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EFFECTS OF DIETARY THYMOQUINONE INCLUSION ON ANTIOXIDATIVE, OXIDATIVE, PRO-INFLAMMATORY RESPONSES, SEMEN ATTRIBUTES AND TESTICULAR CHANGES IN HEAT-STRESSED RABBIT BUCKS

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Abstract

Heat stress (HS) is considered a severe concern for rabbit buck's health, welfare and reproductive features. Thymoquinone (THQ) is a natural molecule with robust antioxidant, antimicrobial, and anti-inflammatory properties. Thereafter, three months of feeding research was applied to assess the role of THQ-enriched diets in improving the health, semen attributes and testicular changes of stressed rabbit bucks. Forty rabbit bucks were used in this research, and animals were fed diets containing 0 (THQ0; serve as a heat stress group), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg of THQ for three months continually during the summer season. Outcomes displayed that THQ significantly enhanced FBW (final body weight), DBWG (daily body weight gain), and FI (feed intake) but did not affect FCR (feed conversion ratio). Compared with the THQ0 group, dietary THQ addition significantly decreased creatinine, TB (total bilirubin), TG (triglycerides), and GGT (gamma-glutamyl transferase) levels. Bucks receiving 200 mg of THQ had higher TAC (total antioxidant capacity) and GSH (glutathione) than other groups. At the same time, MDA was significantly shrunk in THQ-treated groups relative to the THQ group. The levels of interferon-gamma (IFN-ᵞ) and interleukin-4 (IL-4) were significantly diminished (P <0.001), but nitric oxide and lysosome activity were significantly increased in bucks given diets containing THQ (200 and 400 mg/kg) as relative to the TH0 group. Libido score and reaction time were significantly improved by THQ dietary inclusion. Semen attributes, including volume, motility, concentration, and vitality, were significantly augmented in THQ100 and THQ200 groups. Histological screening of testicular tissues showed moderate to severe degenerative and necrotic changes in the testicular tissues of bucks exposed to HS. This was enhanced with the supplementation of THQ at different levels. Collectively, THQ (200 or 400 mg/kg diet) can improve health, semen attributes and restore testicular damage in rabbit bucks via boosting antioxidant and reducing inflammatory cytokines.

Key words: thymoquinone, heat stress, rabbit bucks, antioxidant, inflammation, semen quality

In previous eras, global warming associated with substantially raised temperatures has become a menacing issue for the planet, affecting animals, plants and other organisms. This high temperature exhibited numerous undesirable effects on the livestock sector, including the rabbit industry. Rabbits have many advantages, such as rapid growth, prolificacy, and feed efficacy (Oladimeji et al., 2022). Despite those advantages, they suffer from heat stress (HS) during hot seasons, especially in arid and semi-arid regions. The main obstacle for the rabbit industry is their high sensitivity to HS. It harms the feed capacity, growth, immunity system, reproduction and productive potential of rabbits.

Moreover, some rabbit farms could diminish or stop to sustain their production through the summer season. Rabbit bucks also can be affected by the HS condition. Overall, HS caused low sexual behavior, hormonal misbalances, and increased abnormal sperm; decreased sperm production resulted in male infertility (Abdelnour et al., 2021). Also, many blood biochemical changes or misbalances in rabbit bucks were affected by exposure to HS (Hosny et al., 2020). Additionally, HS induces a reduction in the immune status and produces a higher level of inflammatory responses in the blood, which might associate with the apoptosis of sperm in males (Chaidanya et al., 2020; Abdelnour et al., 2021). Moreover, HS induced oxidative stress (OS), which causes damage to tissues via augmenting lipid peroxidation (Hosny et al., 2020).

Discovering a new approach for reducing the testicular inflammation and apoptotic sperm and enhancing sperm function and health is critical for clarification. Several nutritional approaches have received attention in the last eras for enhancing the sexual behavior and reproductive attributes in rabbit bucks and mitigating the negative influences of HS (Hosny et al., 2020; Abdelnour et al., 2021; El-Ratel et al., 2021). After screening the websites, no previous studies reported using thymoquinone (THQ) to alleviate these deleterious effects of HS in rabbit bucks. Using natural compounds as immunomodulatory, anti-HS, anti-apoptotic, anti-inflammatory agents may receive more attention from scientists in the era of free antibiotics. Natural biomolecules are vigorous constituents isolated from herbs that counteract the synthesis of ROS (Abdelnour et al., 2021) induced by several antagonistic factors. One of the most popular herbs is *Nigella sativa*, which has many active components such as THQ (thymoquinone), 4-terpineol, carvacrol, and t-anethole recognized since ancient centuries as a natural strong antioxidant, and anti-inflammatory (Pottoo et al., 2022; Alenezi, 2023). THQ exhibited numerous health-promoting capabilities (Sarkar et al., 2021).

The antioxidant property of THQ may be attributed to its ability to shield tissues alongside OS, which group persuades of free radical mediators and suppression of lipid peroxidation (Pottoo et al., 2022). Moreover, THQ is substantially significant to cosmetic, pharmaceutical, agricultural, and food products (Elmowalid et al., 2022; Pottoo et al., 2022; Alenezi, 2023). Many previous studies on rabbits described that adding *Nigella sativa* in rabbit diets exhibited an immunomodulatory effect and growth promotion via improving the health status, blood profile and immunity (El-Gindy et al., 2020; Elmowalid et al., 2022). Regarding the potential role of THQ in relieving the negative effects of HS in animals, only one experiment on mice has reported the positive effects of THQ in combating HS (Saeed et al., 2011). Multiple previous studies have shown the protection roles of THQ against lead toxicity in mice (Mabrouk and Ben Cheikh, 2016), tartrazine hepatotoxicity (Demircigil et al., 2023), cancer disease (Aslan et al., 2021), kidney toxicity (Kaymak et al., 2022), cadmium toxicity (Ben Mrid et al., 2022), cryodamage of ram sperm (İnanc et al., 2022), and provided a significant defense against *Aeromonas sobria* in fish infections (Ibrahim et al., 2022).

Besides, it was proposed that THQ shows a role in reclaiming the influences of different damaging situations on male fertility, such as HS (Mosbah et al., 2018), attenuation of neuropathic pain (Alenezi, 2023), toluene-persuaded deficiency of spermatogenesis in rats (Kanter, 2011), methotrexate-persuaded testicular impairment in mice (Shikhbahaei et al., 2018), and torsion-detorsion induced ischemic testicular destruction in mice (Gökçe et al., 2010). Moreover, THQ could protect against the testicular changes induced by hypothyroidism, which may cause male dysfunction via enhancing the antioxidative status, reducing the inflammatory indices such as TNF-α (Algaidi et al., 2022). This feature could be associated with its hopeful anti-apoptotic and anti-inflammatory actions. Its highly natural biological assets are an auspicious goal for the planned study. Until now, there appears to be no information existing in the previous research on the protective role of THQ in alleviating HS-triggered stress in rabbit bucks.

Thus, the present study was undertaken to explore the possible protective effect of THQ against HS-induced male reproductive damages in the light of immune dysfunction, liver and kidney imbalances, oxidative stress, as well as testicular damages and inferior sperm attributes in stressed male rabbits.

Material and methods

Ethical statement

The research procedures were accomplished in concurrence with the NIH endorsements. The protocol was sanctioned by the Ethics of Animal Use in Research Committee of Zagazig University, Egypt (ZU-IACUC) (Approval number ZU IACUC/2/F/367/2022). Every effort was made to handle the animals humanely and tackle ethical issues.

Animals, experimental design and diet

Adult healthy male rabbits (n=40), with an average body weight (BW) of 1123±30 g and eight weeks of age, were included in the current investigation. The forty bucks were separated into four groups, ten in each group. The bucks were individually kept in appropriate wire cages (40 × 50 × 35 cm) under naturally ventilated rabbitry. Bucks were given a standard diet to cover their requirements (feed ingredients and composition analysis are presented in Table 1). Animals were randomly allocated into four groups (n = 10) according to the addition levels with dietary thymoquinone (490-91-5, Sigma-Aldrich, Chemie GmbH, Germany). Rabbit bucks in the control treatment (THQ0) were given the basal pellet diet without additives (Saeed et al., 2011) and used as an HS group. For the other three treatments, animals were given basal pellet diets added with THQ at 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg dry matter (DM). The experimental period lasted 12 weeks during the natural condition under Egyptian conditions. To evaluate the HS severity on bucks, THI (temperature humidity index) was calculated based on the ambient temperatures and relative humidity estimation. The THI was intended employing the subsequent equation:

$$THI = db - [(0.31 - 0.31(RH)] \times [(db°C - 14.4)],$$

where RH is relative humidity, and db is the dry bulb temperature in Celsius.

The considered THI values were consequently categorized as follows:

<27.8 = absence of heat stress,

27.8 to 28.9 = moderate heat stress,

28.9 to 30.0 = severe heat stress,

and >30.0 = extremely severe heat stress (Marai et al., 2001).

Growth efficiency

Bucks were weighed every week to determine the DBWG (daily body weight gain), FBW (final body weight), and FI (feed intake) determined daily in each group. Moreover, FCR (feed conversion ratio) was evaluated at the end of the research.

Blood metabolites

After 12 weeks of treatment, six bucks (from each group) were randomly picked up for blood collection. Blood samples were collected from the ear vein and placed in sterilized EDTA tubes according to the Marongiu et al. (2007) protocol. The blood samples were centrifuged at 1198.8×g for 10 min to detach the plasma and then were deposited at −20°C pending analysis. Plasma biochemical parameters for liver functions counting TG (triglycerides), GGT (gamma-glutamyl transferase), TB (total bilirubin), and as well as kidney functions such as creatinine and urea were evaluated using a Hitachi 7020 automatic biochemical analyzer.

Oxidative stress assessment

Valuation of oxidative indices including catalase (CAT; Kit No; A007), reduced glutathione (GSH; Kit No; A005), superoxide dismutase (SOD; Kit No; A001), the total antioxidant capacity (TAC; kit No: A015) and malondialdehyde (MDA; A003) levels in plasma were measured using commercial assay ELISA kits provided by Institute of Biological Engineering of Nanjing Jianchen (Jiangsu, China). The coefficients of intra and inter-assays were 10 and 12%, respectively.

Inflammatory valuation

According to the method of Major et al. (2010), the NO (nitric oxide) was detected in the plasma. The lysozyme activity was analyzed following the de Frutos et al. (2020) method using the turbidometric technique. The values of interferon-gamma (INF-$^{\gamma}$; Cat No. MBS438244) and interleukin-4 (IL4; Cat No. MBS7048460) in rabbit plasma were quantitated according to Bebők et al. (1994) and Olaniyan et al. (2018), respectively, utilizing ELISA kit according to the manufacturer's directions (MyBioSource, San Diego, CA, USA).

Evaluation of sexual behavior and semen attributes

Semen samples were picked up twice weekly after two months of treatment (8−12 weeks). Therefore, around 70−80 ejaculates were assessed for evaluating the sexual behavior, including libido, reaction time, and semen quality variables were performed per treatment (2× 4 ejaculates × 10 bucks). The ejaculates samples were collected using an artificial vagina and a teaser doe. As described by the method of Daader et al. (2003), the reaction time of all bucks was calculated after bucks were introduced to females until the mounting occurred. At the same time, the libido (sexual desire) is expressed as the interval period from the start of mounting on the teaser doe until the buck ejaculates. The semen pH values were recorded via rapid pH test paper (Nantong Hetangcuiyue Medical, Jiangsu, China).

Using a sterilized graduated tube, the ejaculate volumes were documented in each buck after the gel masses removing. Using a light microscope (100× magnification) with a hot stage, sperm motility (forward motility) was assessed by visual inspection in numerous microscopic pitches for each semen ejaculate sample (score ranged between 0 and 100%). Valuations of sperm viability and morphological abnormality were achieved by calculating two hundred sperm cells marked with eosin − a nigrosine blue staining blend as reported in the prior work (Abdelnour et al., 2021). Sperm with partial or complete staining reflected dead spermatozoa, while unstained spermatozoa reflected viable cells. For assessing the sperm concentrations were recorded after the extension of semen (1:10) in PBS solution using an improved Neubauer chamber slide and a light microscope (40× magnification). As indicated by the method of Hosny et al. (2020), the sperm concentration, viable and normal morphology, volume, and proportions of motile sperm cells were utilized for estimating total sperm output (TSO) = semen ejaculate volume (mL) × semen concentration ($\times 10^6$/mL).

Histopathologic study

After the research period, three bucks (from each group) were sacrificed according to the Islamic method for studying the histological changes in testicular tissues after treatments. Testicular tissues were obtained, cleaned in distilled water, and closely fixed in a neutral buffered formalin solution (10%) for two days. After the fixation, administered using the paraffin procedure, partitioned at 4–5 µm, stained with hematoxylin-eosin, and examined microscopically (Suvarna et al., 2018). The stained slides were scrutinized microscopically for morphological alterations.

Data analysis

Data were edited in Microsoft Excel (Microsoft Corporation, USA). A Shapiro–Wilk test was implemented to examine for normality as designated by Razali and Wah (2011) method. Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of the statistical analysis system (PROC ANOVA; SAS Institute Inc., 2012). The subsequent statistical model was employed for the examination of all variables:

$$Y_{ij} = \mu + TRT_i + e_{ij},$$

where, Y_{ij} = observations, μ = overall mean, TRT = effect of ith treatment, e_{ij} = random error. The differences between treatment means were divided by Tukey's studentized range (HSD) test. A significance was recognized at $P<0.05$. Figures were fitted by the GraphPad Prism software 9.0 (GraphPad, USA).

Results

THI values

As depicted in Figure 1, the mean values of THI were 32.3, 30.6 and 29.23 after one, two and three months of treatments, respectively. According to the values of THI, bucks were suffering from severe heat stress. They were not able to dissipate the heat load during hot times.

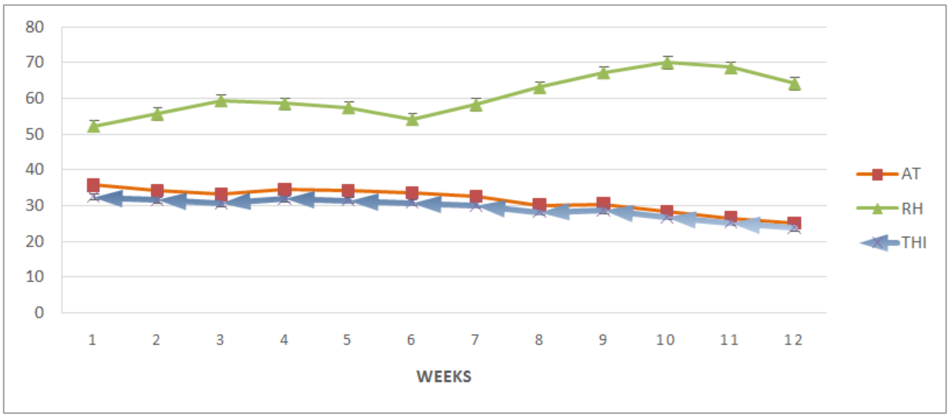

Figure 1. The values of AT (ambient temperatures, °C), THI (temperature humidity index; %), and RH (relative humidity, %) during the thirteen weeks of the experimental period under Egyptian conditions

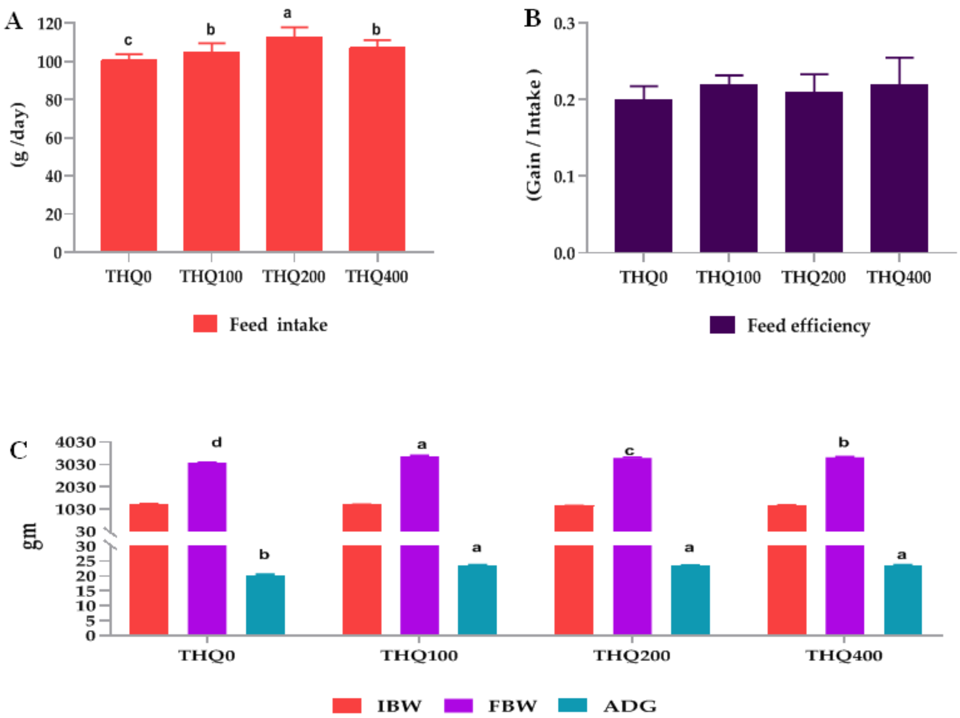

Figure 2. The effects of dietary thymoquinone supplementation at different levels 0, (THQ0), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg diet on (A) feed intake, (B) feed efficiency and (C) IBW (initial body weight), FBW (final body weight) and (ADG) average daily gain of rabbit bucks kept under thermal stress

Growth efficiency

As shown in Figure 2, the THQ0 exhibited a significant reduction (P<0.05) in FBW (final body weight; Figure 2 C) and ADG (average gain daily, Figure 2 C), while those variables were improved significantly by the thymoquinone dietary inclusion (Figure 2 C). In addition, no significant alterations were distinguished among all investigational groups concerning FCR (Figure 2 B). The best results for FI (feed intake, Figure 2 A) and FBW (Figure 2 C) were with THQ200 and THQ100 groups, respectively. Dietary inclusion of THQ boosted the growth indices of rabbit bucks kept under hot conditions.

Blood metabolites

For liver function, as depicted in Figure 3, all THQ treatments diminished total bilirubin (TB; P<0.001; Figure 3 A) and GGT levels (Figure 3 B; P<0.01) when compared with the THQ0 group. Concerning the TG (total glycerides; Figure 3 A), THQ200 and THQ400 displayed the lowest values relative to THQ0 treatment. Kidney functions (Figure 3 C), such as circulating creatinine, were affected by the THQ dietary addition, where the values of creatinine were diminished considerably (P<0.05) in all THQ groups related to the THQ0 one. Bucks treated with THQ exhibited insignificant effects on urea levels (Figure 3 C).

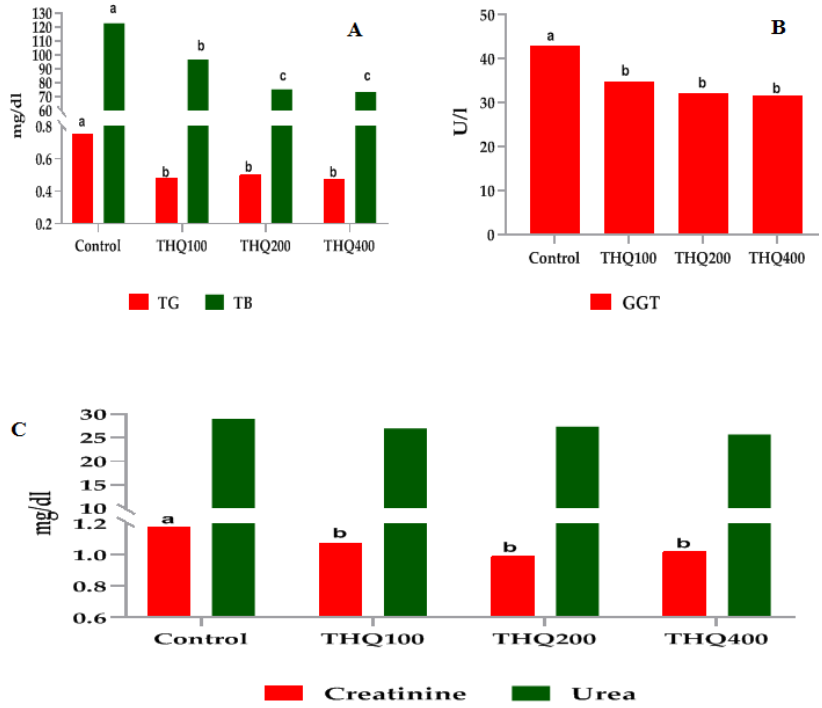

Figure 3. The effects of dietary thymoquinone supplementation at different levels 0, (THQ0), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg diet on liver function (A) triglycerides and total bilirubin, (B) GGT (gamma-glutamyl transferase) and (C) kidney function including creatine and urea in the plasma of rabbit bucks kept under thermal stress

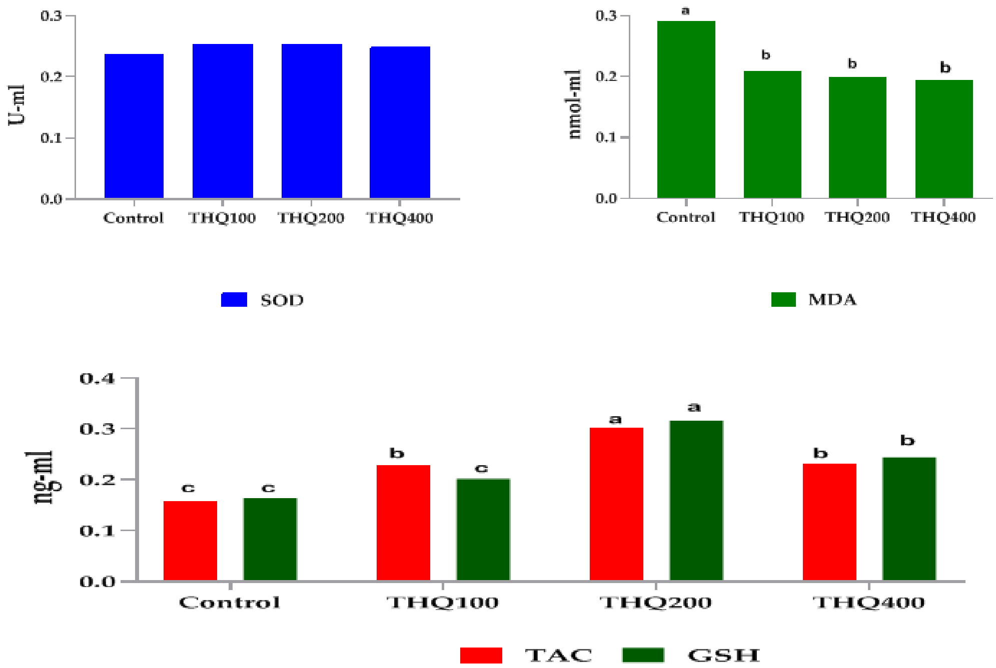

Figure 4. The effects of dietary thymoquinone supplementation at different levels 0, (THQ0), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg diet on oxidative indices (A) SOD, (B) MDA and (C) TAC and GSH activities in the plasma of rabbit bucks kept under thermal stress

Redox status

As described in Figure 4, bucks fed with THQ (100, 200 and 400 mg/kg diet) had significantly dropped the MDA levels in relation to the THQ0 group (Figure 4 B). Relative to the THQ0 group, stressed bucks that received THQ exhibited no statistical changes in the SOD levels (Figure 4 A). The highest value of TAC and GSH levels (Figure 4 C) were detected in the bucks given THQ200 in their diets (0.301 and 0.317 ng/l, respectively) under HS situations. Furthermore, THQ100 and THQ400 groups significantly improved the activities of TAC and GSH compared to the THQ0 group (Figure 4 C).

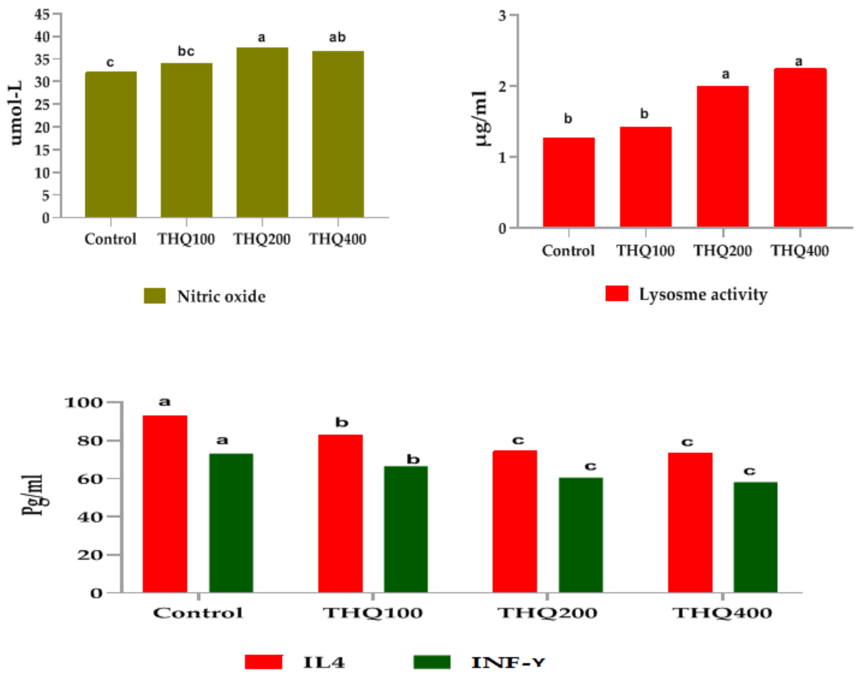

Figure 5. The effects of dietary thymoquinone supplementation at different levels 0, (THQ0), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg diet on inflammatory indices (A) nitric oxide, (B) lysosome activity and (C) interleukin-4 (IL-4) and interferon-gamma (IFN-γ) activities in the plasma of rabbit bucks kept under thermal stress

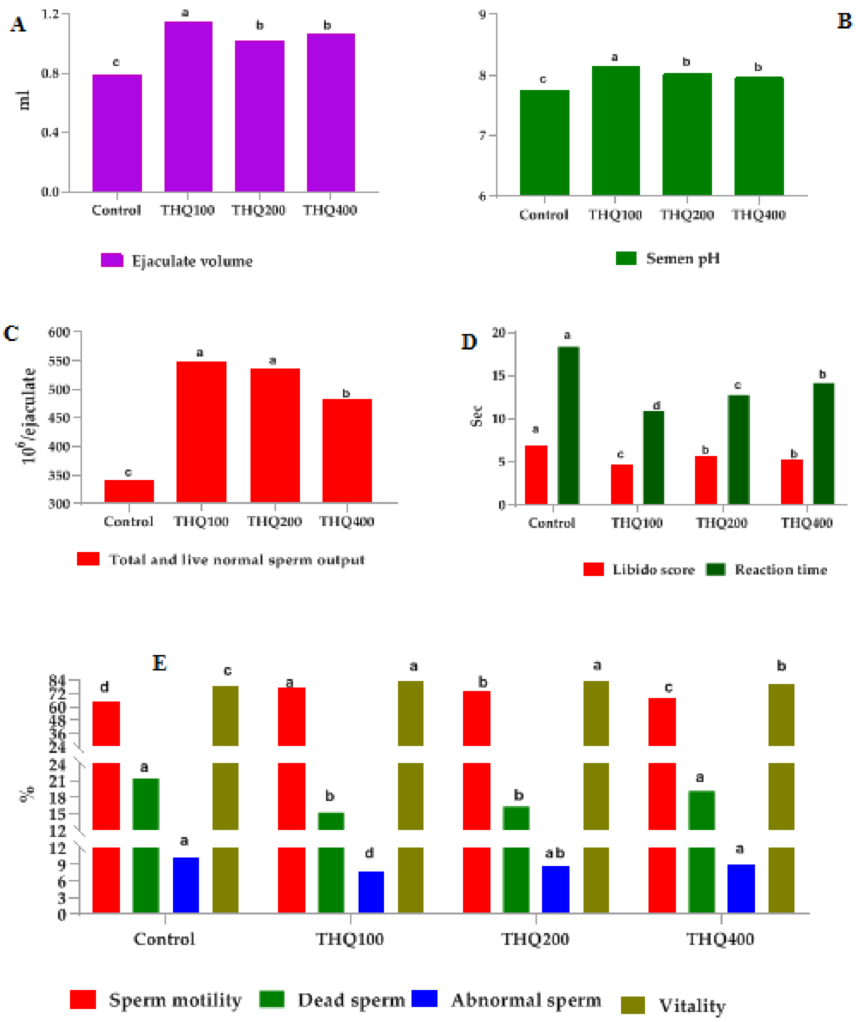

Figure 6. The effects of dietary thymoquinone supplementation at different levels 0, (THQ0), 100 (THQ100), 200 (THQ200), and 400 (THQ400) mg/kg diet on sexual behavior and semen attributes (A) ejaculate volume (ml), (B) semen pH, (C) total live and normal sperm output, (D) libido and reaction time (sec.) and (E) sperm motility, dead sperm, abnormalities and vitality of rabbit bucks kept under thermal stress

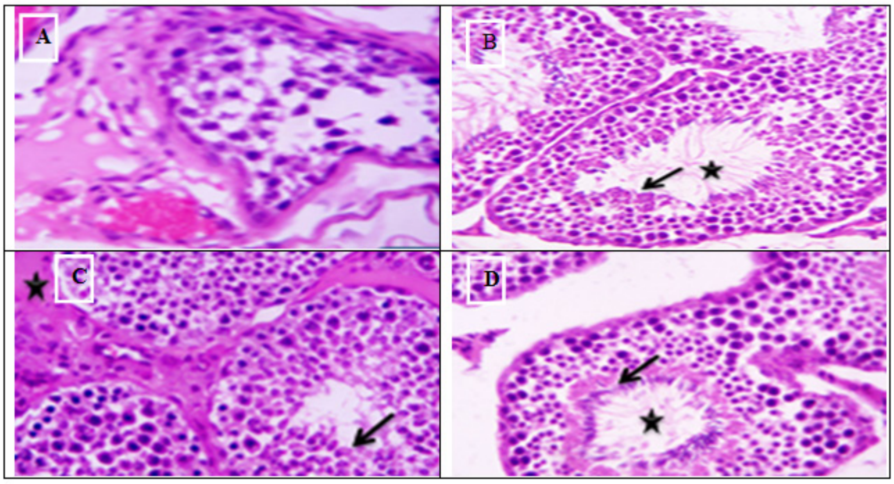

Figure 7. Photomicrographs showing testicular changes (H&E stain) of rabbit buck fed diets fortified with different levels of thymoquinone 0 (A), 100 (B), 200 (C), and 400 (D) mg/kg diet

Pro-inflammatory response

As depicted in Figure 5, the maximum (P<0.05) NO levels (Figure 5 A) were noticed in bucks treated with 200 mg of THQ in parallel with the other groups. The LYZ (lysosome activity; Figure 5 B) was augmented considerably in the THQ200 and THQ400 groups compared to that in the THQ100 and THQ0 treatments. Generally, adding 100 mg of THQ to HS bucks did not affect the values of LYZ and NO of blood plasma compared with untreated one. As shown in Figure 5 C, the dietary addition of THQ diminished the values of INF-$^{\gamma}$ and IL-4 compared to the untreated one.

Sexual behavior and semen quality

Figure 6 shows the effects of THQ on sexual behavior and semen attributes of HS bucks. Dietary inclusion of THQ significantly increased the ejaculate volume with the best values in THQ100 group (Figure 6 A). The same trend was detected for semen pH (Figure 6 B). Both THQ100 and THQ200 treatments presented high levels (P<0.001) of total and live sperm output compared with THQ100 and THQ0 groups (Figure 6 C). For sexual behavior, libido score and reaction times recorded the lowest values (P<0.001) with THQ100 compared to other groups. There was a significant increase in the time of both libido score and reaction in THQ0 compared with the other treated groups (Figure 6 D). Sperm motility and vitality were significantly increased and the lowest values of dead and abnormal sperm were noticed in the THQ100 group (Figure 6 E). Both THQ200 and THQ400 groups exhibited intermediate values for all semen attributes with a significant difference with the THQ0 group except for abnormal sperm.

Testicular changes

As represented in Figure 7, HS exposed buck testicular tissues revealed damaged spermatogenic cells with interstitial edema and congested blood vessels (Figure 7 A). On the other hand, testicular tissues of bucks that received various THQ dietary inclusion showed moderate degenerative changes of spermatocytes and improved the number of spermatids. Moreover, no edema or congested blood vessels were detected in seminiferous tubes (Figure 7 B, C and D).

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

Item	Amount
Ingredients (g/kg)	
alfalfa hay	330
barley grain	250
wheat bran	250
soybean meal	150
sodium chloride	3
limestone	12
minerals mixture*	2
vitamins mixture**	1
DL-Methionine	2
total	1000
Chemical analysis (% on DM basis)	
organic matter	90.82
crude protein	18.20
crude fiber	12.17
ether extract	3.26
nitrogen free extract	57.19
ash	9.18
calculated values	
calcium (%)	0.85
phosphorus (%)	0.5
calcium/phosphorus	1.68

*Each 1.5 kg contains manganese 80 mg, zinc 60 mg, iron 30 mg, copper 4 mg, iodine 0.5 mg, selenium 0.1 mg and cobalt 0.1 mg. **Each 1 kg contains: vitamin A 12000000 IU, vitamin D_3 3000000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, biotin 75 mg, folic acid 1000 mg, nicotinic acid 30000 mg and pantothenic acid 10000 mg.

Discussion

The existing investigations revealed that the dietary inclusion of THQ in buck rabbits enhanced the growth efficiency, blood biochemical parameters, immunity, and reduced the inflammatory mediators IL-4 and IFN-$^{\gamma}$, suggesting a considerable improvement in semen attributes and sperm functionality and health. Moreover, as reported by previous authors, including Naseer et al. (2020) and Hu et al. (2023), induced testicular damage such as severe degenerative and necrotic alterations, while the THQ administration significantly attenuated these damages due to the antioxidant, anti-inflammatory and anti-apophatic effects of THQ. This study is the first work to assess the potentiality of using THQ as an anti-HS mediator in rabbit bucks. HS is accepted to have deleterious effects on rabbit health, welfare and potential fertility outcomes and these effects may be aggravated in the era of climate change. Moreover, HS diminishes the immune responses, persuades cellular impairment via augmentation of OS and antioxidants discrepancy (Abdelnour et al., 2021) and increases the synthesis of circulating pro-inflammatory mediators such as IL-4 and IFNγ (Aderao et al., 2023).

In this sense, using natural molecules to target this dysfunction such as immune, inflammation and anti-oxidative disturbances can be a great strategy for alleviating the HS effects in bucks. In other words, improving the redox status and immunity by dietary inclusion in rabbit bucks is of great significance to the rabbit trade for improving the reproductive competence of bucks during summer seasons in the tropical regions such as Egypt. For detecting the severity of HS in animals, the THI is usually revealed as the utmost reliable stress indicator for a rabbit, as pointed out previously by Marai et al. (2001) method. Figure 1 depicts the values of THI during the experimental period, where it was 32.3, 30.6 and 29.23 after one, two and three months of treatments indicating bucks were suffering from severe HS. Hence, the THQ addition of stressed bucks showed considerable improvement in the growth, immunity, reduced inflammatory marks and maintained the testicular tissues from damages as well as improved sexual behavior, semen attributes and sperm health of stressed bucks. Generally, the fortification of THQ to the buck diets during HS condition improved the liver function, immunity, antioxidant profile, testicular structure, sperm function, semen attributes, and decreased oxidative stress (MDA) and the inflammation process and lastly efficiently utilized for vanquishing the destructive impacts of HS. There were doubts about the fruitful commercialization of natural active compounds as growth promoters or anti-HS mediators that may have potential speculation supplies for animal breeders (Aderao et al., 2023). The existing outcomes also displayed that bucks' diets fortified with THQ significantly improved FBW, daily DBWG and FCR but did not affect FI. As indicated in our results, the inclusion of THQ had the most noticeable part in prompting greater growth variables and enhancing the feed utilization competence in rabbit bucks exposed to HS. Many papers clarified that the dietary inclusion of *Nigella sativa* has significant effects on the growth indices in rabbits (Hammed and Amao, 2022). Even though the effects of *Nigella sativa* on the growth marks have been explored in many livestock species, the defensive effects of its active molecules, such as THQ in stressed bucks are unexplored.

Further examination is desirable to clarify the potential roles of the THQ in improving the reproductive competence of animals. Following our findings, THQ addition to fish had greater growth-supporting properties in Nile tilapia (Ibrahim et al., 2022). Currently, THQ has been broadly hired as considerable additives or complements in the pharmaceuticals, and food industry (Algaidi et al., 2022; Kaymak et al., 2022) and recently in livestock (El-Gindy et al., 2020) and aquaculture (Ibrahim et al., 2022) sectors. Of late, the affinity to hire natural molecules as feed additives for enhancing growth, immunity, health status, well-being, and care have been increasingly extended under adversative situations. HS triggered changes in the hepatic metabolism and may accumulate the lipid, thus supporting the acceleration of the synthesis of OS. Our previous work revealed that HS decreased ATP generation, greater OS, alterations in the precursor source for gluconeogenesis, and accumulation of the hepatic lipids in the hepatocytes that may participate in the fatty liver ailment in stressed animals (Abdelnour et al., 2021).

Treating stressed bucks with THQ had greater stability of the hepatic functionality by diminishing the levels of TB, TG, and GGT activity, as clarified in our present study. In line with our data, El-Gindy et al. (2020) described that the *Nigella sativa* added to rabbit diets produced significantly lower total lipids, TG, and LDL levels, and significantly increased the HDL level. High levels of those previous marks, such as GGT in the plasma, indicate liver cell damage by HS. As reported in this work, a considerable improvement in hepatic function, which might be associated with the THQ, has a pharmacological role as an anti-lipolytic mediator (Pottoo et al., 2022; Demircigil et al., 2023). Authors suggested that THQ could promote hepatic lipolysis via promoting the *SIRT1* signaling (Algaidi et al., 2022) and mitigate hepatic lipid aggregation. In the present data, THQ inclusion in a stressed buck's diet can conquer metabolic lipid disorder and protect the hepatocytes from damage HS-triggered via and sustain GGT and TG at the normal levels. According to this theory, El-Gindy et al. (2020) proposed that dietary *Nigella sativa* addition successfully relieves fat gathering in the liver of growing rabbits. Concerning kidney task, creatinine levels were meaningfully diminished owing to the THQ added to buck's diets exposed to HS, but the levels of urea were not affected by the treatments. In the structure of this antioxidant ability of THQ, and in respect of their competence to trick OS-triggered kidney damage, THQ has presented robust health-promoting effects (Kaymak et al., 2022). As a few studies have been implemented on the potential effects of THQ as an active compound, so we did not find enough studies to compare with them.

Exposing animals to various environmental issues, such as HS, triggers to produce a high level of ROS, which may constrain the antioxidant structure in the cellular system. In this meaning, fortification of diets with powerful antioxidants might be useful for sustaining the body's homeostasis and can improve an animal's health and productivity during adverse environmental stressors (Hosny et al., 2020; Abdelnour et al., 2021). The antioxidative system in the body consists of GSH and TAC, which are the main mediators for counteracting the high levels of OS in blood plasma, and therefore the capability of plasma to stalk OS twisted from oxidation pathways in the body (Oladimeji et al., 2022).

As shown in this experiment, the THQ can increase the antioxidative indices and thus reduce lipid peroxidation such as MDA. This significant improvement could reflect the robust antioxidant properties of THQ, which several previous works have evidenced (Hammed and Amao, 2022; Ibrahim et al., 2022). Thenceforth, oxidative impairment of the brain is generated by numerous types of machinery, for instance, higher lipid peroxidation, protein unfolding, fluctuations in pH, mitochondrial disturbance, DNA injury, and the intracellular calcium brought about the elevation of cell death (Ben Mrid et al., 2022). Aboubakr et al. (2021) suggested THQ alleviates chlorpyrifos-induced neuronal injury in rats via enhancing the SOD, CAT and GSH and reduced the MDA levels significantly. This study demonstrated the roles of THQ, such as anti-inflammatory and anti-apoptotic effects. The discrepancy in the antioxidant system is furthermore the reason for MDA. Lipid peroxidation weakens cell membrane and makes it vulnerable to more oxidation by free radicals (Ben Mrid et al., 2022).

Our findings also detected that HS persuaded a considerable increase in MDA levels. On the other hand, THQ considerably decreases the level of MDA creation and therefore avoids the defeat of cellular integrity. This feature could be associated with improved testicular function observed based on the histological examination. Overall, adding *Nigella sativa* as a source of THQ was safe for rabbits and improved the action of antioxidant defenses in different animals and reduced lipid peroxidation (El-Gindy et al., 2020; Elmowalid et al., 2022). In mice subjected to HS, Saeed et al. (2011) reported that the male mice receiving THQ (5 mg/kg BW) improved the hepato-renal functions and sustained or protected testicular function from HS damages. Studies have clarified that THQ demonstrated a favorable influence in lessening the risk of serious health complications thanks to their antioxidant possessions (Kaymak et al., 2022; Sarkar et al., 2021). IL-4 and INF-$^{\gamma}$ were decreased ($P<0.001$) in THQ200 and THQ400 treatments related to those in the other groups. Interleukins are a cluster of cytokines that first seemed in leukocytes. Sheiha et al. (2020) reported that HS produced superior IL-4, representing the destruction of immunity. It is accepted that the intake of anti-inflammatory compounds improves health status and aids in avoiding ailments, and supports healing. Furthermore, studies have revealed THQ as natural molecules have possible anti-inflammatory activity that follows via the suppression of interleukin groups such as IL-6, and TNF-α and reducing apoptosis (Aboubakr et al., 2021; Alenezi, 2023).

In the study of Demircigil et al. (2023), it has been shown that the THQ has protected hepatotoxicity induced by tartrazine via supporting the hepatic antioxidant indices such as GSH, CAT, and TAC activities. Moreover, the anti-inflammatory activities of THQ were evidenced reducing the IL-6 and TNF-α, and the anti-apoptotic effects were proved by reducing the caspase 3 (Demircigil et al., 2023). Henceforth, the existing trial shows that THQ has a multiplicity of biological possessions that noticeably reduce inflammatory marks, boost health status and amend immune activity. Additional anti-inflammatory machinery of THQ is desirable in various animal species. The nutritional management with THQ as novel active molecules is a new policy for augmenting health, well-being and immunity by dropping the pro-inflammatory reactions to vanquish the unfavorable HS effects.

As presented in this research, we detected that THQ significantly amplified the NO and lysozyme activity levels in stressed bucks. The previous studies of Abdelnour et al. (2021) reported that the greater NO levels might be connected with the escalation of blood flow to the skin, thus boosting the bucks to dissipate heat and escalating heat loss. Interestingly, adding THQ to bucks' diets may support the animals to sustain their body homeostasis by stimulating endogenous cellular defense machinery to accomplish hepatic oxidation and inflammation caused by HS. Additional insights on various livestock species should be explored with a growing interest connecting THQ as a reliable substitute source of feed supplement employment in rabbit production under adverse environment. Improving the sexual behavior and reproductive capacity during the summer might support rabbit production's sustainability through the year (Kanter, 2011; Hosny et al., 2020). In the present study, the libido score and reaction time were significantly improved by THQ dietary inclusion. Sperm attributes such as volume, motility, concentration, vitality and testosterone levels were significantly augmented in THQ100 and THQ200 groups. Histological showing of testicular tissues discovered moderate to stark degenerative and necrotic changes in bucks exposed to HS, which is improved with the addition of THQ at various doses. THQ can improve rabbit bucks' heat tolerance via boosting antioxidant and reducing inflammatory cytokines. Similar to our data, Algaidi et al. (2022) indicated that THQ significantly downregulated TNF-α transcription while it considerably upregulated SIRT1 expression in the testicular tissues of hypothyroid rats. This feature may reflect the protective role of testicular tissues from oxidative stress (Algaidi et al., 2022). The study of Hammed and Amao (2022) reported that bucks receiving 1.0% black seed supplementation produced high growth efficiency and improved testicular and epididymal characteristics.

Moreover, several natural compounds are used to relieve the negative effects of HS in rabbit bucks via promoting sperm function and health (El-Ratel et al., 2021). The adding of THQ evidenced this improvement to freezing media in ram semen. The previous study found that the THQ significantly improved sperm function after post-thawing (İnanc et al., 2022) via maintaining the DNA sperm integrity and plasma membrane. Further studies will be required on this topic to validate our hypothesis.

Conclusions

Our findings concluded how THQ (200 or 400 mg/kg diet) based diets positively reduced the negative influences of stressed bucks via promoting growth, antioxidant capacity, sexual behavior, sperm function and health, and oxidative stress. Here, histological data for testicular integrity, semen quality and antioxidant provided an enhanced implement for recognition of the mode of actions of THQ that maintained their favorable purposes during high temperatures. Lastly, the favorable possessions of THQ incorporation have opened up plentiful roads during climate changes for their uses as novel dietary supplements

Author contributions

All authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The research procedures were accomplished in concurrence with the NIH endorsements. The protocol was sanctioned by the Ethics of Animal Use in Research Committee of Zagazig University, Egypt (ZU-IACUC) (Approval number ZU IACUC/2/F/367/2022). Every effort was made to handle the animals humanely and to tackle ethical issues.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Conflicts of interest

The authors declare no conflict of interest.

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