03	0.228
NFE	68.59	71.16	68.77	69.22	0.56	0.347
Nutritive values						
DCP (%)	13.44^{c}	14.33^{a}	13.77^{bc}	13.93^{ab}	0.11	0.007
TDN (%)	61.75^{b}	65.09^{a}	62.59^{b}	62.96^{ab}	0.47	0.039
DE (Kcal)[a]	2740^{b}	2890^{a}	2779^{b}	2796^{ab}	20.77	0.034

Means in the same row bearing different letters differ significantly ($p < 0.05$)

SEM standard error of the means; NFE nitrogen free extract; DCP digestible crude protein; TDN Total digestible nutrients; DE digestible energy.

[a] Calculated according to Schieman et al. (1972)

Table 8 Effect of feed additives (EVOO, extra virgin olive oil; GA, gallic acid; LGEO, lemongrass essential oil) on hematologic variables in growing New Zealand White rabbits ($n = 4$)

	Experimental diets				SEM	*p* value
	Control	EVOO	GA	LGEO		
Erythrogram						
RBCs (10^6/mm^3)	4.27	4.26	4.37	4.50	0.10	0.855
Hb (gm/dl)	10.15	9.98	10.40	11.00	0.18	0.191
PCV%	28.68	28.55	29.85	30.58	0.77	0.796
MCV(Fl)	67.19	67.13	68.33	67.83	0.44	0.773
MCHC (%)	35.76	35.01	34.91	36.17	0.53	0.842
Platelets	225	232	300	238	23.43	0.701
Leukogram						
WBCs (10^3/mm^3)	2.89^b	5.67^a	2.92^b	2.95^b	0.42	0.027
Lymphocyte (10^3/mm^3)	2.68^b	5.07^a	2.68^b	2.74^b	0.36	0.025
Heterophil (10^3/mm^3)	0.06^b	0.24^a	0.08^b	0.07^b	0.03	0.043

Means in the same row bearing different letters differ significantly ($p < 0.05$)

SEM standard error of the means

membrane integrity which might be responsible, at least in part, on its positive effect on performance. Also possibly, the improvement of growth performance achieved with GA could be related to its inhibitory role in the production of both prostaglandins and the enzymes involved in glucocorticoid production, corticosterone, which negatively affects growth of stressed animals (Hsu et al. 2007; Seo et al. 2016).

In the current trial, the highest daily water consumption of the control group is compatible with the outcome of Badr (2015) in rabbit does. This could be a way through which rabbits compensate water loss in respiratory vaporization (Habeeb et al. 1993). On the contrary, the notable reduction of water consumption in response to supplementation of EVOO, GA, or LGEO reduction could be possibly related to the ameliorative effect of the tested additives on animal's

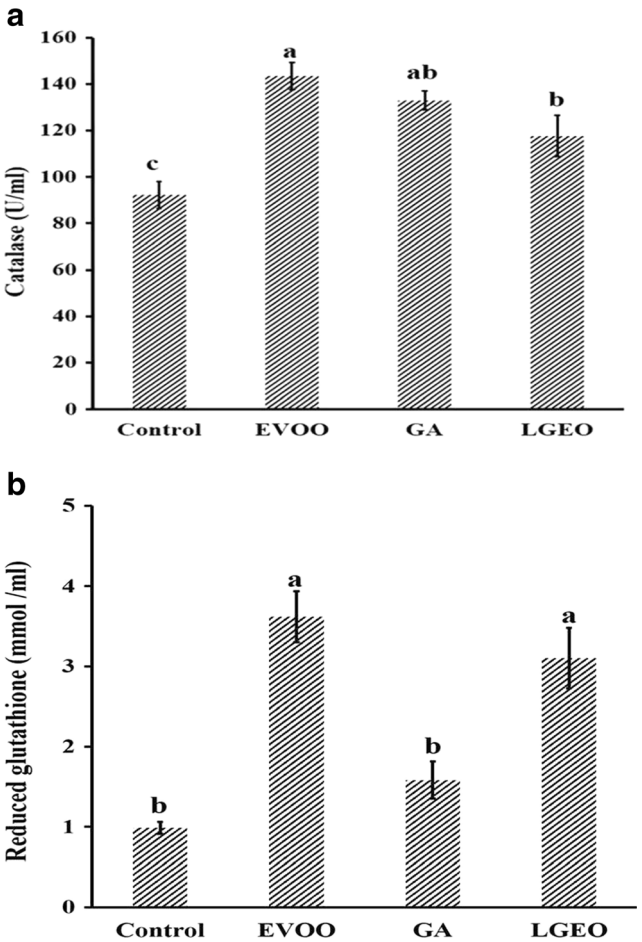

Fig. 4 Effect of feed additives (*EVOO* extra virgin olive oil, *GA* gallic acid, *LGEO* lemongrass essential oil) on catalase (**a**) and reduced glutathione (**b**) concentration in growing New Zealand White rabbits. The values shown are the means ± SE ($n = 3$). *Bars* with different letters significantly differ from one another ($p < 0.05$)

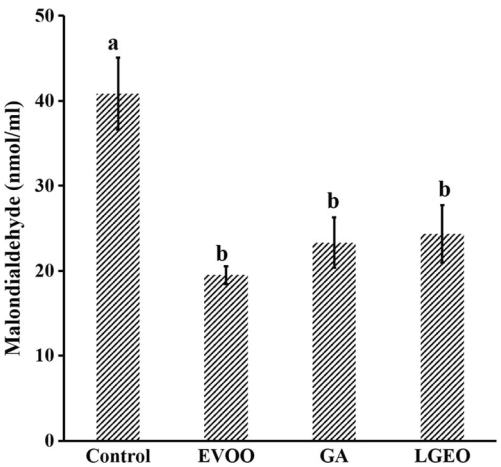

Fig. 5 Effect of feed additives (*EVOO* extra virgin olive oil, *GA* gallic acid, *LGEO* lemongrass essential oil) on malondialdehyde level in growing New Zealand White rabbits. The values shown are the means ± SE ($n = 3$). *Bars* with different letters significantly differ from one another ($p < 0.05$)

rectal, ear, and skin temperatures (unpublished data). On the other hand, there is not any significant effect of EVOO, GA, or LGEO on carcass traits except for an appreciated increase in med parts percentage relative to slaughter weight in response to all tested additives. Similarly, the dietary inclusion of increasing levels of chestnut hydrolysable tannin (200, 400, and 600 g/100 kg), GA is one of its metabolites, in growing rabbits for 9 weeks does not provide any improvements in carcass traits (Dalle Zotte et al. 2012).

In the present study, EVOO supplementation resulted in significant increases in lymphocytes and heterophils count. Such elevations might be due to activation of gut-associated lymphoid tissue in response to the diet supplemented with EVOO or its cytoprotective activity against free radical-induced injury during heat stress (Khalil et al. 2013).

In hot climates, an ample of oxygen-derived free radicals is generated as a part of the heat stress syndrome causing oxidative damage of macromolecules (Ganaie et al. 2013; Sahin and Kucuk 2003). Additionally, catecholamines and corticosteroids are released in excess inducing lipid peroxidative damage (Bahrami et al. 2012). Hence, in the current study, to clarify the underlying mechanism of the tested feed additives in ameliorating heat stress impacts in growing rabbits, antioxidants and lipid peroxidation assays were performed. Primarily, a sharp decline in GSH content and CAT activity concomitant with a rise in the MDA level was evident in the control growing rabbits as a result of heat stress induced oxidative stress and lipid peroxidation. CAT present in high concentrations within the cells and is considered the first line of defense against ROS (Speranza et al. 1993). GSH is a major non-protein thiol present in erythrocytes and is commonly used to defend the cells against oxidative stress (Storey 1996). The decline of GSH content and CAT activity could be due to their consumption during combating the generated heat stress-induced ROS to maintain the steady state concentrations of generated free radicals (Belhadj Slimen et al. 2016). Also, these findings are in parallel with the results of the previous literature of Altan et al. (2000) who found that MDA concentrations were greater in birds exposed to high temperatures than unexposed one.

Controversially, heat-stressed growing rabbits fed diets supplemented with EVOO, GA, or LGEO had higher concentrations of CAT and GSH indicating a notable improvement of their oxidative status. Similar findings have been observed by Cicerale et al. (2010) and Guimarães et al. (2011) who demonstrated that EVOO and LGEO phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, and inflammatory markers. Also, Saber et al. (2015) confirmed that EVOO combat oxidative stress by elevating the activity of antioxidant enzymes and reducing MDA level. Also, data from literature reported that GA has a positive effect on the antioxidative properties, as it may directly combine with free radicals and lead to their inactivation, which in turn may decrease the intracellular concentration of free radicals (Kim 2007; Priscilla and Prince 2009). As GA has tri-hydroxyl groups in its molecular structure, thereby Lu et al. (2006) postulated that the hydroxyl group at the *para* position to the carboxylic group is chiefly effectual for GA antioxidant activity.

Conclusion

Based on the data presented above, it could be concluded that, during hot seasons in the subtropics, dietary supplements such as EVOO, GA, and LGEO, particularly EVOO, offers an easily applicable additive for enhancing growth performance, nutrient digestibility, lipid peroxidation, and antioxidative status of growing rabbits without probable side effects. Future studies are needed to evaluate the effects dietary inclusion of the aforementioned additives or their combinations on rabbit performance under normal thermal temperature.

Compliance with ethical standards The Ethics of Animal Use in Research Committee (EAURC) of Zagazig University approved all protocols involving animals here. All experimental procedures were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

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