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# Thyme leaves as an eco-friendly feed additive improves both the productive and reproductive performance of rabbits under hot climatic conditions

Ahmed Ezzat Ahmed[1,2]*, Mohammed Ali Alkahtani[1], Ahmed Aboubaker Abdel-Moniem Abdel-Wareth[3]

[1]Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia
[2]Department of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[3]Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, Qena, Egypt

*Corresponding author: aabdelrahman@kku.edu.sa; ahmed.ezzat@vet.svu.edu.eg

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**Abstract:** The present study examined the efficiency of thyme in improving the productive and reproductive performances in male rabbits living in hot climates with the further lowering of the faecal ammonia and adverse heat stress. One hundred and twenty-five Zealand-White male rabbits were assigned to five dietary treatments [age: 60-day-old; body weight (b.w.): 1 362 ± 20 g] ($n$ = 25). The basal diet was supplemented with either 0 (control), 4, 8, 12 or 16 g/kg of thyme leaves. The experiment lasted for 90 days. The feed and water were provided *ad libitum*. The animals were housed in an open system (39 °C ambient-temperature and 30–35% relative-humidity). The dietary thyme leaf levels significantly improved the appetite, body weight gain and growth performance compared to the control ($P$ < 0.001). The weight gain and feed conversion ratio were directly proportional to the thyme leaves intake. The faecal ammonia was markedly lowered in response to the feeding with thyme leaves. The thyme leaves significantly improved the liver and kidney functions as indicated by their biomarkers. The testosterone concentrations and semen characteristics were also significantly improved in the thyme leaves-treated groups compared to the control ($P$ < 0.01). In conclusion, thyme leaves, at an optimum dose of 16 g/kg of the diet, could be an efficient feed additive for rabbits surviving under hot climatic conditions.

**Keywords:** ammonia; weight gain; testosterone; semen quality; thymol

Recently, animal production, in general, and rabbit production, in particular, have developed rapidly, most notably to meet an increased demand in fresh meat for human consumption as well as extra income for families or small farmers (Abdel-Wareth et al. 2015). Food safety and environmental conditions are two major challenges facing rabbit production. EU regulations [Regulation (EC) No. 1831/2003]

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have banned the use of antibiotics as growth promoters when feeding animals, due to the possible resistance to antibiotics and the presence of antibiotic residues in products of animal origin intended for human consumption. Subsequently, several research articles have been conducted to explore the use of phytogenics as alternate feed additives in rabbit nutrition. Herbs and herbal products were incorporated in rabbit diets to stimulate the productive and reproductive performance of rabbits. Phytogenic substances have generally been known as being safe and have frequently been used in food and feed industries (Raskovic et al. 2015). Moreover, herb extracts could have a beneficial impact on the animal performance, health status, and welfare under hot environmental conditions (Attia et al. 2016). Thyme (*Thymus vulgaris* L.) has traditionally been used in herbal medicine. The active constituents of thyme have shown to have antimicrobial and antioxidant properties which promote the appetite and growth performance in rabbits (Raskovic et al. 2015; Abdel-Wareth et al. 2018; Abdel-Wareth et al. 2019). Likewise, the supplementation of 0.5 g/kg thyme oil significantly improved the gut integrity and antioxidant status of rabbits (Placha et al. 2013). The main components of thyme oil are thymol, carvacrol, *p*-cymene, γ-terpinene, linalool, β-myrcene, and terpinen-4-ol (Lee et al. 2005; Abdel-Wareth et al. 2018). Those active components have antioxidant properties (Rota et al. 2008) reducing the serum cholesterol and the carcass fat (Abdulkarimi et al. 2011). Most of the research on dietary thyme oil supplementation was carried out using rabbits (Placha et al. 2013; Abdel-Wareth et al. 2018) and has indicated a role in stimulating the feed intake, body weight gain, meat quality, antioxidants, gut integrity and thus improving the overall feed conversion ratio.

However, no information is available about the effects of thyme leaves on the productive and reproductive performance in male rabbits under hot environmental conditions.

Thus, the present study aimed to clarify the effects of thyme leaf supplementation on the male productive and reproductive performance in rabbits in hot environments, including: the feed conversion and growth rates, kidney and liver functions, faecal ammonia concentrations, semen quality and testosterone levels in response to different concentrations of thyme leaves in the diet of male rabbits, such as those living in Upper Egypt during the summer.

## MATERIAL AND METHODS

The Institutional Animal Care and Use Committee of the South Valley University approved the experimental protocol (No. of the ethical approval – SVUAGR62018) used in this study in accordance with the guidelines of Egyptian Research Ethics Committee and the guidelines contained in the Guide for the Care and Use of Laboratory Animals (NRC 2011).

### Thyme leaf powder preparation and analysis

The ingredients and chemical composition of the basal diets and rations given to the rabbits are listed in Table 1.

The thyme leaves were purchased from a local seller of medicinal herbs (Spices and Herbs Seada El-Attar company, Qena, Egypt). The thyme leaves were ground into particles of less than 1 mm in size and were stored in air-tight containers at room temperature until used. The essential oil percentage of the thyme leaves were extracted by hydro-distilla-

Table 1. Ingredients and chemical composition (as-fed basis) of the control rations fed to the rabbits throughout the experimental periods

| Ingredients | % | Chemical analysis | % |
|---|---|---|---|
| Yellow maize grain | 32.00 | Dry matter | 91.40 |
| Wheat bran | 20.00 | Ash | 9.80 |
| Soybean meal (44% CP) | 18.00 | Crude protein | 17.00 |
| Wheat straw | 12.00 | Crude fibre | 12.60 |
| Lucerne hay | 5.00 | Ether extract | 2.90 |
| Rice bran | 5.00 | Digestible energy (MJ/kg) | 9.42 |
| Linseed straw | 2.80 | Calcium | 1.30 |
| Sunflower meal | 2.50 | Phosphorus | 0.86 |
| Lime stone | 2.00 | Lysine | 0.60 |
| Sodium chloride | 0.30 | Methionine | 0.41 |
| Vitamin-mineral premix[1] | 0.30 | | |
| DL-Methionine | 0.10 | | |

[1]Per kg of rations: vitamin A 10 000 IU, vitamin $D_3$ 900 IU, vitamin E 50.0 mg, vitamin K 2.0 mg, vitamin $B_1$ 2.0 mg, folic acid 5.0 mg, pantothenic acid 20.0 mg, vitamin $B_6$ 2.0 mg, choline 1 200 mg, vitamin $B_{12}$ 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

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tion in a Clevenger-type (Laboratoire PhytoChemia, Québec, Canada) apparatus for 3 h according to the method of Abozid and Asker (2013).

The essential oil extracted was dried over anhydrous sodium sulfate and analysed by gas chromatography using a Delsi 121C gas chromatograph (GC) model fitted with a CPWA × 52 CB column (30 m × 0.32 mm; film thickness 0.25 μm). The oven temperature was programmed to increase from 50 °C to 300 °C at 4 °C/min and both the injector and detector temperatures were set at 240 °C and 255 °C, respectively. The carrier gas was nitrogen at a flow rate of 1 ml/minute. The constituents were identified by a coupling gas chromatography with mass spectrometry (GC-MS), using a Sigma 300 apparatus attached to an HP 5970 300 mass spectrometer.

The ingredients of the thyme leaves detected by the GC-MS showed various compounds and polyphenolic thymol was the most detected ingredient of the thyme leaves (50.85%) as exhibited in Table 2.

Table 2. Gas chromatography-mass spectrometry (GC-MS) based on the active components in the thyme leaves

| RT | Area (%) | Compounds |
|---|---|---|
| 4.40 | 1.84 | α-pinene |
| 4.82 | 1.74 | camphene |
| 5.81 | 0.97 | β-myrcene |
| 6.66 | 0.59 | α-humulene |
| 6.96 | 30.36 | cymol |
| 7.16 | 1.31 | 1, 8-cineole |
| 8.02 | 3.01 | γ-terpinene |
| 9.62 | 0.80 | linalool |
| 11.57 | 0.90 | camphor |
| 12.52 | 2.77 | endo-borneol |
| 12.87 | 0.38 | 4-terpineol |
| 14.84 | 0.53 | thymol methyl ether |
| 17.68 | 50.85 | thymol |
| 22.42 | 1.53 | *trans*-caryophyllene |
| 29.05 | 1.05 | caryophyllene oxide |
| 31.47 | 0.38 | *tau*-cadinol |

RT = retention time

## Experimental animals, design and management

The rabbits were housed under similar managerial, hygienic, and environmental conditions during the total experimental period of 90 days. Throughout the trial, the rabbits were handled according to the principles of experimental animal care (Lebas et al. 1984), and approved by the Ethics Committee of the Animal and Poultry Production Department of the South Valley University. During the experiment, the animals were regularly inspected with regards to their health status and general body condition. Assessment of the body condition was carried out by touching the ribs, pelvis, and spine.

One hundred and twenty-five, 60-day-old New Zealand White male rabbits (average body weight 1 362 ± 20 g) were assigned to five dietary treatments. The basal diet was supplemented with thyme leaves at: 0 (control), 4, 8, 12 or 16 g/kg of the diet. The diets were formulated according to the requirements of growing rabbits as recommended by the National Research Council (NRC 1977). The ingredients, chemical composition and energy value of the experimental diets are presented in Table 1. During the whole experiment, all the animals in all the treatments received the pelleted diet for an *ad libitum* consumption.

The animals were individually kept in cages (44 cm × 50 cm × 35 cm) of galvanised wire net, equipped with a manual feeder and automatic drinker.

The rabbits were kept in an open house system (naturally ventilated room by windows and ceiling fans) with an average temperature of 39.5 ± 2 °C and a relative humidity ranging from 30–35% during the experimental period.

The rabbits were kept in a photoperiod of 16 h of light and 8 h of darkness. Fresh municipal water was available *ad libitum* via stainless steel nipples located inside each cage.

The body weight and feed intakes (g/rabbit) were recorded throughout the experimental periods of 60–90, 90–120, 120–150 and 60–150 days of age. The feed conversion ratio was calculated by dividing the feed consumption by the average body weight gain during the experimental periods of 60–90, 90–120, 120–150 and 60–150 days of age. Signs of diarrhoea were daily documented and mortality was recorded if it occurred.

## Feed and faeces chemical analysis

Samples of the feed and faeces were analysed for moisture by oven drying (method number 930.15), for ash by incineration (942.05), for protein by the

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Kjeldahl method (984.13), and for ether extract by a Soxhlet fat analysis (954.02), for calcium (927.02) and phosphorous (935.59) as described by the Association of Official Agricultural Chemists International (AOAC 2006). The gross energy was determined using adiabatic bomb calorimetry (Parr Instrument Company, IL, USA). At the end of the experiment, fresh faecal samples from all the rabbits were collected then stored at −20 °C until being analysed for ammonia. Ammonia-N (mg NH3-N/30 ml) from both the blank and syringes containing a substrate (NH3-N sample) was measured by distillation (Vapodest 50s carousel; Gerhardt, Königswinter, Germany).

### Semen collection and evaluation

As the biological material, the quality of the semen ejaculates of the sexually mature and healthy male New Zealand White rabbits (145 days of age) were assessed as described by El-Desoky et al. (2017) and Abdel-Wareth et al. (2019). Semen was collected from twenty rabbits (one ejaculate per rabbit) per treatment at the end of 150 days. The ejaculates were collected using an artificial vagina. The volume of each ejaculate was recorded using a graduated collection tube after removal of the gel mass. The assessment of the live, dead, and abnormal sperm was performed by counting 200 sperm cells using an eosin-nigrosine blue staining mixture. The complete or partial purple-stained sperm cells were considered non-viable, whereas the non-stained cells were viable. The percentage of the motile sperm (progressive forward motility %) was performed in several microscopic fields in the semen samples under × 100 magnification using a light microscope with a hot stage and subjectively assessed from 0% to 100%.

### Blood indicators

Fifteen representative rabbits from each treatment group were subjected to anaesthesia by an intramuscular injection of ketamine and xylazine, and then 10 ml of blood was withdrawn from the ear vein of each animal. The serum was obtained by centrifugation of the coagulated blood at 1 372 *g* for 15 min and the serum was collected and stored at −20 °C until used in the biochemical assays. The serum testosterone concentrations were colorimetrically assayed by using immunoassay commercial kits (Monobind Inc., Lake Forest, CA, USA). The liver enzymes, aspartate transaminase (AST) and alanine transaminase (ALT), as well as kidney function biomarkers, i.e., urea and creatinine were determined using standard diagnostic kits (Monobind Inc., Lake Forest, CA, USA).

### Statistical analysis

A statistical analysis was performed using a completely randomised design and the general linear model (GLM) procedure of SAS v9.2 (SAS Institute, NC, USA). The model only included the level of the thyme leaf supplementation. An individual rabbit was the experimental unit for all the analyses. Orthogonal polynomial contrasts were also used to determine the linear and quadratic effects of the levels of the thyme leaves.

The differences between the groups were considered significant at $P < 0.05$.

## RESULTS

### Feed intake and growth performance

The general health status and welfare of the rabbits were good during the experimental period. No signs of diarrhoea, sickness or deaths were observed in the treatment groups, indicating that the health status of the rabbits was not compromised by the heat stress or experimental treatments. Various differences were observed in the body weight gain, feed intake and feed conversion ratio among the treatments (Table 3).

The body weight gain was significantly increased (linear, $P < 0.001$) and the feed conversion ratios were significantly improved (linear, $P < 0.001$) with an increasing level of thyme leaves compared to the control during the periods of 90–120, 120–150 and 60–150 days of age under the hot environmental conditions ($P < 0.001$). The body weight gain was linearly and quadratically increased (linear, $P < 0.001$) and the feed conversion ratios were significantly improved (linear and quadratic, $P < 0.001$) with an increasing amount of thyme leaves compared to the control during the period of 60–90 days of age. Considering the whole growing pe-

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Table 3. Effects of the thyme leaf supplementation at the different concentrations; 4, 8, 12 and 16 g/kg of the diet, on the biological performance of the New Zealand White male rabbits ($n$ = 25) throughout the feeding periods

| Items | Thyme leaf levels (g/kg) | | | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|---|---|
| | CTL | 4 | 8 | 12 | 16 | | lin | quad |
| Body weight gain (g) | | | | | | | | |
| 60–90 days | 408 | 498 | 524 | 539 | 548 | 7.78 | < 0.001 | < 0.001 |
| 90–120 days | 586 | 605 | 620 | 621 | 634 | 12.21 | 0.008 | 0.564 |
| 120–150 days | 612 | 621 | 688 | 708 | 760 | 17.07 | < 0.001 | 0.755 |
| 60–150 days | 1 605 | 1 724 | 1 848 | 1 851 | 1 942 | 18.53 | < 0.001 | 0.016 |
| Daily feed intake (g) | | | | | | | | |
| 60–90 days | 1 566 | 1 653 | 1 705 | 1 738 | 1 747 | 22.90 | < 0.001 | 0.055 |
| 90–120 days | 1 956 | 1 883 | 1 891 | 1 934 | 1 937 | 18.69 | 0.812 | 0.015 |
| 120–150 days | 2 249 | 2 286 | 2 243 | 2 336 | 2 365 | 30.57 | 0.066 | 0.819 |
| 60–150 days | 5 771 | 5 822 | 5 916 | 5 932 | 6 050 | 48.65 | < 0.001 | 0.835 |
| Feed conversion ratio (g feed/g gain) | | | | | | | | |
| 60–90 days | 3.853 | 3.321 | 3.256 | 3.228 | 3.191 | 0.074 | < 0.001 | 0.001 |
| 90–120 days | 3.345 | 3.112 | 3.054 | 3.115 | 3.058 | 0.057 | 0.005 | 0.041 |
| 120–150 days | 3.686 | 3.691 | 3.302 | 3.272 | 3.117 | 0.077 | < 0.001 | 0.895 |
| 60–150 days | 3.597 | 3.379 | 3.204 | 3.202 | 3.114 | 0.035 | < 0.001 | 0.004 |

CTL = control; lin and quad = linear and quadratic responses, respectively, to the supplementation levels; SEM = standard error of the mean

riod (60–150 days of age), the supplemented groups also showed a higher feed intake (linear, $P < 0.001$) compared to the control. The highest performance including the body weight gain and feed conversion ratio was recorded for the thyme leaf levels at 4, 8, 12 or 16 g/kg of the diet compared with the control.

## Semen characteristics

The effect of the thyme leaf supplementation on the semen quality up to 150 days of age is shown in Table 4. The supplementation of the thyme leaves to the rabbit diets exhibited a significant increase in the ejaculate volume, sperm viability, and sperm motility at the end of the experimental period under the hot climatic conditions. The ejaculate volume was linearly and quadratically increased ($P < 0.01$) with the increased levels of thyme leaves compared to the control group. No significant effect of the thyme leaves was recorded on the semen pH value. The abnormal sperm percentage was significantly lower in thyme leaves-supplemented diet group compared to the non-supplemented diet one (lin-

Table 4. Effects of the thyme leaves on the semen quality of the New Zealand White male rabbits

| Items | Thyme leaf levels (g/kg) | | | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|---|---|
| | CTL | 4 | 8 | 12 | 16 | | lin | quad |
| Semen quality | | | | | | | | |
| Semen volume (ml) | 0.49 | 0.59 | 0.60 | 0.63 | 0.63 | 0.01 | < 0.001 | < 0.001 |
| Abnormal sperm (%) | 17.10 | 14.30 | 13.90 | 12.89 | 12.05 | 0.41 | < 0.001 | 0.062 |
| Live sperm (%) | 74.40 | 77.40 | 79.00 | 81.40 | 83.80 | 1.65 | < 0.001 | 0.871 |
| Semen pH (value) | 7.05 | 7.07 | 7.06 | 7.07 | 7.06 | 0.02 | 0.812 | 0.615 |
| Forward motility (%) | 54.70 | 61.70 | 67.90 | 70.91 | 71.80 | 1.12 | < 0.001 | 0.052 |

CTL = control; lin and quad = linear and quadratic responses, respectively, to the supplementation levels; SEM = standard error of the mean

The values in each row are the means of 25 replicates for each treatment ($n$ = 25)

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ear, $P < 0.01$). The male rabbits fed on a diet supplemented with various thyme levels; 4, 8, 12 and 16 g/kg significantly increased the sperm viability and sperm forward motility percentage (linear, $P < 0.001$) compared to those of the control.

## Ammonia faecal concentrations

The effects of the thyme leaf levels on the ammonia faecal concentration of the male rabbits under the hot climatic conditions are summarised in Figure 1. The present results showed that the ammonia faecal concentration was significantly decreased (linear and quadratic, $P < 0.001$) with an increase in the dietary thyme leaves at 4, 8, 12 and 16 g/kg under the hot climatic conditions. Overall, the best faecal ammonia concentration in the rabbits was observed at 16 g/kg under the hot climatic conditions.

## Blood biochemical constituents

The effects of the dietary supplemental thyme leaves on the liver and kidney function tests as well as the serum testosterone concentrations of the male New Zealand White rabbits is presented in Figure 2, 3 and 4, respectively. The rabbits fed on the diets supplemented with thyme leaves at 4, 8, 12 and 16 g/kg

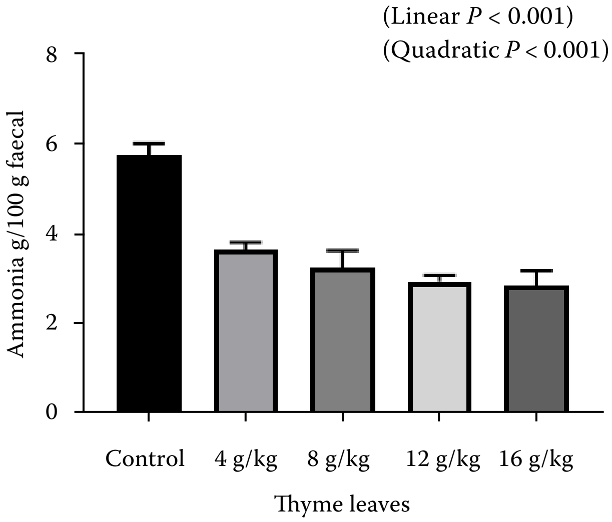


Figure 1. Ammonia faecal concentration g/100 g faeces of male rabbits in response to the thyme leaf supplementation of a 0 g/kg (control), 4, 8, 12 and 16 g/kg at 150 days of age

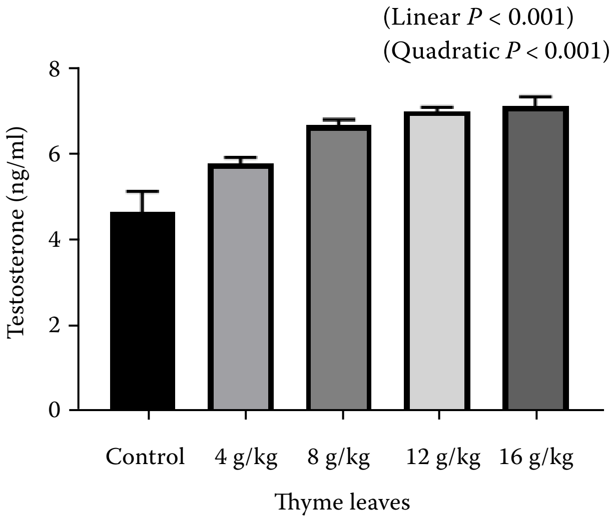


Figure 2. Testosterone levels ng/ml of the male rabbits in response to the thyme leaf supplementation of 0 g/kg (control), 4, 8, 12 and 16 g/kg at 150 days of age

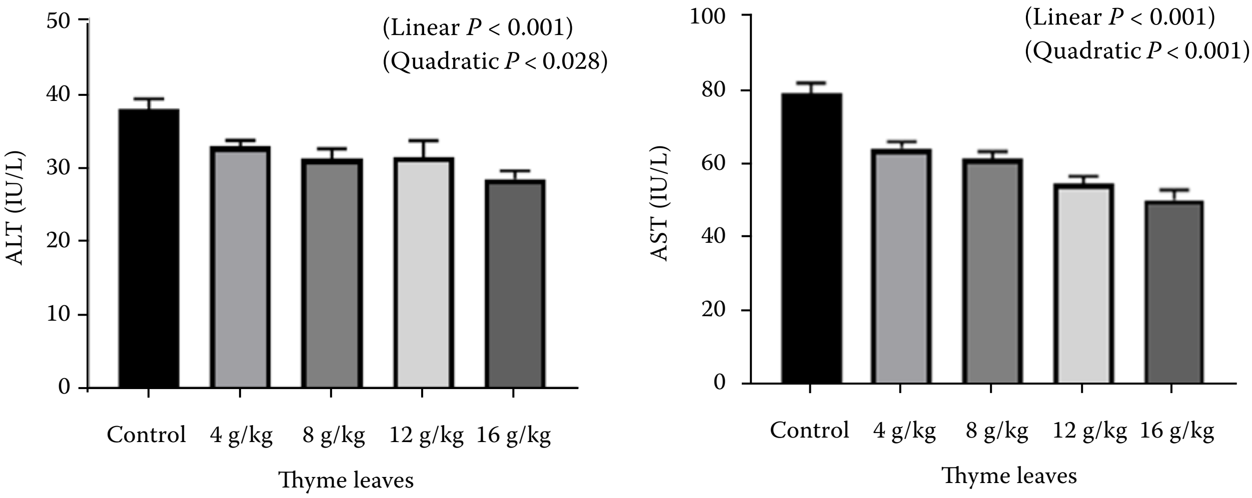


Figure 3. Liver enzyme (ALT and AST) levels of the male rabbits in response to the thyme leaf supplementation of 0 g/kg (control), 4, 8, 12 and 16 g/kg at 150 days of age

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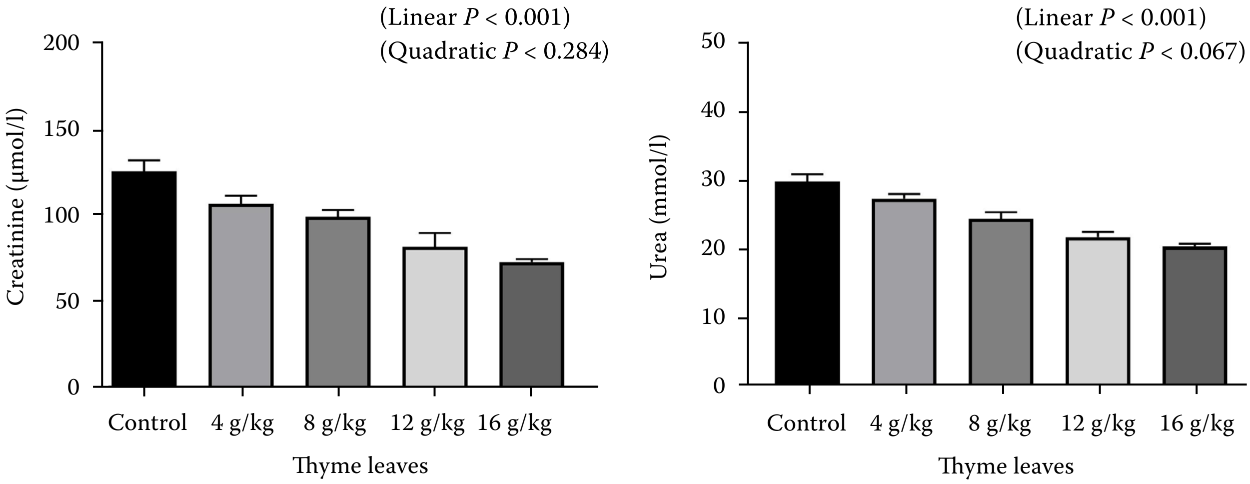


Figure 4. Serum creatinine μmol/l and urea mmol/l levels of the male rabbits in response to the thyme leaf supplementation of 0 g/kg (control), 4, 8, 12 and 16 g/kg at 150 days of age

significantly lowered the concentrations of the serum ALT and AST (linear and quadratic, $P <$ 0.001) compared to the non-supplemented group (Figure 2). Moreover, the serum urea and creatinine decreased linearly ($P < 0.001$) after supplementing the diets with thyme leaves compared to the non-supplemented counterpart (Figure 3). Interestingly, the serum testosterone concentrations significantly increased (linear and quadratic, $P < 0.001$) in the thyme leaf-supplemented group compared to those fed on the control diet under the hot climatic conditions (Figure 4).

## DISCUSSION

Due to the scarcity of available data on the effect of thyme leaves on sustaining productive and reproductive male rabbits under hot environmental conditions, a comparison was performed with other studies that used a thyme extract, essential oils, and active compounds either in rabbits or other farm animals. It is necessary to determine the chemical composition of the thyme leaves to identify their optimal composition, and the determination of the chemical composition of the thyme leaves prior to their use may provide useful information about the effect on male rabbits. The present study showed that the thyme leaves contained 1.58% essential oils of which thymol constitutes 50.1% relative to its analysed composition. The active component of thyme was consistent with those previously reported (Lee et al. 2005; Abdel-Wareth et al. 2018). Thymol 85.5%, carvacrol 6.81%, linalool 4.71%, α-terpineol 2.91%, and 1, 8-cineole 2.45% (Lee et al. 2005) were the major constituents of the thyme leaf oil extract. Accordingly, our findings also presented thymol as the major constituent of the thyme leaves (50.85%).

Polyphenolic thymol is known as an appetiser substance and has antimicrobial activity (Abdel-Wareth et al. 2012). Several studies have reported that thymol could enhance the digestive enzyme activities and improve the immune response (Hashemipour et al. 2013; Abd El-Hack et al. 2016). Furthermore, it stimulates the antioxidant activity and ameliorates the oxidative stress, hyperlipidaemia, and alleviates the inflammation-related responses (Yu et al. 2016). The general health status of the male New Zealand White rabbits was apparently good during the trial period, indicating that the environmental conditions were not detrimental to the rabbits' health. However, our findings indicated that the body weight gain and feed conversion ratio linearly improved ($P < 0.001$) in the rabbits fed a basal diet supplemented with thyme leaves under the hot environmental conditions during the periods of 90–120, 120–150 and 60–150 days of age. The feed intake linearly improved ($P < 0.001$) in the rabbits fed a basal diet supplemented with thyme leaves during the periods of 60–90 and 60–150 days of age under the hot environmental conditions. An improved feed intake with an improved weight gain in the thyme leaf levels suggested that the thyme leaves had an economic value especially in terms of its improved feed conversion efficiency. The highest performance, including body weight gain and feed conversion ratio, was recorded for

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the thyme leaf levels at 4, 8, 12 or 16 g/kg of the diet compared with the control. The improvements in the body weight gain and feed conversion ratio could be related to the improved feed utilisation efficiency and thyme leaf content of the thymol in a concentration dependent manner. Those findings agreed with our previous study on male Californian rabbits (Abdel-Wareth et al. 2018) where we reported that thyme oil had a beneficial impact on the animal performance and general health status in high temperature environments. Moreover, phytogenic additives to diets of broilers (Hippenstiel et al. 2011; Abdel-Wareth et al. 2012; Abdel-Wareth 2016; Abdel-Wareth et al. 2019) and rabbits (Cardinali et al. 2015; Abdel-Wareth et al. 2018) have helped to increase the body weight gain, feed intake and feed conversion ratio. Furthermore, phytogenics were often supposed to improve the flavour and, indirectly, the palatability of feeds, thus increasing the voluntary feed intake which, in turn, results in an improved body weight gain and feed conversion ratio (Cardinali et al. 2015). The current study showed that the feed conversion ratio could be improved by the thyme leaf supplementation under the hot environmental conditions in a dose-dependent manner. Those results may be attributed to the positive effect of the active thyme leaf component on the efficiency of the digestive tract and improving the feed conversion ratio (Gerencser et al. 2014; Zeng et al. 2015).

Consistent with our study, the administration of an aqueous thyme extract at 50 mg/kg b.w. in male rabbits was found to significantly improve the body weight gain, feed intake and feed conversion ratio compared to the control ($P < 0.001$) (Kandeil et al. 2019).

Thyme is used as a feed supplement in farm animal nutrition because of its contents, such as thymol, which has a positive response on the growth and feed conversion ratio (Abd El-Azeem et al. 2019). The improvement in the rabbit performance could be attributed to the parallel improvement in the appetite, absorption, metabolism of the nutrients and thyme's ability to modulate the gut microbiota (Abd El-Azeem et al. 2019).

The essential oil of thyme are defined based on the enhancement with antimicrobial activity of the gut health of an animal. However, the quantification of the active compounds, as well as elucidating upon their mechanisms is a difficult task, thus creating a standardisation will be imprecise at best (Hippenstiel et al. 2011; Bozkurt et al. 2014). Therefore, more studies under a standardisation platform are needed to explore the mode of action of the different phytogenics on the rabbit production. In the present study, dietary thyme leaves at 4, 8, 12, 16 g/kg of the total diet resulted in a positive response in terms of the production performance on the male rabbits in the hot environment, which can be attributed to active components of thyme leaves.

Interestingly, thyme leaves linearly decreased the ammonia faecal concentration of the male rabbits. Essential oils control the pathogenic bacteria and stimulate the intestinal enzyme activity which, in turn, increase the nitrogen absorption and control the liberated odour and ammonia content (Sethiya 2016). There is a lack of information in this direction. Those results agreed with previous studies that stated that a thyme extract significantly decreased the concentration of ammonia in the caecum content of rabbits compared to those of the control (Kandeil et al. 2019). Furthermore, Abd El-Azeem et al. (2019) reported that thyme oil significantly decreased the ammonia-N concentration in the caecum content of rabbits compared to those of the control.

Thyme leaves exhibited a linear ($P < 0.001$) significant increase in the ejaculate volume, sperm viability, and sperm motility in the semen collected at the end of the experimental period. The improvements that occurred in the semen quality, body weight and testes could be associated to each other in response to feeding on thyme leaves, and the antioxidant activity of the thyme components could play a pivotal role in improving the sperm and semen quality. Sperm motility is an important indicator of the reproductive performance, as it reflects the ability of the sperm cells to move across the female's genital tract and reflects the sperm power of the fertilisation and ovum penetration. The present findings agreed with other studies (Shanoon and Jassim 2012; Kandeil et al. 2019) who reported that aqueous extracts of thyme significantly increased the semen ejaculate volume, sperm concentration, sperm motility, sperm viability and normal morphology in rabbit bucks. Coincidentally, our results showed an increased testosterone concentration in response to feeding on thyme leaves together with an increased body weight, semen volume, testes mass and sperm motility index in the male rabbits that could reflect the positive effect of the thyme leaf components

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on the spermatogenesis and sexual desire (Ruiz-Olvera et al. 2017). Our results agreed with a previous study (Khosravinia 2014) which reported that serum testosterone was significantly increased in broilers that received carvacrol, the main thyme constituent, at doses greater than 0.2 g/l compared to the controls which propose the possibility of testosterone-coupled hypolipidemic properties for carvacrol, and that the increase in the testosterone concentration confirmed its action as an anabolic hormone which obviously resulted in the feed conversion and rates in our results.

Supplementing the feed with thyme leaves for male rabbits significantly decreased the serum concentrations of the urea, creatinine, ALT and AST compared to those of the control (Abdel-Gabbar et al. 2019). Those findings suggest that being fed a diet supplemented with thyme leaves could improve the kidney and liver functions, and thus mitigate the adverse effects of the high ambient temperature on the biological activities of the living body. Abu Raghif et al. (2015) found that an aqueous thyme extract significantly reduced the serum levels of the liver enzymes in rabbits. Moreover, thyme extracts at 100 mg/kg significantly decreased all kidney markers, i.e., creatinine and urea, as well as decreased the levels of the ALT and AST liver enzymes (Abdel-Gabbar et al. 2019). Salem (2015) indicated that feeding rats on diets supplemented with 2.5% thyme leaves significantly decreased the urea and creatinine concentrations in the kidney secretion and further stimulated the antioxidant activity in neutralising the free radicals which cause inflammation in kidney tissues (Hasan Mohammed 2015).

In conclusion, our results confirmed the chemical composition of thyme leaves according to previous studies. It is recommended, as a feed additive, in an optimum dose of up to 16 g/kg of the diet for male New-Zealand rabbits under hot environmental conditions to improve both the productive and reproductive performances. Those improvements included: the growth rate, kidney/liver functions, and semen quality and testosterone levels, in addition to lowering the faecal ammonia as well.

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## Conflict of interest

The authors declare no conflict of interest.

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