Effects of dietary phytogenic supplementation on performance, semen quality and serum biochemical of rabbits

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Research

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Abstract

Background

Recently, restrict the use of antibiotic growth promoters by several countries and the menace of antibiotic resistant bacteria has forced to look for alternatives in rabbits. This study aimed to assess the efficiency of thyme essential oil (TEO) as a novel feed additive to dietary antibiotic to improve productive, reproductive performance and serum biochemical of male rabbit.

Methods

A total of one hundred and fifty 70-day-old male Californian rabbits were assigned to five dietary treatments including a basal diet as a negative control (NC), a basal diet was supplemented with an antibiotic as positive control (PC) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO. The experiment period lasted for 60 days.

Results

Supplementation of TEO levels significantly (P < 0.01) increased body weight gain (BWG) and improved feed conversion ratio (FCR) of male Californian rabbits compared to NC and PC groups. Likewise, the TEO significantly improved semen characteristics of rabbits compared to NC and PC groups. Serum testosterone, aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine were improved by supplementations of thyme essential compared to NC and PC.

Conclusions

Based on the study results, TEO levels up to 180 mg/kg have the potential to improve productive and reproductive performances of Californian male rabbits.

1. Introduction

Recently, rabbits industry becomes an important role to meet the high requirements of animal protein for human consumption and a source of the national economies in Egypt [1, 2]. Rabbit meat is characterized by a high protein and low fat and cholesterol content [3], these nutritional meat qualities are of great value for the meat industry and consumers. Ban on antibiotic growth promoters by several countries and the menace of antibiotic resistant bacteria has forced to look for alternatives for improving efficiency in animal production [4]. Due to this ban, much research has been conducted to explore the use of phytogenic as alternate feed additives in animal nutrition [5, 6]. Phytogenic substances are generally regarded as safe and are frequently used in the food and feed industries [7]. The impact of phytogenic on the intestinal health, antioxidant status and antimicrobial activity is considered essential for the
biological activities. Moreover, the thyme oil as phytogenic feed additives can have a beneficial impact on rabbit performance, health status, and welfare [8]. Furthermore, dietary Thyme extract supplementation at 0.5 g/kg significantly improved gut integrity and antioxidant status of rabbits [9] which mainly due to thyme active components. The main components of thyme essential oil (TEO) are thymol, carvacrol, γ-terpinene, p-cymene, β-myrcene, linalool and terpinen-4-ol [2, 10]. These active components had the best oxidative status [11] and may decrease serum cholesterol [12] which could enhance reproductive performances of rabbits.

El-Ratel et al. [13] reported that oral administration of phytobiotic improved liver function of rabbits compared with the control. Likewise, supplementation of 2.5% of thyme leaves to rabbit diets significantly improved kidney function by decreased urea and creatinine levels [14]. However, no information is available about effects of TEO as alternatives to dietary antibiotics growth promoters on performance semen quality, kidney and liver functions and testosterone levels in male rabbits.

To explore the effects of TEO on productive and reproductive performance of male rabbits, we investigated the efficiency of TEO as alternatives to dietary antibiotics for improving productive, reproductive performances, liver and kidneys functions of male rabbit.

2. Materials And Methods

This trial was carried out at Agricultural Research Centre, Rabbit Farm, South Valley University, Egypt. The Institutional Ethics Committee of the South Valley approved the experimental procedures regarding the handling and care of rabbits. All procedures by this study were in accordance with international ethical standards.

2.1. Experimental Animals, Design and Management

A total of 150, seventy-day-old male Californian rabbits (BW 1250±30 g) were assigned to five dietary treatments (n = 30 per dietary treatment). Dietary treatments included a basal diet as a negative control (NC), a basal diet was supplemented with an antibiotic at 500 mg/kg oxytetracycline as a positive control (PC) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO. The experimental rations ingredients and chemical composition are given in Table 1. The diets were formulated to contain adequate levels of nutrients for rabbits according to National Research Council (NRC) [15].

Rabbits were housed separately in cages of galvanised wire net (width × length × height: 60 cm × 60 cm × 40 cm), equipped with an automatic drinker and a manual feeder. Farm temperature was maintained at 23°C and cycle of 16 h of light and 8 h of dark was used during the experimental period.

Animal initial and final body weights (BW) was recorded and BW gain was calculated. The daily feed intake (g/rabbit) of male Californian rabbits was measured. The feed conversion ratio (FCR) was calculated by dividing feed intake, g/kg by average BW gain g/kg. Mortality was recorded as it occurred.
2.2. Thyme Essential Oil Preparation and Analysis

The chemical composition of hydrodistilled TEO (Table 2) was analyzed using a GC/MS system according to Abozid and Asker [16]. The TEO was analyzed by gas chromatography (Delsi 121C gas chromatograph). Constituents were identified by coupling gas chromatography with mass spectrometry (GC/MS), using a Sigma 300 apparatus attached to a HP 5970 300 mass spectrometer.

2.3. Chemical analysis

The feed were analyzed for moisture by oven drying (Method no. 930.15), ash by incineration (Method no. 942.05), protein by Kjeldahl (Method no. 984.13), ether extract by Soxhlet fat analysis (Method no. 920.39), calcium and phosphorus (Ca and P; Method no. 999.10) as described by the AOAC International [17]. Gross energy was measured with an adiabatic bomb calorimeter (Parr Instrument Company, IL, USA).

2.4. Semen Characterizations

Semen characterizations including volume of each ejaculate, sperm livability, sperm motility, abnormal sperm and sperm forward motility of sexually mature and healthy rabbits (130 days of age) of the male rabbits were assessed as described previously by by Abdel-Wareth et al. [5] and El-Desoky et al. [18].

2.5. Blood biochemical

At the end of the experiment, fifteen rabbits were used to collect blood samples from ear vein of in each treatment in non-heparinized sterile tubes. The samples were allowed to coagulate at room temperature for 30 min and then centrifuged at 3000 rpm for 15 min and the serum was harvested and stored at -20°C until used in the biochemical analysis as described previously by Abdel-Wareth et al [5]. Serum testosterone, aspartate transaminase (AST) and alanine transaminase (ALT), urea and creatinine were assessed conferring to the manufacturer's instructions by the spectrophotometric technique using commercial diagnostic kits (Monobind Inc. Lake Forest; CA 92630, USA).

2.6. Statistical Analysis

The statistical analysis was analysed by ANOVA followed by Duncan's test using SAS software [19]. The cage was the experimental unit for each parameter. The significance effects were declared at (P<0.05).
Orthogonal polynomial contrasts were also used to determine linear and quadratic effects of levels of TEO inclusion considering only negative control (0 mg/kg TEO) as a control, and positive control was not included in this analysis.

3. Results

3.1. Growth Performance and Health Status

The effects of TEO levels as alternatives to dietary antibiotic on growth performance are presented in Table 3. TEO levels significantly increased (P<0.01) BW gain and improved FCR of male Californian rabbits compared to PC and NC groups during the periods of 70-100, 100-130 and 70-130 days of age. On the other hand, the supplemented TEO groups did not affect feed intake (P≥0.05) compared to control groups.

3.2. Semen characteristics

Effects of TEO on semen characteristics were presented in (Table 4). Results showed that TEO increased the sperm livability, sperm motility and ejaculate volume compared to PC and NC groups at the end of treatments. Abnormal sperm was decreased (P<0.01) with increasing TEO, compared to PC and NC groups. Moreover, supplementation of TEO up to 180 mg/kg to male rabbit diets significantly increased (P<0.001) sperm forward motility% and sperm livability% compared to control groups, however there was no significant difference in the semen pH value between treatments. Over all, the PC (oxytetracycline) exhibited a significant increase in the semen quality compared to NC.

3.3. Blood biochemical constituents

The effects of dietary supplemental TEO on blood serum constituents of rabbit male are showed in Figure 1, 2 and 3, respectively. Rabbits fed the diets supplemented with TEO at 60, 120 and 180 mg/kg significantly (P<0.001) decrease serum urea and creatinine compared to PC and NC groups. Moreover, activity of serum ALT and AST were significantly (P<0.05) decreased by supplementation of TEO to male rabbits diets compared with PC and NC groups. Furthermore, male rabbits fed diets supplemented with the TEO up to 180 mg/kg showed the highest improvement serum testosterone concentrations (P<0.05) compared to PC and NC groups.

4. Discussion

The TEO phytogenic feed additive as alternatives to dietary antibiotics high safety and could be used to improve semen quality and health conditions. The impact of phytogenic on the intestinal health, antioxidant status and antimicrobial activity is considered essential for the biological activities; however,
studies lack determination of active components and explore their mechanisms are still not clear [20,21,22]. Therefore more studies under standardization are needed to explore the mechanisms of herbal essential oils on rabbit production and reproduction. In current study, the main active component of TEO was thymol which constitutes 40% relative of its analysed composition. The composition of TEO used in the present study was consistent with those reported in the literature [2, 10]. The current study showed that BW gain and FCR were improved in rabbits fed a control diet supplemented with TEO up to 180 mg/kg compared to PC and NC groups. These improvements may be due to the active compounds stimulated digestive enzymes and therefore leads to improve nutrient digestibility [6, 23]. Similar results were reported supplementation of TEO with olive oil improved growth performance of male Californian rabbits under high temperature environments [2]. Likewise, oral administration of aqueous thyme extract with 50 mg/kg BW improved (P < 0.001) BWG, feed intake and FCR of rabbits compared to control [24].

The TEO levels significantly increased reproductive performance of rabbit male at the end of the treatments period (Table 4). Interestingly the improvements in the reproductive performance of rabbit male were in parallel with testosterone concentration (Fig. 1). Ruiz-Olvera et al. [25] reported that semen volume and sperm motility reflects to serum testosterone concentrations. Semen quality of rabbit was significantly increased by supplementation of thyme aqueous extracts [26]. Also, rabbit fed on extracts of thyme significantly increased (P < 0.05) semen volume, sperm motility, sperm concentration and sperm livability compared with the control [24].

In the current study, supplementation of TEO to male rabbits significantly decreased serum urea, creatinine, ALT and AST activity, as well as increased serum testosterone levels compared to PC and NC groups. These results are in accordance with those of El-Ratel et al. [13] reported that the activity of AST and ALT of rabbits were significantly improved by oral administration of phytogenic (5 or 10 mg allicin/BW) compared with the control. Supplementation of 2.5% of thyme leaves to rabbit diets significantly decreased urea and creatinine [14]. Likewise, Abdel-Gabbar et al. [27] reported that thyme extracts at 100 mg/kg induced significant (P < 0.001) decrease in creatinine and urea as well as ALT and AST of rabbits compared to control group. Therefore, the effect of the TEO on productive and reproductive of rabbits is better known. It increased both productive and reproductive production, as well as the health status, as observed in this study.

5. Conclusions

In view of the above findings, thyme essential oil levels up to 180 mg/kg can play an important role, as an alternative to dietary antibiotic, to improve productive performance, semen quality and testosterone levels as well as kidney and liver functions in California male rabbits.

Abbreviations

TEO: Thyme essential oil; NC: negative control; PC: positive control; BW: body weight; BWG: body weight gain; FCR: feed conversion ratio; AST: aspartate transaminase; ALT: alanine transaminase.
Declarations

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Author Contributions:

A.A.A-W was responsible for the design, conduction of animal feeding experiments, semen quality analysis and drafted the article. A.E.M blood samples analysis and the experiment of thyme oil GS-Ms analysis and revised this manuscript critically for important intellectual content. Both authors read and approved the final manuscript

Availability of data and materials:

All data that support the findings of this study are included in this article.

Ethics approval and consent to participate:

All animal care and use procedures for this study were approved by the Institutional Animal Care and Use Committee at South Valley University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sheiha AM, Abdelnour SA, Abd El-Hack ME, Khafaga AF, Metwally KA, Ajarem JS, Maodaa SN, Allam AA, El-Saadony MT. Effects of Dietary Biological or Chemical-Synthesized Nano-Selenium
Supplementation on Growing Rabbits Exposed to Thermal Stress. *Animals* 2020, 10, 430.

2. Abdel-Wareth AAA, Taha EMM, Südekum K-H, Lohakare J 2018. Thyme oil inclusion levels in a rabbit ration: Evaluation of productive performance, carcass criteria and meat quality under hot environmental conditions. *Animal Nutrition*, 4, 410-416. [https://doi.org/10.1016/j.aninu.2018.02.004](https://doi.org/10.1016/j.aninu.2018.02.004)

3. Dalle Zotte A, Szendro Z. The role of rabbit meat as functional food. *Meat Sci.*, 2011. 88, 319-331.

4. Bovera F, Marono S, Di Meo C, Piccolo G, Iannaccone F, Nizza A. Effect of mannanoligosaccharides supplementation on caecal microbial activity of rabbits. *Animal* 2010, 9, 1522-1527. [https://doi.org/10.1017/S1751731110000558](https://doi.org/10.1017/S1751731110000558)

5. Abdel-Wareth AAA, Ahmed AE, Hassan HA, Abd El-Sadek MS, Ghazalah AA, Lohakare. Nutritional impact of nano-selenium, garlic oil, and their combination on growth and reproductive performance of male Californian rabbits. *Animal Feed Science and Technology* 2019, 249, 37-45. [https://doi.org/10.1016/j.anifeedsci.2019.01.016](https://doi.org/10.1016/j.anifeedsci.2019.01.016)

6. Abouelezz K, Abou-Hadied M, Yuan J, Elokil A, Wang G, Wang S, Wang J, Bian G. Nutritional impacts of dietary oreg-ano and Enviva essential oils on the performance, gut microbiota and blood biochemistry of growing ducks. *Animal* 2019, 13, 2216-2222. [https://doi.org/10.1017/S1751731119000508](https://doi.org/10.1017/S1751731119000508)

7. Rašković A, Pavlović N, Kvrgić M, Sudji J, Mitić Ġ, Čapo I, Mikov M. Effects of pharmaceutical formulations containing thyme on carbon tetrachloride induced liver injury in rats. *BMC Complementary and Alternative Medicine* 2015, 15, 442-453. [https://doi.org/10.1186/s12906-015-0966-z](https://doi.org/10.1186/s12906-015-0966-z)

8. Attia YA, Bakhashwain AA, Bertu NK. Thyme oil (Thyme vulgaris L.) as a natural growth promoter for broiler chickens reared under hot climate. *Italian Journal of Animal Science* 2016, 16, 275-82. [https://doi.org/10.1080/1828051X.2016.1245594](https://doi.org/10.1080/1828051X.2016.1245594)

9. Placha I, Chrastinova L, Laukova A, Cobanova K, Takacova J, Strompfova V, Chrenkova M, Formelova Z, Faix S. Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. *Acta Veterinaria Hungarica* 2013, 61, 197-208. [https://doi.org/10.1556/AVet.2013.012](https://doi.org/10.1556/AVet.2013.012)

10. Lee S, Umano K, Shibamoto T, Lee K. Identification of volatile components in basil (Ocimumbasilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. *Food Chemistry* 2005, 91, 131-137. [https://doi.org/10.1016/j.foodchem.2004.05.056](https://doi.org/10.1016/j.foodchem.2004.05.056)

11. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and *Thymus hyemalis* essential oils. *Food Control* 2008, 19, 681-687. [https://doi.org/10.1016/j.foodcont.2007.07.007](https://doi.org/10.1016/j.foodcont.2007.07.007)

12. Abdulkarimi R, Daneshyar M, Aghazadeh A. Thyme (Thymus vulgaris) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Italian Journal of Animal Science* 2011, 10, 101-105. [https://doi.org/10.4081/ijas.2011.e20](https://doi.org/10.4081/ijas.2011.e20)

13. El-Ratel IT, Abdel-Khalek AE, Gabr SA, Hammad ME, El-Morsy HL. Influence of allicin administration on reproductive efficiency, immunity and lipid peroxidation of rabbit does under high ambient
temperature. *Journal of Animal Physiology and Animal Nutrition* 2020, 104, 539-548. doi:10.1111/jpn.13316

14. Salem AA. Effect of feeding on olive oil and thyme on pregnancy and lactation periods. *International Journal of Nutrition and Food Sciences* 2015, 4(1), 19-28. https://doi.org/10.11648/j.ijnfs.20150

15. Nutrient requirements of rabbits: 1977. National Academies Press.

16. Abozid MM, Asker MMS. Chemical composition, antioxidant and antimicrobial activity of the essential oil of the thyme and rosemary. *International Journal of Academic Research and Development* 2013, 5, 186-195. https://doi.org/10.7813/2075-4124.2013/5-3/A.26

17. Official methods of analysis 17th edition', Assoc. Off. Anal. Chem, Arlington, Virginia, USA, 2006.

18. El-Desoky NI, Hashem NM, Elkomy A, Abo-elezz ZR. Physiological response and semen quality of rabbit bucks supplemented with Moringa leaves ethanolic extract during summer season. *Animal 2017, 11*, 1549-1557. https://doi.org/10.1017/S1751731117000088

19. SAS Institute. SAS/STAT® 9.1 User's Guide. 2nd ed. Cary, NC: SAS Institute Inc.

20. Bozkurt M, Hippenstiel F, Abdel-Wareth AAA, Kehraus S, Küçükyılmaz K, Südekum K-H. Effects of selected herbs and essential oils on performance, egg quality and some metabolic activities in laying hens-A review. *European Poultry Science 2014, 78*, 15pp. doi:10.1399/eps.2014.49

21. Hafeez A, Männer K, Schieder C, Zentek J. Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. *Poultry Science 2016, 95*, 622-629. doi:10.3382/ps/pev368

22. Hippenstiel F, Abdel-Wareth AAA, Kehraus S, Südekum K-H. Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers-A review. *Archiv fur Geflugelkunde 2011*, 75, 226-234.

23. Abd El-Hack M, Alagawany M, Ragab FM, Tiwari R, Karthik K, Dhama K Zorriehzahra J, Adel M. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *Journal of Essential Oil Research 2016, 28*, 365-382. https://doi.org/10.1080/10412905.2016.1153002

24. Kandeil MA, Mohamed AH, Abdel Gabbar M, Ahmed RR, Ali SM. Ameliorative effects of oral ginger and/or thyme aqueous extracts on productive and reproductive performance of V-line male rabbits. *Journal of Animal Physiology and Animal Nutrition 2019, 103*, 1437-1446. https://doi.org/10.1111/jpn.13147

25. Ruiz-Olvera SF, Rajmil O, Sanchez-Curbelo JR, Vinay J, Rodriguez-Espinosa J. Association of serum testosterone levels and testicular volume in adult patients. *Andrologia* 2018; 50: e12933.

26. Shanoon AK, Jassim MS. Effects of Thymus vulgaris and Zingiber officinale aqueous on semen parameters, testes weight and histology measurements of broiler breeder male. *International Journal of Poultry Science 2012, 11(9)*, 594-598. https://doi.org/10.3923/ijps.2012.594.598

27. Abdel-Gabbar M, Kandeil MA, Mohamed AH, Ahmed RR, Ali SM. Administration of ginger and/or thyme has ameliorative effects on liver and kidney functions of V-line rabbits: Histological and
biochemical studies. *Journal of Animal Physiology and Animal Nutrition* 2019, 103, 1758-1767. [https://doi.org/10.1111/jpn.13166](https://doi.org/10.1111/jpn.13166)

**Tables**

**Table 1** Ingredient and chemical composition (as-fed basis) of the control diet fed to 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 fiber       | 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<sup>1</sup> | 0.30  |                   |      |
| DL-Methionine                      | 0.10  |                   |      |

<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.

**Table 2** The chemical composition of hydrodistilled thyme essential oil
| Compounds            | Rt.  | Area % |
|----------------------|------|--------|
| p-Cymene             | 6.99 | 23.59  |
| β -linalool          | 9.61 | 0.74   |
| Carvone              | 15.70| 9.80   |
| Anethole             | 17.49| 2.50   |
| Thymol               | 17.70| 39.45  |
| Carvacrol            | 18.09| 2.07   |
| trans-Caryophyllene  | 22.46| 0.98   |
| γ-terpinene          | 25.14| 12.49  |
| Aromadenrene         | 34.84| 2.12   |
| Ledol                | 48.66| 2.24   |

**Table 3** Effects of thyme essential oils on productive performance of male rabbits
| Items                          | NC¹  | PC²  | 60 mg | 120 mg | 180 mg | SEM³ | P-value |
|-------------------------------|------|------|-------|--------|--------|------|---------|
|                               | TEO⁴ | Lin⁵ | Quad⁶ |
| Body weight gain, g           |      |      |       |        |        |      |         |
| 70-100 d                      | 445ᵈ | 492ᶜ | 549ᵇ  | 570ᵃ  | 582ᵃ  | 6.89 | <0.001 |
| 100-130 d                     | 587  | 603  | 620   | 622   | 634   | 11.48| 0.075 |
|                               |      |      |       |        |        |      | 0.006  |
|                               |      |      |       |        |        |      | 0.640  |
| 70-130 d                      | 103³ᵈ| 1095ᶜ| 1170ᵇ | 1192ᵃᵇ| 1216ᵃ | 14.84| <0.001 |
|                               |      |      |       |        |        |      | <0.001 |
|                               |      |      |       |        |        |      | 0.033  |
| Feed intake, g                |      |      |       |        |        |      |         |
| 70-100 d                      | 163⁴ᶜ| 166³ᶜ| 172⁹ᵇ | 175⁸ᵃᵇ| 178⁹ᵃ | 15.42| <0.001 |
| 100-130 d                     | 195³ᵃ| 188³ᵇ| 189¹ᵇ | 193⁴ᵃᵇ| 194¹ᵇᵃ | 19.2¹| 0.05² |
|                               |      |      |       |        |        |      | 0.7¹⁸ |
|                               |      |      |       |        |        |      | 0.013  |
| 70-130 d                      | 35⁹³ᶜ| 35⁴³ᶜ| 36²⁰ᵇᶜ| 36⁹²ᵃᵇ| 37³⁰ᵃ | 25.9²| <0.00¹ |
|                               |      |      |       |        |        |      | <0.00¹ |
|                               |      |      |       |        |        |      | 0.1¹⁰ |
| Feed conversion ratio         |      |      |       |        |        |      |         |
| 70-100 d                      | 3.6⁷ᵃ| 3.3⁸ᵇ| 3.1⁴⁷ᶜ| 3.0⁸³ᶜ| 3.0⁷³ᶜ | 0.0⁴⁸| 0.0⁰¹ |
| 100-130 d                     | 3.³³ᵃ| 3.¹₂ᵇ| 3.⁰⁵ᵇ| 3.¹¹ᵇ| 3.⁰⁶ᵇ | 0.0⁵⁶| 0.0¹⁴ |
|                               |      |      |       |        |        |      | 0.0⁰⁶ |
|                               |      |      |       |        |        |      | 0.0⁴⁶ |
| 70-130 d                      | 3.⁴⁷ᵃ| 3.²³ᵇ| 3.⁰⁹⁶ᶜ| 3.⁰⁹³ᶜ| 3.⁰⁶⁷ᶜ | 0.0⁴²| 0.0⁰¹ |
|                               |      |      |       |        |        |      | <0.00¹ |
|                               |      |      |       |        |        |      | 0.0⁰² |

a,b Values within a row with different superscripts differ significantly at $P<0.05$.

¹NC; basal diet as a negative control

²PC; a basal diet was supplemented with an antibiotic as positive control

³SEM; standard error of means

⁴TEO; Thyme essential oil treatments


Lin and Quad; Linear and quadratic responses, respectively, to supplementation levels.

Table 4 Effects of thyme essential oil on semen quality of male rabbits

| Items          | Thyme essential oil (TEO) | SEM<sup>1</sup> | P-value |
|----------------|----------------------------|-----------------|---------|
|                | NC<sup>1</sup> | PC<sup>2</sup> | 60 mg | 120 mg | 180 mg | TEO<sup>4</sup> | Lin<sup>5</sup> | Quad<sup>6</sup> |
| Sperm Volume, ml | 0.52<sup>d</sup> | 0.56<sup>c</sup> | 0.61<sup>b</sup> | 0.64<sup>a</sup> | 0.65<sup>a</sup> | 0.09 | 0.001 | 0.001 | 0.004 |
| Abnormal sperm, % | 16.7<sup>a</sup> | 15.4<sup>ab</sup> | 12.6<sup>bc</sup> | 11.9<sup>cd</sup> | 11.2<sup>d</sup> | 0.41 | 0.001 | 0.001 | 0.432 |
| Live sperm, % | 75.0<sup>d</sup> | 75.4<sup>d</sup> | 79.2<sup>c</sup> | 81.6<sup>b</sup> | 83.6<sup>a</sup> | 0.63 | 0.001 | 0.001 | 0.458 |
| pH, value | 7.11 | 7.09 | 7.10 | 7.11 | 7.10 | 0.03 | 0.990 | 0.965 | 0.853 |
| Forward motility, % | 55.1<sup>c</sup> | 55.3<sup>c</sup> | 65.7<sup>b</sup> | 69.5<sup>a</sup> | 70.8<sup>a</sup> | 0.57 | 0.001 | 0.001 | 0.056 |

<sup>a,b</sup> Values within a row with different superscripts differ significantly at <i>P</i><0.05.

<sup>1</sup>NC; basal diet as a negative control

<sup>2</sup>PC; a basal diet was supplemented with an antibiotic as positive control

<sup>3</sup>SEM; standard error of means

<sup>4</sup>TEO; Thyme essential oil treatments

<sup>5-6</sup>Lin and Quad; Linear and quadratic responses, respectively, to supplementation levels.

**Figures**
Testosterone ng/ml of male rabbits in response to dietary treatments included a basal diet (negative control), a basal diet was supplemented with an antibiotic at 500 mg/kg oxytetracyclin (positive control) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO at 130 days of age.

Figure 1

Testosterone ng/ml of male rabbits in response to dietary treatments included a basal diet (negative control), a basal diet was supplemented with an antibiotic at 500 mg/kg oxytetracyclin (positive control) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO at 130 days of age.
Figure 2
Liver enzyme (ALT and AST) levels of male rabbits in response to dietary treatments included a basal diet (negative control), a basal diet was supplemented with an antibiotic at 500 mg/kg oxytetracyclin (positive control) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO at 130 days of age.

Figure 3
Serum creatinine mg/dl and urea mg/dl of male rabbits in response to dietary treatments included a basal diet (negative control), a basal diet was supplemented with an antibiotic at 500 mg/kg oxytetracyclin (positive control) and the basal diet was supplemented with 60, 120 or 180 mg/kg of TEO at 130 days of age.