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Original Article

# Antioxidant effect of black seed oil (*Nigella sativa*) on blood and meat in rabbits simultaneously stressed by transport and heat

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## ABSTRACT

Transportation and heat are crucial stressors that affect animal welfare and homeostasis, as well as the organoleptic and sensory quality of meat in livestock animals. This study evaluated the antioxidant effect of *Nigella* oil in rabbits exposed simultaneously to transport and heat. Five groups (Gr) of 5 rabbits were acclimatized at 22-24°C for 60 days. During this period, Gr1, Gr2, and Gr3 received orally at 10am 2.5mL NaCl (0.9%.kg$^{-1}$day$^{-1}$), while in the same way Gr4 and Gr5 received 2 and 3mL NOkg$^{-1}$, respectively. At the end of the treatment, Gr 1 was left exposed to 22-24°C, and Gr2 was exposed to 33-34°C for 1h and 30mins, and then non-transported. Meanwhile, Gr3, Gr4, and Gr5 were transported (80km) at 33±1°C. Blood was collected to analyze Neutrophil/Lymphocyte ratio (NLR), glucose, malondialdehyde (MDA) and Catalase (CAT), and superoxide dismutase (SOD). Muscle samples (*longissimus dorsi*) were collected to analyze the ultimate pH (pHu), MDA, and cooking loss (CL). In non-pretreated and non-transported rabbits, those exposed to heat (Gr2) showed a significant ($p < .05$) increase in NLR, glucose, MDA, pHu, and CL, associated with a significant ($p < .05$) decrease in SOD and CAT, compared to those exposed to thermal neutrality (Gr1). In non-pretreated rabbits, transport association with heat (Gr3) significantly increased ($p < .05$) NLR, glucose, MDA, pHu, and CL, and significantly decreased ($p < .05$) CAT and SOD compared to the heat alone group (Gr2). In transported rabbits under heat (Gr4), the pretreatment (2mL NOkg$^{-1}$d$^{-1}$) significantly reduced ($p < .05$) NLR, glucose, MDA, pHu, and CL, and increased ($p < .05$) CAT and SOD, compared to non-pretreated animals (Gr3). In conclusion, NO attenuated the oxidation induced by transport-associated heat in a dose-dependent manner, suggesting that *Nigella* seeds could be used as a dietary supplement against preslaughter stress in rabbits.

**Keywords**: stress, transport, heat, *Nigella*, rabbit, Morocco

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## INTRODUCTION

Rabbits are ideal animals for meat production that are widely farmed worldwide, characterized by prolific production, short gestation intervals, and high feed conversion rates (Rasinska et al., 2019). In Morocco, rabbit farming is a growing sector, primarily focused on meat production, with strong demand and profitable opportunities, although the majority of farms are traditional (Bouzekraoui, 2002). Rabbit meat is very popular and widely consumed in numerous Mediterranean countries (El-Adawy et al., 2020; Morshdy et al., 2021). It is low in fat and rich in protein, unsaturated fatty acids, conjugated linoleic acid, selenium, antioxidant vitamins, and polyamines. It is also tender, lean, and flavorful, hence it has good sensory properties (Dalle Zotte, 2002). These strengths and advantages require mastery of breeding and feeding conditions for rabbit production, as well as careful environmental management (Hernández & Dalle Zotte, 2010). However, they could be negatively impacted by exposure to environmental heat and road transport.

Rabbits are homeothermic animals that are particularly sensitive to heat (Marai et al., 2007), which can alter the animal's homeostasis and increase oxidative stress (OS) and reactive oxygen species (ROS) production (Matics et al., 2021). In addition, in rabbits, transport is another stressful factor that can compromise their health, welfare, and meat quality (Voslarova et al., 2018; Nielsen et al., 2020). The primary transport stress factors include capture and loading into the vehicle, stocking density, ambient temperature, humidity, transport duration, and deprivation of water and food. To these factors, the mechanical condition of the vehicle, road topography, and the driver's behavior (Simova et al., 2017; Cockram & Dulal, 2018; Nielsen et al., 2020). For example, at the end of rabbit transport, Suba-Bokodi et al. (2024) noted a significantly higher mean total fecal cortisol, the most reliable indicator of stress, compared to the measurement before transport (90.85µg $g^{-1}$ vs. 6.71µg $g^{-1}$).

In rabbits, stress induced by either transport or heat becomes significantly more intense when the two stressors are combined, affecting their health and production performance. Under the effect of simultaneous exposure to transport stress and heat, a large number of ROS and their metabolites are released into the blood of rabbits (Jimoh et al., 2017). In this situation, the concentration and activity of SOD and CAT in rabbits decreased (Sabés-Alsina et al., 2016; Jimoh et al., 2017; Kuang et al., 2021), while the serum level of MDA increased (Garner et al., 2020).

The plant kingdom, and more specifically aromatic plants, remains an effective and highly sought-after means of obtaining natural antioxidants (Cunha et al., 2018). These plants are capable of biosynthesizing odorous molecules characteristic of their fixed oils, which reveal various therapeutic activities that have long been used in traditional medicine. Among the aromatic plants with multiple biological activities, we can mention black cumin (*Nigella sativa* L.) (NS), an annual herbaceous plant belonging to the Ranunculaceae family. The seeds of this plant possess important curative properties, enabling them to have a wide spectrum of medicinal applications, as they are antioxidant, immunostimulant, and antitumor (Pop et al., 2020). Indeed, in rats, it has been reported that *Nigella* oil (NO) could contribute to increasing the levels of testosterone, luteinizing hormone, and follicle-stimulating hormone (El Khasmi et al., 2011), reducing depression and

anxiety, and stimulating memory abilities (Farh et al., 2017). Furthermore, the addition of cold-pressed black cumin seed oil to dromedary meat protected the meat against lipid and protein oxidations induced by refrigerated storage time or heat exposure (Rachchad et al., 2024; Rachchad et al., 2025). This protection resulted in a significant decrease in the levels of thiobarbituric acid reactive substances (TBARS), carbonyls, and thioles, as well as a significant increase in the activities of antioxidant enzymes, including CAT, SOD, and glutathione peroxidase. Due to its remarkable antioxidant activities, however, to our knowledge, no work has evaluated the protective effect of black cumin against pre-slaughter stress in rabbits. Thus, the present study aimed to evaluate the protective effect of NO on the antioxidant status of the blood and meat of rabbits exposed simultaneously to road transport and heat.

## MATERIALS AND METHODS

### *Experimental Design*

The study was approved by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy of Casablanca, Morocco (code 10/2025). Twenty-five male New Zealand rabbits aged between 5 and 6 months were used in this study. They were purchased from a market in Casablanca, Morocco, and fed ad libitum a diet consisting of lettuce, carrots, and barley flour. The animals were randomly divided by body weight into five groups (Gr) of 5 rabbits per cage, and were acclimated to the pet store conditions for the duration of the treatment (22–24°C, 50–60% relative humidity, 12h of light from 8AM to 8PM, and 12h of darkness) . While respecting the regulations in force in the country, Gr1, Gr2, and Gr3 received orally at 10AM 2.5mL NaCl 0.9%/kg BW/day, for 60 days, while in the same way Gr4 and Gr5 received 2 and 3mL NO/kg BW, respectively. At the end of the 60-day treatment period, Gr1 was left exposed to 22–24°C and Gr2 was exposed to 33-34°C for 1h 30mins without undergoing any transport, while Gr3, Gr4, and Gr5 were subjected to road transport (80km for 1h 30mins) at an ambient temperature of 33-34°C.

### *Sample Collection*

Blood was drawn by puncture of the ear vein and was collected in two tubes, one EDTA tube (2mL) and one dry tube (4mL). Samples were collected once from Gr1 and once after heat exposure from Gr2, and twice –before and after – exposure to heat and transport from Gr3, Gr4, and Gr5. Samples were analyzed for Neutrophil/Lymphocyte ratio (NLR), levels of glucose and malondialdehyde (MDA), and activities of Catalase (CAT) and superoxide dismutase (SOD).

All animals were then anesthetized by an injection of sodium pentobarbital (25mg $kg^{-1}$ BW) into the ear vein. After loss of sensation, they were immediately sacrificed by exsanguination of the jugular veins. The back muscle (*Longissimus dorsi*) was removed and stored at 4±1°C to determine the pH (pHu), the MDA levels, and the cooking loss (CL) at the 24h postmortem stage.

After evaluation of the NLR, pHu, and CL, the sera obtained after centrifugation at 750g for 15mins, and the prepared meat extracts were aliquoted and stored at -80°C until subsequent analyses of glucose, MDA, CAT, and SOD.

***Neutrophil/Lymphocyte Ratio Determination***

The differential distribution of leukocytes (%) was determined using May-Grunwald-Giemsa-stained blood smears. The percentage of neutrophils and lymphocytes, as well as the NLR, was determined on 100 leukocytes.

***Meat pH and Cooking Loss***

Extracted meat samples were ground and 2g of the meat sample were homogenized using a porcelain mortar with 20mL of neutralized 5mM sodium iodoacetate. The pH was measured directly at 18-20°C using a pH meter equipped with a lance-type electrode.

To determine CL, meat samples were placed in polyethylene bags and fully immersed in a water bath without the addition of other ingredients until an internal temperature of 80°C was reached for 90mins. The internal temperature was monitored using a stainless steel probe thermometer. After cooking, the bags were removed and immediately immersed in an ice bath for 15mins to cool the samples in their exuded liquids. After cooling, the samples were removed, dried on filter paper, and reweighed. Cooking losses were calculated as the difference in weight before and after cooking, expressed as a percentage of the initial weight.

***Biochemical Analyses***

For the serum, glucose was analyzed colorimetrically (JENWAY 6320D spectrophotometer, model 6320D) using commercial kits (CHRONOLAB, Switzerland) according to the manufacturer's recommendations. For the serum and meat, TBARS levels were measured using the methods of Botsoglou et al. (1994), while CAT and SOD were measured using the methods of Sinha (1972) and Paoletti et al. (1986), respectively. Finally, protein levels were calculated by measuring absorbance at 280nm and comparing it to that obtained with bovine serum proteins as a standard.

***Sensory Profile***

Five sensory evaluators specialized in food science participated in the evaluation of the meat sensory profile using a descriptive hedonic scale from 1 to 9 (9 being the highest sensory quality, while one corresponds to the lowest sensory quality) (Li et al., 2013). Sensory scores were assigned to the six sensory parameters (odor, color, elasticity, viscosity, and texture) of the cooked meat samples from each rabbit group.

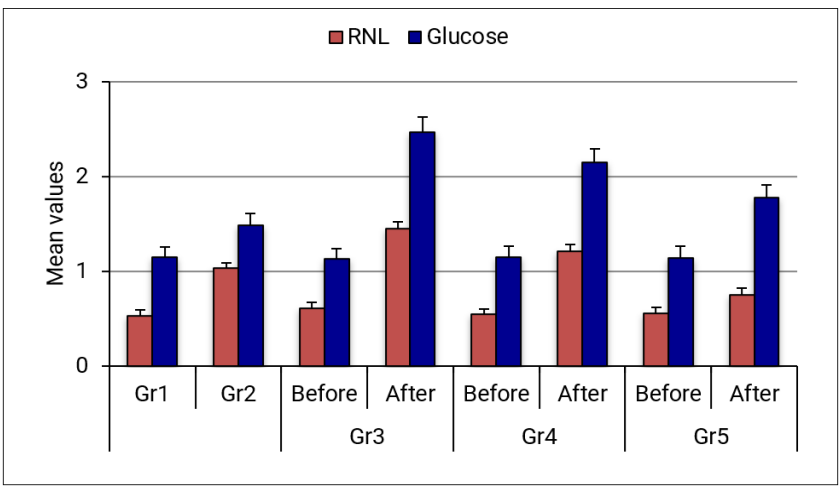
***Statistical Analysis***

All analyses were performed in duplicate, and results were presented as means ± standard deviation. Origin 7.0 software was used for data analysis. Significant differences were analyzed using Duncan's multiple-range test, and a p-value < .05 indicated significant differences.

## RESULTS

### *Neutrophil/lymphocyte Ratio and Blood Glucose*

In non-pretreated with NO and non-transported animals, those that had been exposed to heat (Gr2) for 90mins showed a significant increase ($p < .05$) in NLR and serum levels of glucose compared to those that had been left exposed to thermal neutrality temperature (Gr1) (1.03±0.06 vs 0.53±0.06 and 1.49±0.12g $L^{-1}$ vs 1.15±0.11g $L^{-1}$, respectively) (Figure 1). In the absence of NO pretreatment, the association between heat and transport (Gr3) significantly increased ($p < .05$) NLR and serum levels of glucose compared to heat alone (Gr2) (1.45±0.07 vs 1.03±0.06 and 2.47±0.16g $L^{-1}$ vs 1.49±0.12g $L^{-1}$, respectively) (Figure 1). In transported animals under heat (Gr4), NO pretreatment (2mL NO/kgBW/d) significantly reduced ($p < .05$) NLR and serum levels of glucose compared to non-pretreated and transported animals under heat (Gr3) (1.21±0.07 vs 1.45±0.07 and 2.15±0.14g $L^{-1}$ vs 2.47±0.16g $L^{-1}$, respectively) (Figure 1). Compared to Gr4, animals pretreated with 3mL NO/kgBW/d (Gr5) then exposed simultaneously to transport and heat showed lower values ($p < .05$) of NLR and glucose (1.21±0.07 vs 0.75±0.07 and 2.15±0.14g $L^{-1}$ vs 1.78±0.13g $L^{-1}$, respectively) (Figure 1).

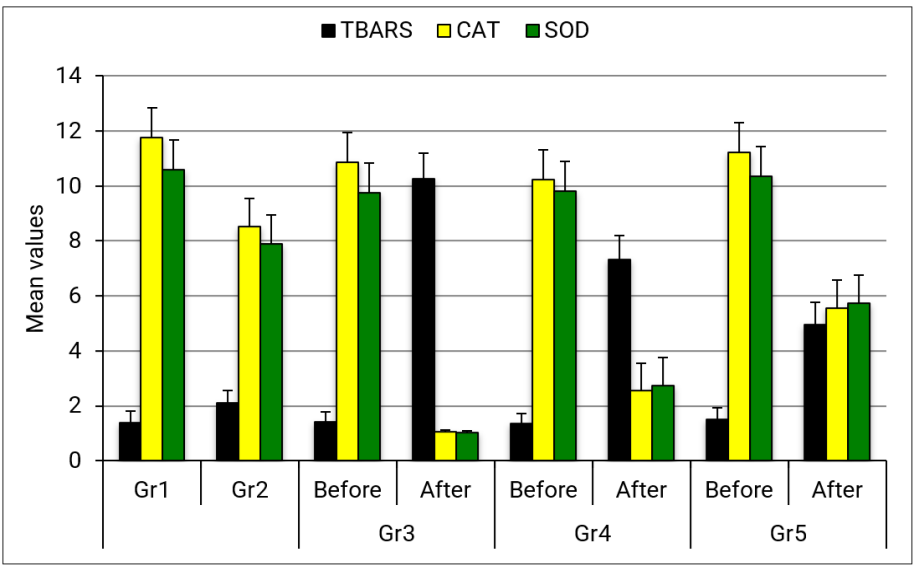



**Legend:**
Gr1 = No pre-treatment, no heat stress (22-24°C)
Gr2 = No pre-treatment, plus heat stress (33-34°C)
Gr3 = No pre-treatment, plus heat (33-34°C), plus transport
Gr4 = Pre-treatment 2mL/kg/day NO, plus heat (33-34°C), plus transport
Gr5 = Pre-treatment 3mL/kg/day NO, plus heat (33-34°C), plus transport

**Figure 1.** Neutrophil-to-lymphocyte ratio (NLR) and blood glucose (g/L) in rabbits before and after simultaneous exposure to transport and heat, after oral pretreatment with black seed oil (NO).

***Serum Thiobarbituric Acid and Enzyme Activities***

Gr2 showed a significant increase ($p < .05$) in serum levels of TBARS (nmol malondialdehyde/mg protein) and a significant ($p < .05$) decrease in activities of serum CAT (µmol $H_2O_2$/min/mg protein) and SOD (µmol/min/mg protein), compared to those observed in Gr1 ([TBARS] 2.11 ±0.45 vs 1.39 ±0.41, [CAT] 8.53 ±1.02 vs 11.75 ±1.08 and [SOD] 7.89 ±1.06 vs 10.59 ±1.07, respectively) (Figure 2). In Gr3, after transport under heat, serum levels of TBARS were significantly ($p < .05$) higher and serum activities of CAT and SOD were significantly ($p < .05$) lower than those measured in Gr2 (TBARS) 10.27 ±0.91 vs 2.11 ±0.45, (CAT) 1.05 ±0.06 vs 8.53 ±1.02, and (SOD) 1.04 ±1.05 vs 7.89 ±1.06, respectively) (Figure 2). Pretreated (2mL NO/kg BW/d) and transported animals under heat (Gr4), showed low serum levels of TBARS and high serum activities of CAT and SOD compared to Gr3 ([TBARS] 7.32 ±0.87 vs 10.27 ±0.91, [CAT] 2.55 ±1.01 vs 1.05 ±0.06, and [SOD] 2.74 ±1.01 vs 1.04 ±1.05, respectively; $p < .05$) (Figure 2). Compared to transported animals under heat (Gr4), those pretreated with 3mL NO/kg BW/d (Gr5) then exposed to transport and heat showed lower values of TBARS and higher activities of CAT and SOD ([TBARS] 7.32 ±0.87 vs 4.95 ±0.81, [CAT] 2.55 ±1.01 vs 5.55 ±1.02 and [SOD] 2.74 ±1.01 vs 5.74 ±1.02, respectively; $p < .05$) (Figure 2).

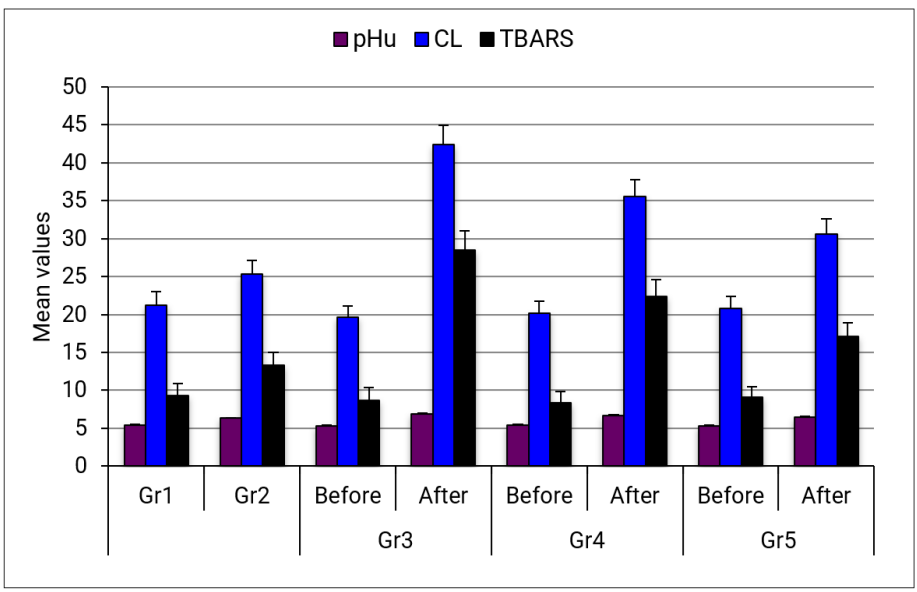



**Legend:**
Gr1 = No pre-treatment, no heat stress (22-24°C)
Gr2 = No pre-treatment, plus heat stress (33-34°C)
Gr3 = No pre-treatment, plus heat (33-34°C), plus transport
Gr4 = Pre-treatment 2mL/kg/day NO, plus heat (33-34°C), plus transport
Gr5 = Pre-treatment 3mL/kg/day NO, plus heat (33-34°C), plus transport

**Figure 2.** Thiobarbituric acid (TBARS) content (nmol malondialdehyde/mg protein), catalase (CAT) (µmol $H_2O_2$/min/mg protein), and superoxide dismutase (SOD) (µmol/min/mg protein) activity in rabbits before and after simultaneous exposure to transport and heat, after oral pretreatment with black seed oil (NO).

### *Meat Ultimate pH, Cooking loss, and Thiobarbituric Acid*

Concerning the meat at the 24h postmortem stage, ultimate pH (pHu), cooking loss (CL) (%) and thiobarbituric acid (TBARS) content (nmol malondialdehyde/mg protein) Gr2 showed a significant increase ($p < .05$) in these parameters compared to Gr1 ([pH] 6.31 ±0.05 vs 5.46 ±0.04, [CL] 25.31 ±1.83 vs 21.26 ±1.74, [TBARS] 13.31 ±1.67 vs 9.34 ±1.51, respectively) (Figure 3). In Gr3, after transport under heat, pHu, CL, and TBARS were significantly ($p < .05$) higher than those measured in Gr2 ([pH] 6.87 ±0.08 vs 6.31 ±0.05, [CL] 42.37 ±2.57 vs 25.31 ±1.83, and [TBARS] 28.53 ±2.52 vs 13.31 ±1.67, respectively) (Figure 3). Pretreated (2mL NO/kg BW/d) then transported animals under heat (Gr4), showed low pHu, CL, and TBARS compared to Gr3 ( [pHu] 6.71 ±0.07 vs 6.87 ±0.08, [CL] 35.52 ±2.21 vs 42.37 ±2.57, and [TBARS] 22.35 ±2.24 vs 28.53 ±2.52, respectively, $p < .05$) (Figure 3). Compared to transported rabbits under heat (Gr4), those pretreated with 3mL NO/kg BW/d (Gr5) then exposed to transport and heat showed lower values of pHu, CL and TBARS ([pHu] 6.71 ±0.07 vs 6.51 ±0.07, [CL] 35.52 ±2.21 vs 30.65 ±1.91, and [TBARS] 22.35 ±2.24 vs 17.11 ±1.85, respectively; $p < .05$) (Figure 3).

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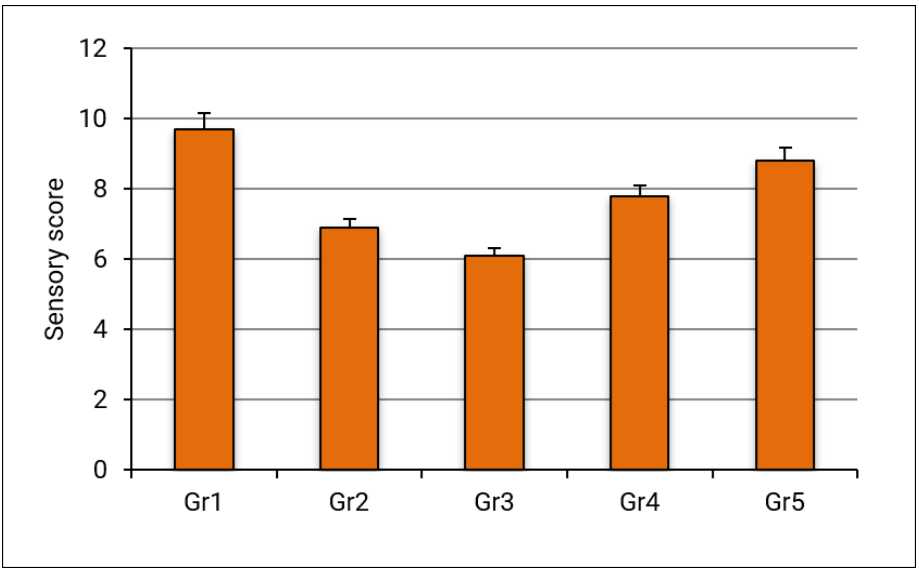
Gr2 = No pre-treatment, plus heat stress (33-34°C)
Gr3 = No pre-treatment, plus heat (33-34°C), plus transport
Gr4 = Pre-treatment 2mL/kg/day NO, plus heat (33-34°C), plus transport
Gr5 = Pre-treatment 3mL/kg/day NO, plus heat (33-34°C), plus transport

**Figure 3.** Ultimate pH, cooking loss (CL) (%), and thiobarbituric acid (TBARS) content (nmol malondialdehyde/mg protein) in rabbits before and after simultaneous exposure to transport and heat, after oral pretreatment with black seed oil (NO).

### *Sensory Profile*

Overall acceptability of cooked meat at the 24h postmortem stage was significantly lower ($p < .05$) in Gr2 compared to Gr1 (6.9 ±0.26 vs 9.7 ±0.45) (Figure 4). In Gr3, after transport under heat, overall acceptability was significantly lower ($p < .05$) than that measured in Gr2 (6.1 ±0.21 vs 6.9 ±0.26) (Figure 4). However, in pretreated (2mL NO/kg BW/d) then transported rabbits under heat (Gr4), this parameter was higher than in Gr3 (7.8 ±0.31 vs 6.1 ±0.21; $p < .05$) (Figure 4). Furthermore, compared to transported rabbits under heat (Gr4), those pretreated with 3mL NO/kg BW/d (Gr5) then exposed simultaneously to transport and heat showed higher overall acceptability (7.8 ±0.31 vs 8.8 ±0.37; $p < .05$) (Figure 4).

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Gr4 = Pre-treatment 2mL/kg/day NO, plus heat (33-34°C), plus transport
Gr5 = Pre-treatment 3mL/kg/day NO, plus heat (33-34°C), plus transport

**Figure 4.** Sensory score of cooked meat in rabbits before and after simultaneous exposure to transport and heat, after oral pretreatment with black seed oil (NO).

## DISCUSSION

In the present study, the impact of heat-induced stress alone and heat-induced stress associated with transport was evaluated in rabbits by analyzing NLR, serum glucose levels, TBARS, CAT, and SOD, as well as pHu, CL, TBARS content, and the sensory profile of the meat. The protective effect of black cumin (*Nigella sativa*) oil (NO) on the antioxidant status of blood and meat in this species was also evaluated. In rabbits, heat exposure increased significantly NLR, serum levels of glucose and TBARS, and meat pHu, CL, and TBARS, and decreased

significantly serum CAT and SOD, and meat overall acceptability. In the simultaneous presence of transport and ambient heat, the stress responses in non-pretreated rabbits with NO became significantly more pronounced, while those in pretreated rabbits showed considerable alleviation according to a dose-dependent effect.

NLR and serum glucose levels increased significantly following the exposure of rabbits to transport stress associated with heat stress. Indeed, this situation activates the hypothalamic-pituitary-adrenal axis, which stimulates the adrenal cortex to promote the synthesis and secretion of glucocorticoids (Harbuz et al., 1995). These hormones lead to a significant decrease in overall immune function by reducing the number of lymphocytes, thereby making animals more vulnerable to pathogens (Amici et al., 2000; Liang et al., 2022; Bellavance & Rivest, 2014). The increase in glucocorticoid secretion during heat stress also promotes hyperglycemia following the process of gluconeogenesis (Kumari & Nath, 2018).

In rabbits, the normal body temperature ranges from 38.5 to 39.5°C, and heat stress occurs when the ambient temperature exceeds 30°C (Nielsen et al., 2020; Dalle Zotte et al., 2025). Heat stress results from the interaction of high ambient temperature, humidity, radiant heat, and air velocity (Lara & Rostagno, 2013), and leads to oxidative stress, endocrine regulation disorders, decreased immune function and production performance, reduced daily weight gain, increased mortality rate, and impaired meat quality and carcass characteristics (Marai et al., 2007; Liang et al., 2022). The alteration of rabbit meat quality due to heat stress was characterized by a reduction in color and juiciness, accompanied by an increase in cooking losses (Zeferino et al., 2013). In rabbits, chronic exposure to heat stress has been responsible for the profound impairment of their welfare and performance due to overproduction of free radicals and ROS (Islam et al., 2021; El-Tarabany et al., 2021). According to Li et al. (2010), SOD and CAT activity in the pituitary and hypothalamus of New Zealand rabbits decreased with increasing temperature and duration of heat exposure.

Furthermore, chronic heat stress has been shown to trigger increased serum corticosterone production in many livestock associated with the production of meat rich in water and heme iron, which promotes the formation of ROS (Gonzalez-Rivas et al., 2020; El-Tarabany et al., 2021; Islam et al., 2021). More recently, Rachchad et al. (2025) showed in vitro on the one hand, that the exposure of rabbit and dromedary meat samples to heat until reaching an internal temperature of 80°C induced an increase in drip loss, CL and TBARS and carbonyl contents, associated with a decrease in CAT and SOD activities, and on the other hand, that the pretreatment of these samples with NO attenuated their oxidations and water losses due to heat stress.

In rabbits, transport-induced stress becomes significantly more intense when combined with heat exposure, which affects their health and production performance, making them more susceptible to heat stress (Marai et al., 2007) due to the absence of sweat glands (Chiericato et al., 1992; Nielsen et al., 2020). Due to the effect of simultaneous exposure to transport stress and heat, a large number of ROS and their metabolites are released into the blood of rabbits (Jimoh et al., 2017). In this situation, the concentration and activity of SOD and CAT in rabbits decreased (Sabés-Alsina et al., 2016 ; Jimoh et al., 2017; Kuang et al., 2021), while the serum level of MDA increased (Garner et al., 2020).

Used as additives in rabbit feed, oils extracted from medicinal plants have

demonstrated numerous benefits, including antimicrobial and antioxidant properties (Nasr et al., 2022). In rabbits, grape seed oil (0.5 to 1.5mL $kg^{-1}$) stimulated growth (Abdel-Wareth et al., 2018), while supplementation with extra virgin olive oil (30mg $kg^{-1}$) reduced plasma MDA levels during exposure to heat stress (El-Ratel et al., 2021). In addition, other studies have been conducted to preserve the quality of rabbit meat using natural antioxidants, such as rosemary essential oil (El Bayomi et al., 2023) and nigella oil (Morshdy et al., 2021; Rachchad et al., 2025). According to Abdelnour et al. (2022), dietary supplementation with thyme essential oil (100 to 150mg $kg^{-1}$) increased the number of lymphocytes and reduced blood MDA levels. Cherif et al. (2018) reported that dietary supplementation in lambs with black cumin seeds (1%) for 3 months increased the resistance of the meat to lipid peroxidation. At the same time, Odhaib et al. (2018) reported similar findings in the long dorsal and the semitendinosus muscles of the lamb. Similarly, in a group of broiler chickens supplemented with black cumin seeds (1-2%), a reduction in TBARS content was observed, accompanied by an increase in SOD activity in the meat (Rahman & Kim, 2016). Furthermore, in Japanese quail, Asghar et al. (2022) found that the addition of black cumin seeds (1 to 4%) to the diet induced an increase in the shelf life of the meat by reducing lipid peroxidation. Furthermore, following exposure to heat stress in rabbits (Al Garadi et al., 2024) and broiler chickens (Apalowo et al., 2024) supplemented with black cumin seeds, TBARS levels were reduced, and CAT and SOD activities in serum and liver were elevated, suggesting a reduction in the impact of heat stress by black cumin.

Other in vitro studies have reported the protective effect of NO against meat oxidation. Morshdy et al. (2021) noted that rabbit meat pretreated with different doses (0.1%, 0.25%, and 0.5%) of NO showed a reduction in lipid peroxidation during 12-day refrigerated storage. On the other hand, the addition of NO to camel meat protected the latter against lipid and protein oxidation induced by the duration of its storage in the refrigerator or by its exposure to heat, by significantly decreasing the levels of TBARS, carbonyls and thiols, and by significantly increasing the activities of antioxidant enzymes namely, CAT and SOD (Rachchad et al., 2024; Rachchad et al., 2025). More recently, Rachchad et al. (2025) found that the concentrations of TBARS and carbonyls in rabbit and camel meat samples, pretreated with 1mL of NO/100g and then cooked to reach 80°C, were significantly lower than those of untreated samples, and decreased further when the dose of NO increased (2mL/100g). In NO-treated samples, the activities of both CAT and SOD enzymes were significantly higher than in untreated samples, and they increased with increasing NO dose in both species.

The most important free radicals generated by stress are oxygen derivatives, particularly superoxide anion and hydroxyl radical. In the present study, the improvement of lipid, immune and anti-oxidant status, as well as the sensory profile stability by NO in rabbits that were subjected simultaneously to heat and transport stress, could be explained by the richness of this oil in different bioactive and antioxidant compounds such as thymoquinone, α-pinene, carvacrol, p-cymene, β-pinene, α-thujene, longifolene, tocopherols, ascorbic acid, flavonoids, thymol, tannins, magnesium and zinc (Alberts et al., 2024), which can directly scavenge free radicals, inhibit the production of pro-oxidant nitric oxide and modulate the activity of endogenous antioxidant systems (Pop et al., 2020).

## CONCLUSION

In rabbits, transport stress resulted in an increase in oxidative stress, affecting both blood and meat quality. This was indicated by elevated levels of TBARS, glucose, CL, and NLR, along with a decrease in pHu, sensory scores, and the enzymatic activities of CAT and SOD. This effect is even more pronounced when transport stress occurs in conjunction with heat stress. The study highlights the protective role of NO against heat stress combined with transport stress, which leads to reduced lipid oxidation and circulating lipids, as well as stabilization of antioxidant enzyme activities, pHu, NLR, and sensory characteristics. The results of the study allow us to consider using black cumin seeds as a dietary supplement for a few weeks before slaughter to minimize the impact of transport and heat and to preserve homeostasis and the organoleptic and sensory characteristics of meat in rabbits.

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The authors appreciate everyone who contributed to the success of this research.

**Author Contributions**

EM conceived and conceptualized the idea of the research and wrote the draft the manuscript. FM interpreted the data and prepared the final manuscript. RK performed data collection and processing, performed literature searches, and sourced plant materials. All authors read and approved the final manuscript.

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**Availability of Data and Materials**

Upon reasonable request, supporting data for the study are obtainable from the corresponding authors.

**Ethical Considerations**

The study was approved by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy of Casablanca, Morocco (code 10/2025).

**Competing Interest**

The authors do not have any conflict of interest to declare.

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