



## Original Research Article

# Thyme oil inclusion levels in a rabbit ration: Evaluation of productive performance, carcass criteria and meat quality under hot environmental conditions



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## ARTICLE INFO

## Article history:

Received 29 August 2017

Received in revised form

20 January 2018

Accepted 28 February 2018

Available online 6 March 2018

## Keywords:

Antioxidant

Essential oil

Meat characteristics

Nutrition

Production

## ABSTRACT

The aim of this study was to determine the impact of thyme essential oil supplementation in rabbit rations on performance, carcass criteria, and meat quality under hot environmental conditions. A total of 75, 4-week-old Californian male rabbits were assigned to 5 dietary treatments until 12 weeks of age. The rabbits were reared in an open house system (38 °C average ambient temperature and 26% to 35% relative humidity). Treatments were as follows: unsupplemented standard ration, negative control (CON); standard ration supplemented with 1.50 g/kg olive oil as carrier, positive control (POS); POS + 50 mg/kg thyme oil (TO1); POS + 100 mg/kg thyme oil (TO2); and POS + 150 mg/kg thyme oil (TO3). Dietary thyme oil up to 150 mg/kg improved ( $P < 0.001$ ) feed intake and growth performance in comparison to CON. The highest average daily gain and most efficient feed conversion ratio (linear,  $P < 0.001$  and quadratic,  $P < 0.001$ ) were found for TO1 followed by TO2, TO3 and POS, respectively. The incorporation of thyme oil improved ( $P < 0.001$ ) carcass criteria and decreased ( $P < 0.001$ ) perirenal and scapular fat without any side effects on internal organs. Notably, the water holding capacity of rabbit meat was greater ( $P < 0.001$ ), and the lipid oxidation was lower ( $P < 0.01$ ) in rabbits fed treated rations compared with CON. Differences were also recorded in oxy-myoglobin and metmyoglobin contents in rabbit meat among treatments. In conclusion, thyme oil of a specified composition and to be added to a rabbit ration up to 100 mg/kg using 1.50 g/kg olive oil as a carrier can be used as an efficient feed additive for improving productive performance of rabbits under hot environmental conditions.

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## 1. Introduction

Recently, rabbit production in Egypt has developed rapidly, most notably to meet an increased demand of fresh meat for human consumption as well as a source of extra income for families or small farmers in Upper Egypt (Abdel-Wareth et al., 2015). Food security and environmental conditions remain 2 major challenges facing meat production in Egypt. A common nutritional strategy to increase food security and total income as well as to reduce hot environmental conditions is to use phyto-genic substances as feed additives. Rabbits are very sensitive to high temperatures because they have few functional sweat glands and, therefore, have difficulty in eliminating excess body heat (Marai et al., 2002) which can

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



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impact on metabolism, performance, carcass criteria and meat quality (Zeferino et al., 2013).

Phytochemicals are generally regarded as safe and are frequently used in the food and feed industries (Rašković et al., 2015). Thyme (*Thymus vulgaris* L.) is a flavourful evergreen herb cultivated in many other parts of the world and has traditionally been used in herbal medicine. Thyme has antimicrobial and antioxidant properties and, mainly due to its active components, enhances appetite and has been reported to promote growth performance (Hippenstiel et al., 2011; Abdel-Wareth et al., 2012; Rašković et al., 2015). Moreover, thyme oil can have a beneficial impact on animal performance, health status, and welfare under hot environmental conditions (Attia et al., 2017). Accordingly, dietary supplementation with 0.5 g/kg thyme oil improved intestinal integrity and total antioxidant status of rabbits (Placha et al., 2013), likely mediated through natural antioxidants that can increase meat quality and shelf-life mainly by inhibiting lipid oxidation.

The main components of thyme oil are thymol, carvacrol, p-cymene,  $\gamma$ -terpinene, linalool,  $\beta$ -myrcene, terpinen-4-ol (Lee et al., 2005). These constituents are known to have antioxidant properties (Rota et al., 2008) and may lower blood cholesterol and abdominal fat accumulation (Abdulkarimi et al., 2011). Most of the research on dietary thyme supplementation was carried out using broilers (Abdel-Wareth et al., 2012) and rabbits (Placha et al., 2013) and has indicated a role in stimulating feed intake, increasing body weight (BW), BW gain, and intestinal integrity, as a consequence, improving overall feed conversion ratio (FCR).

However, no information is available about effects of graded inclusion levels of a specified thyme essential oil on performance and meat quality of growing rabbits under hot environmental (i.e., elevated temperatures) conditions. To explore the mechanism of the effects of thyme essential oil supplementation on rabbit productive performance under hot environmental conditions generally found, e.g., in Upper Egypt during summer, we investigated the effects of varied inclusion levels of a specified thyme oil in a rabbit ration on growth performance, carcass criteria, antioxidant properties and meat quality.

## 2. Materials and methods

### 2.1. Experimental animals, design and management

The present study was conducted at the rabbit research farm of the Department of Animal and Poultry Production, Faculty of Agriculture of the South Valley University, Qena, Egypt. Rabbits were housed under similar managerial, hygienic, and environmental conditions during the total experimental period of 56 d. Throughout the trial, the rabbits were handled according to the principles for the care of experimental animals (Lebas et al., 1984), and the experiment was approved by the Committee of Ethics of the Animal and Poultry Production Department of the South Valley University. During the experiment, rabbits were subjected to regular inspections for body condition and health. The assessments of body condition were carried out by touching the ribs, pelvis, and spine of the rabbits.

After weaning at the age of 4 weeks, a total of 75 male Californian rabbits (average BW  $523 \pm 14$  g) were randomly assigned to 5 dietary treatment groups of 15 rabbits each. Treatments consisted of an unsupplemented standard ration, negative control (CON); standard ration supplemented with 1.50 g/kg olive oil as carrier, positive control (POS); POS + 50 mg/kg thyme essential oil (TO1); POS + 100 mg/kg thyme essential oil (TO2); and POS + 150 mg/kg thyme essential oils (TO3) in the ration. The POS and TO rations were prepared individually by supplementing the exact dosage of olive oil and thyme essential oil to 3-kg batches of the CON ration.

The experiment lasted for 56 days. Ingredients, chemical composition and the energy value of the pelleted CON ration are presented in Table 1, assuming that POS and TO rations had the same chemical composition and energy value given the low supplementation levels.

The chemical composition of hydro-distilled thyme essential oil was analysed using a GC/MS system according to Abozid and Asker (2013). The main components of thyme oil were thymol (39.4%), p-cymene (23.6%),  $\gamma$ -terpinene (12.5%), ledol (2.24%) and aromadene (2.12%), which together accounted for approximately 80% of the total essential oil.

Rabbits were individually reared in cages (measuring width  $\times$  length  $\times$  height: 44 cm  $\times$  50 cm  $\times$  35 cm) of galvanized wire net, equipped with an automatic drinker and a manual feeder. The rabbits were reared in an open house system (naturally ventilated room by windows and ceiling fans) with average temperature of  $38.8 \pm 2$  °C and a relative humidity ranging from 26% to 35% during the whole experimental period with a 16 h light and 8 h dark regime. Fresh tap water was available for *ad libitum* consumption via stainless steel nipples located inside each cage. Individual feed intake was recorded daily at 08:00. Each rabbit was weighed weekly on the same day at 07:00. The FCR was calculated by dividing daily feed consumption by average daily BW gain. Signs of diarrhoea were documented daily and mortality was recorded as it occurred.

### 2.2. Chemical analysis

Chemical analysis of the CON ration was carried out at the Institute of Animal Science, University of Bonn, Germany. The ration

**Table 1**  
Ingredients and chemical composition (as-fed basis) of the CON ration fed to growing rabbits throughout the 8-week experimental period.

Item	Content
Ingredients, %	
Yellow maize grain	32.00
Wheat bran	20.00
Soybean meal (44% CP)	18.00
Wheat straw	12.00
Lucerne hay	5.00
Rice bran	5.00
Linseed straw	2.80
Sunflower meal	2.50
Lime stone	2.00
Sodium chloride	0.30
Vitamin-mineral premix <sup>1</sup>	0.30
DL-methionine	0.10
Chemical composition analysed, %	
Dry matter	91.40
Ash	9.80
Crude protein	17.00
aNDFom	30.60
ADFom	16.70
Ether extract	2.90
Sugar	4.00
Starch	30.50
Digestible energy, MJ/kg	9.66
Calcium	1.30
Phosphorus	0.86
Lysine	0.60

CON = negative control; aNDFom = neutral detergent fibre amylase treated, exclusive residual ash; ADFom = acid detergent fibre, exclusive residual ash.

<sup>1</sup> Per kg of ration: vitamin A 10,000 IU, vitamin D<sub>3</sub> 900 IU, vitamin E 50.0 mg, vitamin K 2.0 mg, vitamin B<sub>1</sub> 2.0 mg, folic acid 5.0 mg, pantothenic acid 20.0 mg, vitamin B<sub>6</sub> 2.0 mg, choline 1200 mg, vitamin B<sub>12</sub> 0.01 mg, niacin 50 mg, biotin 0.2 mg, Cu 0.1 mg, Fe 75.0 mg, Mn 8.5 mg, Zn 70 mg.

was ground to pass a 1-mm screen using a centrifugal mill (KG type ZM1, Retsch, Haan, Germany) and analysed according to the methods of VDLUFA (2012; method numbers in parentheses). Dry matter was determined by oven-drying at 100 °C for 24 h (3.1). Total nitrogen (N) was estimated by combustion assay (4.1.2; Rapid N cube, Elementar Analysensysteme, Hanau, Germany) and crude protein was expressed as N × 6.25. The contents of neutral detergent fibre (assayed with a heat stable amylase and without sodium sulphite, aNDFom; 6.5.1) and acid detergent fibre (ADFom; 6.5.2), were analysed using Ankom<sup>2000</sup> Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) and were both expressed exclusive of residual ash. The content of ether extract was assayed with the Soxhlet method after HCl digestion (5.1.1). The gross energy contents of the ration and faeces were measured using an adiabatic bomb calorimeter (model C 200; IKA, Staufen, Germany). Additionally, contents of ash (8.1), sugar (7.1.2) and starch (7.2.1) were quantified in the ration. Calcium was measured using atomic absorption spectrometry and phosphorus was analysed using a colorimetric method (10.6.1). Lysine and, after performic acid oxidation, methionine in the ration were analysed using an amino acid analyser (4.11.1).

### 2.3. Carcass measurements

At 12 weeks of age, all rabbits were weighed at the experimental farm. Ten representative rabbits ( $n = 10$ ) from each group were selected for carcass and meat quality analysis representing the average weight and variability of each group. The slaughter procedure was conducted at the Department of Animal and Poultry Production research abattoir, Faculty of Agriculture, South Valley University, Egypt. The animals were humanely slaughtered according to halal slaughter procedure. The procedure involved severing the carotid artery, jugular vein, trachea and oesophagus. The slaughtered rabbits were bled, and then the skin, genitals, urinary bladder, gastrointestinal tract, and the distal part of legs were removed. Carcasses (with head, lungs, liver, kidneys, heart, perirenal and scapular fat) were weighed (warm carcass), then chilled at 4 °C for 24 h in a ventilated room and the chilled carcasses were weighed again. The head, heart, lungs, liver and kidneys were removed from each carcass to obtain the reference carcass, which included meat, bones and fat depots. The internal organs included heart, lungs, liver and kidneys were weighed and the outer shape examined. The carcasses were then cut between the 7th and 8th thoracic vertebra and between the 6th and 7th lumbar vertebra to obtain the fore, mid, and hind parts, which were weighed separately. Also, scapular and perirenal fat was dissected and weighed. The carcass yield, chilled carcass percentage and the ratio of the organs and carcass parts to the chilled carcass weight were calculated as required. The fore, mid, and hind muscle (i.e., meat) of both sides of randomly selected 5 rabbits per experimental group were separated and individually packed in polyethylene bags, sealed, ice-cooled in portable refrigerators and transported to the Department of Food Science and Technology, Faculty of Agriculture, South Valley University, Egypt for meat quality analysis.

### 2.4. Meat quality analysis

The pH values were measured at 24 h post mortem with a Knick digital pH meter after homogenization of raw muscles (1 g) with iodoacetate (Korkeala et al., 1984). The water holding capacity (WHC) of muscle tissue was estimated by centrifuging 1 g of muscle, placed on tissue paper inside a tube, at 1,500 × g for 4 min (Nakamura and Katoh, 1985). The water remaining after centrifugation was quantified by drying the samples at 70 °C overnight. The WHC was calculated as follows:  $WHC (\%) = 100 \times (\text{Weight after}$

centrifugation - Weight after drying)/Initial weight. Lipid oxidation was measured by the thiobarbituric acid-reactive substances (TBARS) method described by Lee et al. (1998). The meat samples (0.5 g) were mixed with 2.5 mL of stock solution (0.37% thiobarbituric acid - 15% trichloroacetic acid - 0.25 nmol/L HCl) and heated in a boiling water bath for 10 min to develop a pink color. These samples were then cooled and centrifuged (4,500 × g, 25 min). Absorbance of the supernatant was measured at 532 nm using a spectrophotometer (U-2450, Shimadzu, Kyoto, Japan). The TBARS were calculated from a standard curve (8 to 50 nmol/L) of malondialdehyde (MDA), and the results were expressed as mg MDA/kg sample. Metmyoglobin (MetMb), and Oxymyoglobin (OMb) analysis was done as follows: raw meat samples (5 g) were homogenized in 25 mL ice-cold 40 mmol/L sodium phosphate buffer (PBS, pH 6.8) for 10 s using an Ultra-Turrax T25 tissue macerator at 16,300 × g (IKA, Staufen, Germany). The homogenate was allowed to stand at 4 °C for 1 h, and centrifuged at 4,500 × g for 30 min at 4 °C. The supernatant was filtered through Whatman # 1 filter paper, and the absorbance was read at 572, 565, 545 and 525 nm spectrophotometrically. The PBS (pH 6.8) served as a blank, and the contents of MetMb and OMb were calculated as described by Krzywicki (1982):

$$\text{MetMb} (\%) = [-2.51(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098] \times 100;$$

$$\text{OMb} (\%) = [0.882(A_{572}/A_{525}) - 1.267(A_{565}/A_{525}) + 0.809(A_{545}/A_{525}) - 0.361] \times 100, \text{ where } A \text{ denotes absorbance.}$$

### 2.5. Statistical analysis

The statistical analysis was performed using a completely randomized design and the general linear models (GLM) procedure of SAS 9.2 (SAS Institute, 2009). The model only included the level of supplementation. The individual rabbit was the experimental unit for all analysis. Data were analysed by one-way ANOVA. Duncan multiple range tests were used to compare means. Significance was declared at  $P < 0.05$ ;  $P$ -values less than 0.001 are expressed as “<0.001” rather than the actual value. Orthogonal polynomial contrasts were also used to determine linear and quadratic effects of levels of thyme oil inclusion considering only POS (0 mg/kg thyme oils) as a control, and CON was not included in this analysis.

## 3. Results

### 3.1. Feed intake and growth performance

During the experimental period (weeks 4 to 12 of age), the general health status and welfare of the rabbits was good. No signs of diarrhea and sickness or deaths were observed across treatment groups, indicating that the health status of the rabbits was not compromised by heat stress or experimental treatments. Improvements were observed ( $P < 0.001$ ) in feed intake, BW, BW gain and FCR in treated groups compared with the CON rabbits (Table 2). The highest ( $P < 0.001$ ) average daily gain and most efficient FCR were found in the TO1 rabbits followed by TO2, TO3 and POS, respectively, under hot environmental conditions. The weakest performance including BW, BW gain, and FCR was recorded for the CON group compared with POS and thyme oil groups. Considering the whole growing period, feed intake in the supplemented groups was similar, but the CON rabbits showed lower feed intake and poorer growth performance ( $P < 0.001$ ).

### 3.2. Carcass criteria

The effects of thyme oil supplementation on carcass criteria of male Californian rabbits at the end of experimental period under

**Table 2**  
Effects of thyme essential oils on performance of growing rabbits.<sup>1</sup>

Item	Treatments (T) <sup>2</sup>					SEM	P-value		
	CON	POS	TO1	TO2	TO3		T	Lin <sup>3</sup>	Quad <sup>3</sup>
Body weight, g									
4 wk	525	515	538	514	523	5	0.604	0.982	0.463
8 wk	1,275 <sup>c</sup>	1,412 <sup>b</sup>	1,539 <sup>a</sup>	1,408 <sup>b</sup>	1,407 <sup>b</sup>	13	<0.001	0.123	0.003
12 wk	2,058 <sup>c</sup>	2,349 <sup>b</sup>	2,475 <sup>a</sup>	2,386 <sup>b</sup>	2,369 <sup>b</sup>	19	<0.001	0.795	0.003
Daily body weight gain, g									
4 to 8 wk	26.79 <sup>c</sup>	32.04 <sup>b</sup>	35.74 <sup>a</sup>	31.94 <sup>b</sup>	31.58 <sup>b</sup>	0.44	<0.001	0.075	0.002
8 to 12 wk	27.97 <sup>b</sup>	33.45 <sup>a</sup>	33.45 <sup>a</sup>	34.93 <sup>a</sup>	34.36 <sup>a</sup>	0.42	<0.001	0.197	0.698
4 to 12 wk	27.38 <sup>c</sup>	32.74 <sup>b</sup>	34.60 <sup>a</sup>	33.44 <sup>b</sup>	32.98 <sup>b</sup>	0.34	<0.001	0.803	0.007
Daily feed intake, g									
4 to 8 wk	82.37 <sup>b</sup>	84.94 <sup>ab</sup>	87.63 <sup>a</sup>	87.67 <sup>a</sup>	87.67 <sup>a</sup>	0.53	0.002	0.112	0.279
8 to 12 wk	123.7 <sup>d</sup>	132.4 <sup>c</sup>	134.8 <sup>ab</sup>	135.7 <sup>a</sup>	133.7 <sup>bc</sup>	0.56	<0.001	0.036	<0.001
4 to 12 wk	103.0 <sup>c</sup>	108.7 <sup>b</sup>	111.1 <sup>a</sup>	111.7 <sup>a</sup>	110.7 <sup>a</sup>	0.46	<0.001	0.036	0.015
Feed conversion ratio, g feed/g gain									
4 to 8 wk	3.075 <sup>a</sup>	2.651 <sup>b</sup>	2.452 <sup>c</sup>	2.745 <sup>b</sup>	2.776 <sup>b</sup>	0.038	<0.001	0.012	0.046
8 to 12 wk	4.423 <sup>a</sup>	3.958 <sup>b</sup>	4.030 <sup>b</sup>	3.885 <sup>b</sup>	3.891 <sup>b</sup>	0.042	<0.001	0.431	0.601
4 to 12 wk	3.762 <sup>a</sup>	3.320 <sup>bc</sup>	3.211 <sup>c</sup>	3.340 <sup>b</sup>	3.357 <sup>b</sup>	0.029	<0.001	0.192	0.151

<sup>a-c</sup> Means bearing different superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Values in each row are means of 15 replicates for each treatment ( $n = 15$ ).

<sup>2</sup> CON, negative control; POS, positive control, 1.50 g/kg olive oil; TO1, 1.50 g/kg olive oil + 50 mg/kg thyme oil; TO2, 1.50 g/kg olive oil + 100 mg/kg thyme oil; TO3, 1.50 g/kg olive oil + 150 mg/kg thyme oil.

<sup>3</sup> Lin and Quad: linear and quadratic responses, respectively to thyme essential oil inclusion level considering POS (0 mg/kg thyme essential oil) as a control; CON was not included in this analysis.

hot environmental conditions are shown in Table 3. The incorporation of olive oil and thyme essential oil improved ( $P < 0.001$ ) weights of warm carcass, chilled carcass, and reference carcass and chilled carcass percentage compared with CON. Interestingly, fat depots decreased ( $P < 0.001$ ) with increasing levels of incorporated thyme oil when compared with CON. On the contrary, percentage of head, liver, heart, lungs and kidneys of the chilled carcass did not differ among the different experimental groups. Likewise, the percentage of fore, mid and hind parts did not differ among the groups (Table 3).

### 3.3. Meat quality characteristics

The effects of thyme essential oil supplementation on meat quality characteristics of male Californian rabbits at the end of experimental period are shown in Table 4. The WHC covered a wide range, from 52.7% to 63.1% in treatment groups. Thyme oil

supplemented groups had improved WHC ( $P < 0.001$ ) in comparison to CON. The CON rabbits showed the lowest WHC (linear and quadratic,  $P < 0.001$ ). Thyme oil supplementation decreased ( $P < 0.002$ ) pH values of rabbit muscles that ranged between 6.34 and 6.51 (Table 4).

The TO2 and TO3 rabbits showed the lowest values whereas the highest value was recorded for TO1. The TBARS values also differed among rations fed to the rabbits ( $P < 0.001$ ). The lowest TBARS values were found in the POS and TO1 groups, intermediate values were observed for CON and TO2, and TO3 rabbits had the highest values. The Omb content in rabbit meat increased and MetMb decreased ( $P < 0.001$ ) with dietary thyme oil supplementation compared to CON group in fore, mid, and hind carcass. The Omb was higher in TO2 group for both fore and hind parts of carcass, while TO3 group had increased (quadratic,  $P < 0.001$ ) Omb content for mid part of carcass when compared with their counterparts.

**Table 3**  
Effects of thyme essential oils on carcass criteria of growing rabbits.<sup>1</sup>

Item	Treatments (T) <sup>2</sup>					SEM	P-value		
	CON	POS	TO1	TO2	TO3		T	Lin <sup>3</sup>	Quad <sup>3</sup>
Carcass criteria									
Warm carcass, g	1,349 <sup>b</sup>	1,495 <sup>a</sup>	1,528 <sup>a</sup>	1,494 <sup>a</sup>	1,473 <sup>a</sup>	15	<0.001	0.263	0.182
Chilled carcass, g	1,300 <sup>b</sup>	1,448 <sup>a</sup>	1,482 <sup>a</sup>	1,447 <sup>a</sup>	1,426 <sup>a</sup>	16	<0.001	0.256	0.179
Reference carcass, g	1,155 <sup>b</sup>	1,306 <sup>a</sup>	1,352 <sup>a</sup>	1,309 <sup>a</sup>	1,295 <sup>a</sup>	16	<0.001	0.660	0.327
Chilled carcass, %	57.52 <sup>b</sup>	59.57 <sup>a</sup>	60.79 <sup>a</sup>	59.85 <sup>a</sup>	59.28 <sup>a</sup>	0.39	0.032	0.405	0.133
Chilled carcass composition, %									
Abdominal fat	3.28 <sup>a</sup>	1.82 <sup>b</sup>	1.32 <sup>c</sup>	1.68 <sup>b</sup>	1.27 <sup>c</sup>	0.15	<0.001	<0.001	0.487
Head	8.02	8.20	8.01	8.27	8.12	0.04	0.223	0.258	0.456
Fore	27.48	27.49	26.82	27.24	27.28	0.23	0.091	0.749	0.785
Mid	26.30	26.00	25.90	26.04	26.09	0.22	0.060	0.748	0.784
Hind	29.32	31.44	31.77	30.95	30.74	0.38	0.055	0.515	0.534
Liver	3.95	4.074	3.92	4.03	4.07	0.03	0.065	0.705	0.142
Heart	0.76	0.76	0.67	0.75	0.71	0.02	0.199	0.539	0.432
Lungs	0.65	0.66	0.62	0.64	0.64	0.01	0.177	0.772	0.403
Kidneys	1.24	1.28	1.14	1.20	1.25	0.02	0.224	0.919	0.087

<sup>a-c</sup> Means bearing different superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Values in each row are means of 15 replicates for each treatment ( $n = 15$ ).

<sup>2</sup> CON, negative control; POS, positive control, 1.50 g/kg olive oil; TO1, 1.50 g/kg olive oil + 50 mg/kg thyme oil; TO2, 1.50 g/kg olive oil + 100 mg/kg thyme oil; TO3, 1.50 g/kg olive oil + 150 mg/kg thyme oil.

<sup>3</sup> Lin and Quad: linear and quadratic responses, respectively, to thyme essential oil inclusion level considering POS (0 mg/kg thyme essential oil) as a control; CON was not included in this analysis.

**Table 4**  
Effects of thyme essential oil on meat quality characteristics of growing male rabbits.<sup>1</sup>

Item	Treatments (T) <sup>2</sup>					SEM	P-value		
	CON	POS	TO1	TO2	TO3		T	Lin <sup>3</sup>	Quad <sup>3</sup>
Meat quality									
WHC, %	52.65 <sup>c</sup>	61.36 <sup>a</sup>	61.06 <sup>a</sup>	56.34 <sup>b</sup>	63.05 <sup>a</sup>	0.87	<0.001	0.942	0.003
pH	6.44 <sup>ab</sup>	6.41 <sup>bc</sup>	6.51 <sup>a</sup>	6.34 <sup>c</sup>	6.34 <sup>c</sup>	0.02	0.002	<0.001	<0.001
TBARS	1.47 <sup>b</sup>	1.30 <sup>cd</sup>	1.20 <sup>d</sup>	1.34 <sup>c</sup>	1.83 <sup>a</sup>	0.05	<0.001	<0.001	<0.001
Fore pigments									
Metmyoglobin	31.33 <sup>b</sup>	31.47 <sup>b</sup>	32.23 <sup>b</sup>	27.10 <sup>c</sup>	34.06 <sup>a</sup>	0.43	<0.001	0.348	<0.001
Oxymyoglobin	9.46 <sup>b</sup>	8.77 <sup>b</sup>	6.89 <sup>c</sup>	10.52 <sup>a</sup>	8.81 <sup>b</sup>	0.26	<0.001	0.569	0.001
Mid pigments									
Metmyoglobin	24.87 <sup>b</sup>	28.55 <sup>a</sup>	31.61 <sup>a</sup>	30.08 <sup>a</sup>	22.92 <sup>b</sup>	0.78	<0.001	0.471	<0.001
Oxymyoglobin	8.38 <sup>b</sup>	6.34 <sup>d</sup>	5.39 <sup>e</sup>	7.24 <sup>c</sup>	10.68 <sup>a</sup>	0.39	<0.001	<0.001	<0.001
Hind pigments									
Metmyoglobin	32.38 <sup>a</sup>	31.98 <sup>a</sup>	31.61 <sup>a</sup>	24.16 <sup>b</sup>	31.96 <sup>a</sup>	0.72	<0.001	0.002	0.005
Oxymyoglobin	6.24 <sup>c</sup>	7.69 <sup>b</sup>	5.66 <sup>d</sup>	9.57 <sup>a</sup>	6.51 <sup>c</sup>	0.29	<0.001	<0.001	<0.001

WHC = water holding capacity; TBARS = thiobarbituric acid reactive substances.

<sup>a-c</sup> Means bearing different superscripts within a row differ significantly ( $P < 0.05$ ).

<sup>1</sup> Values in each row are means of 15 replicates for each treatment ( $n = 15$ ).

<sup>2</sup> CON, negative control; POS, positive control, 1.50 g/kg olive oil; TO1, 1.50 g/kg olive oil + 50 mg/kg thyme oil; TO2, 1.50 g/kg olive oil + 100 mg/kg thyme oil; TO3, 1.50 g/kg olive oil + 150 mg/kg thyme oil.

<sup>3</sup> Lin and Quad: linear and quadratic responses, respectively, to thyme essential oil inclusion level considering POS (0 mg/kg thyme essential oil) as a control; CON was not included in this analysis.

## 4. Discussion

### 4.1. Productive performance

The present study aimed to explore effects of incremental inclusion levels of thyme oil in a rabbit ration on growth performance, carcass criteria, antioxidant properties and meat quality under hot environmental conditions. Due to scarcity of available data on effect of thyme oil on growing rabbits, comparison was done with studies that used thyme powder or other herbs of the Lamiaceae family, either in rabbits or other farm animals.

Our data indicate that feed intake and growth performance were improved in rabbits fed a standard ration supplemented with olive oil and thyme oil under hot environmental conditions. These observations can be regarded as being mainly due to the active components in thyme oil which have been reported to improve, for broilers, production efficiency and promote general health status in high temperature environments (Attia et al., 2017). Moreover, supplementation of thyme and oregano as phytochemical additives to rations of broilers (Hippenstiel et al., 2011; Abdel-Wareth et al., 2012) and rabbits (Cardinali et al., 2015) have increased feed intake and growth performance. Furthermore, herbs and their constituent essential oils are often supposed to improve flavor and, indirectly, palatability of feeds, thus increasing voluntary feed intake which, in turn, results in improved BW gain and FCR (Zeng et al., 2015). In the current study, FCR was improved by thyme essential oil supplementation under hot environmental conditions in a dose-dependent manner. These results may be attributable to the positive effect of the thyme compounds on digestive efficiency, which leads to improved FCR (Gerencser et al., 2014). Essential oils are often claimed to be digestive enhancers and having antimicrobial activity as well as promote general health, yet studies typically lack quantification of active components and elucidating their mode of action and, thus, standardization will be imprecise (Hippenstiel et al., 2011; Bozkurt et al., 2014). Therefore it is not surprising that other authors (Fallah and Mirzaei, 2016) have reported that thyme leaves at 1, 1.5 and 5 g/kg ration did not affect the feed intake and FCR of broilers, yet active components of thyme leaves were not quantified. In the present study dietary thyme oil yielded a positive response in terms of production performance of Californian rabbits in a hot environment which may be attributable to the active components of thyme essential oil as analysed in the hydro-distilled thyme essential oil of this study.

### 4.2. Carcass characteristics and internal organs

To our knowledge, no publication reports effects of thyme essential oil, using olive oil as carrier, on carcass criteria of growing rabbits under hot environmental conditions.

Overall, carcass weight, yields, dissectible fat, and results on organ percentages were similar to previous results in various environments (Abdel-Wareth et al., 2015; Dalle Zotte et al., 2014b). Interestingly, the incorporation of thyme oil with olive oil linearly decreased fat depots when compared with CON, thus confirming previous data on reduced abdominal fat content in Japanese quails at inclusion levels of 60 or 200 mg/kg thyme essential oil (Denli et al., 2004; Khaksar et al., 2012) and broiler chicken when supplemented at 1 g/kg (Al-Kassie, 2009). Moreover, Dalle Zotte et al. (2014a) reported that supplementation of thyme at 30 g/kg decreased scapular fat content of growing rabbits which is in accordance with our results. Dalle Zotte et al. (2016) recently reviewed the potential biological effects of different combinations of phytochemicals on growth performance, carcass criteria, antioxidant and antibacterial activities in rabbits and stated that these are not fully investigated. It appears that lack of quantitative data of active components also limit progress relative to carcass characteristics.

### 4.3. Selected physicochemical meat quality measurements

At the point of purchase of meat at market, the consumer takes into consideration meat quality, color, safety, price and nutritional quality. Concerning effects of thyme oil on rabbit meat quality criteria under hot environmental conditions, rabbits fed rations containing thyme oil had improved WHC, pigments and oxidative stability in carcass meat. This marked increase in WHC was probably related to the positive effect of thyme oil antioxidants on the integrity of muscle fibres, thereby enhancing their capability to retain water (Dal Bosco et al., 2012). The results are in agreement with Cardinali et al. (2015) who reported that supplementation of oregano and rosemary to rabbit rations increased the WHC. Cheah et al. (1995) mentioned that the beneficial effect of dietary antioxidants on drip loss is due to their ability to stabilize membranes, presumably achieved by inhibiting the phospholipase A2 activity and lowering  $Ca^{2+}$  release, which, in turn, reduces the rate of post-mortem glycolysis with a subsequently higher pH. In this study

muscle pH values were highest for the rabbits fed the ration with the lowest thyme essential oil supplementation level for which no explanation is obvious. Furthermore, many studies were conducted on the effects of essential oils to protect meat and meat products against oxidative processes. Lee et al. (2005) reported an analogous positive action of thyme essential oil on drip loss and antioxidant activities. Essential oil of thyme showed moderate inhibition of lipid oxidation (Rašković et al., 2015).

In the current study, OMB increased and MetMb decreased with dietary thyme oil supplementation compared to CON samples from different parts of the rabbit carcass (fore, mid and hind). These observations support the effectiveness of thyme essential oil components in retarding the conversion of reduced myoglobin and OMB to MetMb, which is in line with improvements observed on Japanese quails for meat oxidative stability and quality (Aksu et al., 2014). The improvement in such physical meat characteristics was probably due to the positive effect of the antioxidants on the integrity of the muscle fibres (Stanley, 1991). Another possible explanation could be related to thyme essential oil components that entered the circulatory system, were distributed and retained in tissues, and thus, were responsible for the exhibited antioxidant properties and improved the color stability of myoglobin by donating electrons or by other unknown mechanisms (Yu et al., 2016; Papuc et al., 2017).

## 5. Conclusions

Based on results of this study, thyme essential oil included up to 100 mg/kg ration with 1.50 g/kg olive oil as carrier as an supplement in the ration of growing rabbits can be used as an effective feed additive to improve performance under hot environmental conditions. These additives may improve the dietary value in a dose-dependent manner and foster the production of rabbit meat, decrease the perirenal and scapular fat content and improve oxidative stability of rabbit meat. Further investigations are needed to evaluate meat quality and oxidative stability of the stored rabbit meat caused by thyme and olive oil supplementation.

## Conflicts of interest

The authors declare that they have no conflict of interests.

## Acknowledgements

The authors gratefully acknowledge the staff of the Experimental Station in the Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, for their generous assistance during the course of the study as well as N. Wahl and P. Jacquemien for their excellent support in the laboratory. Partial financial support was provided by funds allocated to the Institute of Animal Science, University of Bonn.

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