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SCICELL

EFFECT OF ENCAPSULATED ROSEMARY EXTRACT ON TURKEY SEMEN BACTERIAL CONTAMINATION DURING SHORT-TERM LOW TEMPERATURE STORAGE

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ABSTRACT

This study was conducted to define the bacterial community present in turkey semen and evaluate the rosemary essential oil potential in un-encapsulated and encapsulated forms on inhibition of bacterial growth during semen short-term low temperature (4 °C) storage. Semen samples were collected from mature male turkeys and its' bacterial profile was determined through MALDI Biotyper. The semen samples were treated with different concentrations (0, 5, 10 and 25 µg/mL) of un-encapsulated and encapsulated forms of rosemary essential oil. The total bacterial count and sensitivity of various bacterial strains to increasing levels of rosemary extract forms was determined at 0, 2 and 24 h of storage. Rosemary essential oil supplementation significantly reduced the semen bacterial load and most bacterial species showed considerable sensitivity to rosemary essential oil application. The sensitivity of bacterial strains to rosemary extract forms increased in response to extension of storage time and this sensitivity was more profound with encapsulated rosemary extract supplementation. These findings suggest that rosemary essential oil can suppress bacterial growth in turkey semen extenders. Moreover, encapsulation may mitigate the toxicity involved in high essential oil doses and support prolonged inhibition of bacterial growth.

Key words: turkey semen; bacterial contamination; rosemary essential oils; encapsulation

INTRODUCTION

Artificial insemination in commercial farms of turkey is an undeniable prerequisite for optimum fertility as due to extensive genetic selection for bright weight, natural mating possibility was reduced and eliminated for male turkey (Di Iorio *et al.,* 2020). Semen bacterial contamination has been reported to cause sperm agglutination, morphological abnormalities and DNA damage of male gametes, seminal oxidative stress and finally endangered reproductive success in various species (Fraczek & Kurpisz, 2015; Ďuračka & Tvrda, 2018; Duracka *et al.,* 2019). Moreover, sperm progressive motility has been shown to correlate negatively with bacterial load in Lohmann brown rooster semen (Ďuračka *et al.,* 2023). In addition to impaired sperm quality characteristics,

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semen bacterial contamination can detrimentally affect female production either directly through reduced fertility and embryo mortality or indirectly, via causing secondary infections to hens (Roberts *et al.,* 2011). Moreover, semen contamination with *Salmonella*, *Clostridium* or the *Enterobacteriaceae* family can enhance the probability of transmission to human through contaminated products consumed by human (Moyle *et al.,* 2016).

In avian species, the anatomical configuration of the reproductive system, which directs semen through the *vas deferens* into the cloaca, significantly increases the risk of semen contamination with fecal bacterial community (Omprakash & Venkatesh, 2006). The innate immune system of birds has antimicrobial peptides, such as β-defensins and cathelicidins, which can also stimulate cytokines production, thus selectively support the host's immune responses (Zhang & Sunkara, 2014), however might not enough in case of serious contamination with pathogen bacteria. Moreover, short-term liquid low temperature storage of semen is used in avian species commercial farms due to more feasibility, however cryopreservation of avian semen is available to facilitate the management of genetic resources, including the preservation of rare and economically important breeds (Blesbois & Brillard, 2007). Sperm fertilizing capacity is dramatically lost by increasing storage time at 4 ºC harming the insemination efficiency. Semen bacterial contamination during handling, preparation and dilution can be another detrimental factor affecting sperm competence.

Antibiotics are often used to control bacterial load in semen extenders due to their availability and low cost, however they can negatively affect sperm quality, the reproductive tract of inseminated females and the environment (Morrell *et al.,* 2024). Moreover, antibiotic resistance is a serious issue, which made societies to limit antibiotic extended utilization as pathogen bacteria, such as *Escherichia coli*, which is common contamination in avian species showed considerable resistance to antibiotics like ciprofloxacin, tetracycline, streptomycin, ampicillin or erythromycin (Roth *et al.,* 2019). *Rosmarinus officinalis* has been reported to have strong antioxidant, anti-inflammatory and anti-carcinogenic properties due to the presence of considerable amount of polyphenols and volatile oil including carnosic, rosmarinic acids and camphor in it (Di Iorio *et al.,* 2020; Al-jaafreh, 2024). Moreover, *Rosmarinus officinalis* essential oils have been reported to exert antimicrobial effects and can be used in food preservation for controlling bacterial contamination (Kačániová *et al.,* 2023; Adil *et al,* 2024). Supplementation of *R. officinalis* powder in rooster feed (Seyfi *et al.,* 2023) and the diet of rams (Ali *et al.,* 2024) has significantly improved the sperm functional parameters. Moreover, addition of *R. officinalis* essential oil into semen extender showed positive effects on sperm quality characteristics in roosters (Touazi *et al.,* 2018), bulls (Daghigh-Kia *et al.*, 2014) and rams (Motlagh *et al.,* 2014). These positive effects of rosemary extract supplementation in semen quality characteristics can be mediated through the likely inhibition of bacterial growth.

Essential oils are hydrophobic and volatile, which limits their stability and functionality in aqueous environments like semen extenders. Encapsulation offers a solution by enclosing these compounds within biocompatible materials, allowing controlled release and enhanced solubility (Sundar & Parikh, 2023). Therefore, this study aimed to evaluate the efficacy of encapsulating *Rosmarinus officinalis* essential oil (ROEO) in inhibition of turkey semen bacterial community growth during short-term storage at 4 °C.

MATERIALS AND METHODS

Semen sample collection

Semen samples were obtained from the turkey breeding company Branko Nitra, a.s. (Nitra, Slovakia). The samples were collected by cloacal massage from 40 adult Big 6 males and transported to the laboratory in a thermal container (37 °C; M&G Int, Renate, Italy) within 30 min. All ejaculates passed a pre-evaluation for minimum quality criteria and then included in subsequent experiments. Animal handling procedures complied with the ethical guidelines of the Slovak Animal Protection Regulation RD 377/12, in accordance with European Directive 2010/63/EU (Lenický *et al.*, 2021).

Each ejaculate was divided into 8 equal aliquots and each aliquot was diluted either with the control or experimental diluent using a dilution ratio of 1:50 – 1:70 depending on the initial sperm concentration. The SMM extender developed and produced by the Slovak University of Agriculture in Nitra (patent no. 289201) was used for semen dilution. The extender contained saline with an osmolarity of 270 – 320 mOsm.l-1 and a pH in the range of 5.5 to 7.0, with D-levulose, α-D-glucopyranosyl-α-D-glucopyranoside, 1,3,7-trimethylxanthine and 2-aminoethanesulfonic acid. The 8 experimental treatments included 0, 5, 10 and 25 µg/ml of either un-encapsulated or encapsulated forms of ROEO. The semen samples were stored at 4°C and sperm parameters and bacteriological assessments were evaluated at times of 0 (immediately after dilution; control group), 2 and 24 h post-dilution. The semen samples were pre-warmed to 37 °C and 100 µL of sample from each treatments were transferred into a sterile Eppendorf tube and kept at -20 °C for subsequent bacteriological examination (Tvrdá *et al.*, 2022b).

The encapsulation of ROEO was done through formation of the *Rosmarinus officinale* essential oils (ROEO)/β-CD inclusion system prepared by co-precipitation method described by Kotronia *et al.* (2017). Briefly, 500 mg of β-cyclodextrin (β-CD, Sigma Aldrich, Germany) were dissolved in 5 mL of an ethanol/millipore-water mixture (1:2, v/v) under continuous stirring induced by a magnetic stirrer at 55 ± 3 °C until complete dissolution. Then, 125 mg of ROEO (weight ratio of 20:80 of ROEO to β-CD) were added to the β-CD solution at the same temperature and then resulting dispersion was cooled to 4 °C in a refrigerator for 1 hour. The precipitated ROEO/β-CD was collected by vacuum filtration using a Hirsch funnel and washed twice with ethanol to remove the essential oil adsorbed on the surface of β-CD. After drying under vacuum for 3 h at 40 °C, 387 mg of ROEO/β-CD inclusion system was obtained, transferred to a well closed vial and stored at 4 °C until further biological evaluation. The chemical composition of ROEO is presented in Table 1.

Bacteriology

100 µL of each samples were inoculated onto Tryptic soya agar (TSA, Soyabean Casein Digest Agar; Merck, Darmstadt, Germany) or blood agar (BA, Blood Agar Base No. 2; Merck, Darmstadt, Germany) under conditions described by Kačániová *et al.* (2020) for identification and quantification of the bacterial colonies present in semen specimens. Identification of bacterial

Table 1. Chemical composition of *Rosmarinus officinale* essential oils

Components	(%)	Components	(%)	Components	(%)
1,8-cineole	40.4	γ-terpinene	1.2	3-octanone	-
menthol	-	(*Z*)-β-farnesene	-	α-phellandrene	0.2
linalool acetate	-	(*E*)-β-ocimene	0.1	δ-3-carene	0.2
linalool	1.2	hexyl butanoate	-	viridiflorol	-
menthone	0.1	geranyl acetate	-	*n*-amyl isovalerate	-
camphor	11.9	α-humulene	0.7	*n*-hexanol	-
menthyl acetate	-	3-carvomenthenone	-	pinocarvone	0.1
α-pinene	8.7	α-terpinene	0.6	tricyclene	0.1
β-pinene	6.9	caryophyllene oxide	0.6	*p*-cimene	0.1
neo-menthol	-	sabinene	0.4	β-elemene	-
(*E*)-caryophyllene	5.3	β-bourbonene	-	carvone	-
methofuran	-	δ-cadinene	0.3	isopulegol	-
borneol	3.9	α-thujene	0.4	*cis*-3-hexenol	-
camphene	3.5	α-terpinolene	0.4	β-thujone	Tr
isomenthone	-	α-copaene	0.4	α-ylangene	Tr
α-terpineol	2.7	neryl acetate	-	aromadendrene	Tr
α-limonene	2.4	iso-menthyl acetate	-	3-octanol	-
ocimene	2.2	(*E*)-β-farnesene	-	ethyl hexanoate	-
lavandulyl acetate	-	α-amorphene	0.2	*cis*-linalool oxide	-
germacrene D	Tr	hexyl tiglate	-	capryl acetate	-
pulegone	-	α-bisabolol	-	nerol	-
cis-sabinene hydrate	0.2	3-octanol	-	caryophyllene oxide	-
β-myrcene	1.5	isomenthol	-	epi-α-cadinol	-
bornyl acetate	1.4	bicyclogermacrene	-	Total	99.5
4-terpineol	1.1	trans-linalool oxide	-		

species was done by matrix-assisted laser desorption/ionization time of-flight (MALDI-TOF) Biotyper mass spectrometry (Bruker Daltonics, Bremen, Germany) with procedure described by Lenický *et al.* (2021) using Microflex LT instrument, flexControl software version 3.4 and MALDI Biotyper Bruker Taxonomy database (Bruker Daltonics, Bremen, Germany) (Kačániová *et al.,* 2020; Lenický *et al.,* 2021).

Statistical analysis

Data were analyzed by GLM procedure of SAS software in a 3 × 4 × 2 factorial arrangement. The model included the fixed effects of storage time, ROEO supplementation level, type of ROEO and their interaction effects. Pearson correlation coefficients among variables were generated using Proc Corr of SAS. Least-square means were computed and tested for differences by the Tukey's test. Difference between least-squared means was considered to be significant at $P < 0.05$.

RESULTS

Identified bacterial species

There were 12 species of bacteria in turkey semen samples identified by MALDI-TOF method including *Acinetobacter lwoffii*, *Corynebacterium afermentans*, *Enterococcus faecium*, *Enterococcus gallinarium*, *Escherichia coli*, *Micrococcus luteus*, *Proteus mirabilis*, *Serratia marcescens*, *Staphylococcus delphini*, *Staphylo-*

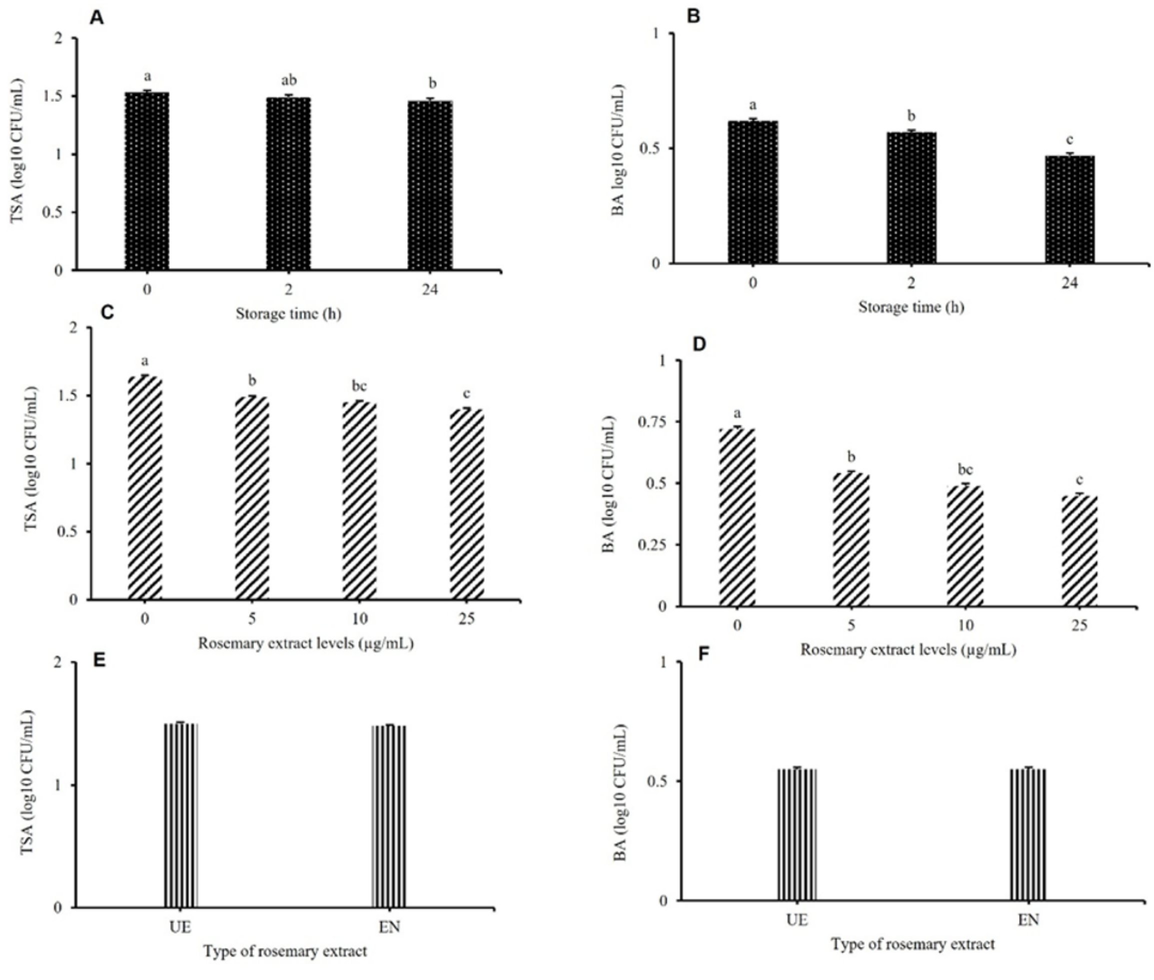

Figure 1. TSA: tryptic soy agar. BA: blood agar. A & B: Effects of storage time (h) on bacterial count respectively in TSA and BA. C & D: Effects of Rosemary extract levels (µg/mL) on bacterial count respectively in TSA and BA. E & F: Effects of the Type of rosemary extract on bacterial count respectively in TSA and BA. a, b, c, d – values in columns with different letters differ significantly (P ≤ 0.0001)

coccus simulans, *Staphylococcus warneri* and *Stenotrophomonas maltophilia* (Figure 1). The identified bacterial species belong to 9 genera and 8 families.

Bacterial load of the semen sample

Bacterial count in blood agar and in Tryptic soy agar was affected by main effects of storage time and ROEO supplementation level but not type of ROEO. Bacterial count in blood agar reduced in response to prolongation of storage time regardless of supplementation level and type of ROEO, however the difference was not statistically significant at 2 h of storage comparing to times 0 and 24 h of storage (Figure 2A). Bacterial count in Tryptic soy agar was reduced gradually in response to prolonging storage time (Figure 2B). Moreover, increasing concentration of ROEO reduced bacterial count in both blood agar and Tryptic soy agar regardless of storage time and type of ROEO (Figure 2C-D). The Bacterial count in Tryptic soy agar and blood agar was affected by interaction of storage time and level of ROEO supplementation (Figure 3A-B). Although 25 µg/mL of ROEO reduced bacterial count in Tryptic soy agar comparing to control group in all storage times, 5 and 10 µg/mL of ROEO were not influential in reducing bacterial count at time 0, whereas 5 µg/mL of ROEO at 2 h and 5 and 10 µg/mL of ROEO at 24 h of storage times, significantly reduced bacterial count comparing to the control group. Although 25 µg/mL of ROEO reduced bacterial count in blood agar in all storage times, levels of 5 and 10 µg/mL of ROEO was influential in reducing bacterial count comparing to control group only at 2 and 24 h of storage times.

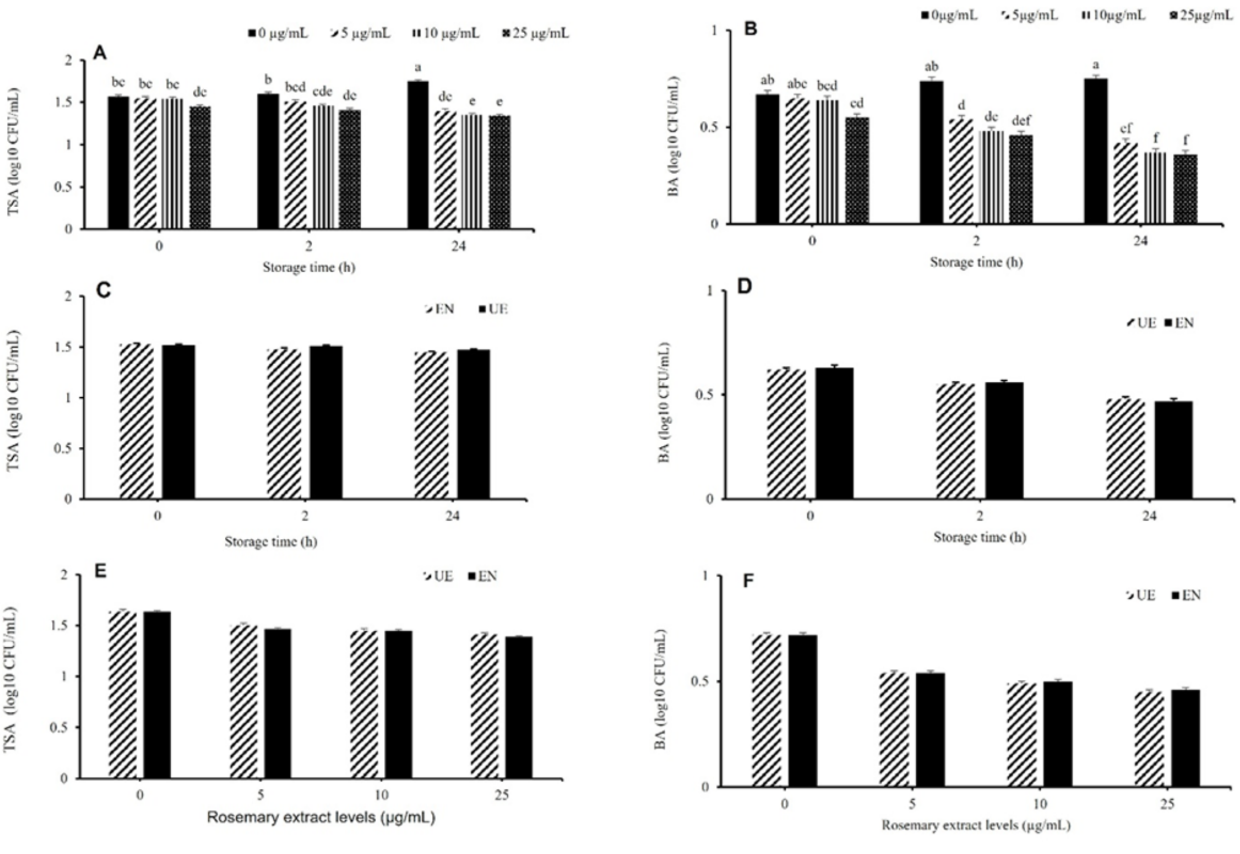

Figure 2. TSA: tryptic soy agar. BA: blood agar. A & B: Interactions of storage time (h) and Rosemary extract levels (µg/mL) on bacterial count respectively in TSA and BA. C & D: Interactions of storage time (h) and type of Rosemary extract on bacterial count respectively in TSA and BA. E & F: Interactions of Rosemary extract levels (µg/mL) and Type Rosemary extract on bacterial count respectively in TSA and BA. UE: Un-encapsulated, EN: Encapsulated. a, b, c, d – values in columns with different letters differ significantly (P ≤ 0.0001).

Figure 3. The krona chart of the identified bacterial species by MALDI-TOF MS Biotyper recovered from turkey semen samples (outermost ring: species, middle ring: genus, and innermost ring: family)

Bacterial species sensitivity

The sensitivity and resistance of the bacterial species present in semen samples to various treatments are presented in Table 2. From the identified bacterial species, *Staphylococcus delphini* and *Staphylococcus simulans* were not affected by level and type of ROEO during the whole storage time. At 0 time of storage, almost all bacterial strains were not affected by treatments except three strains including *Enterococcus faecium*, *Serratia marcescens* and *Staphylococcus warneri*. From these three strains, *Enterococcus faecium* and *Staphylococcus warneri* were affected by all levels and type of ROEO, whereas *Serratia marcescens* was affected only by un-encapsulated form of ROEO in supplementation levels of 10 and 25 µg/mL. After 2 h of storage, six bacterial strains terminated their resistance to treatments and showed sensitivity to either level or type of ROEO. The *Acinetobacter lwoffii* showed sensitivity to both types of ROEO in levels of 10 and 25 µg/mL, whereas *Corynebacterium afermentans* showed sensitivity to all forms and levels of ROEO. Moreover, *Enterococcus gallinarium*, *Proteus mirabilis* and *Stenotrophomonas maltophilia* showed sensitivity to 25 µg/mL of encapsulated and un-encapsulated ROEO forms respectively, whereas *Micrococcus luteus* was affected by 10 and 25 µg/mL of encapsulated ROEO. The *Escherichia coli* was the only strain which was resistance to 5 and 10 µg/mL of either un-encapsulated or encapsulated ROEO and 25 µg/mL of un-encapsulated ROEO, whereas 25 µg/mL of encapsulated ROEO was influential on it after 24 h of storage. The sensitivity of bacterial strains was increased in response to prolonging storage time, increasing supplementation level of ROEO and encapsulation of ROEO.

DISCUSSION

Bacterial contamination of semen has been reported as the contributors of oxidative stress and the subsequent sperm dysfunctions and apoptosis (Fujita *et al.* 2011; Tvrdá *et al.* 2022a). Synthetic antioxidants and antibiotics are used commonly in semen extenders of various species respectively for controlling oxidative stress and pathogen bacterial contamination. Antibiotic utilization is going to be more restricted due to considerable antibiotic resistance of bacterial strains present in semen (Tvrdá *et al.,* 2023). Moreover, there are only a few broad-spectrum antibiotics with the capacity to inhibit growth in all varieties of bacteria present in a medium. Essential oils has been reported to affect a large variety of organisms including bacteria, viruses and fungi (Nieto *et al.,* 2018; Al-Megrin *et al.*, 2020; Kloy *et al.,* 2020), however these compounds can cause strong toxicity against various cell lines such as epithelial cells, fibroblasts, neutrophils, monocytes and also spermatozoa (Bakkali *et al.,* 2008; Paul & Kang, 2012). Rosemary extract with major antibacterial and antioxidant capacity is used in food processing as a natural preservative (Nieto *et al.,* 2018). The rosemary extract as a natural plants source product, has been shown to have strong antimicrobial and antioxidant activities making it a suitable candidate for application in semen extender (Nieto *et al.,* 2018; Olivas-Méndez *et al.,* 2022).

Due to specific anatomy of reproductive system in avian, which lead to sperm passage through the *vas deferens* into the cloaca, the probability of semen contamination with fecal bacteria increase dramatically (Omprakash & Venkatesh, 2006). Semen bacterial contamination can exert varying detrimental effects on sperm quality characteristics including morphological abnormalities, sperm motility, membrane integrity, DNA damage, ROS production and oxidative stress in various species depending on quantity and quality of bacterial community (Fraczek & Kurpisz, 2015; Ďuračka & Tvrda, 2018; Tvrdá *et al.,* 2018; Lenický *et al.,* 2021; Ďuračka *et al.,* 2023). In addition to impaired semen quality and disturbed male fertility, the bacterial contamination

Table 2. Bacterial strains resistance and sensitivity affected by storage time, RE supplementation level and type of rosemary extract

Storage time (h)	0							2							24						
RE levels (µg/mL)	0	5		10		25		0	5		10		25		0	5		10		25	
Type of rosemary extract	C	UE[a]	EN[b]	UE[a]	EN[b]	UE[a]	EN[b]	C	UE[a]	EN[b]	UE[a]	EN[b]	UE[a]	EN[b]	C	UE[a]	EN[b]	UE[a]	EN[b]	UE[a]	EN[b]
Acinetobacter lwoffii	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	S	S	S	S	S
Corynebacterium afermentans	R	R	R	R	R	R	R	R	S	S	R	S	S	S	R	S	S	S	S	S	S
Enterococcus faecium	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
Enterococcus gallinarium	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	S	S
Escherichia coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Micrococcus luteus	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S
Proteus mirabilis	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
Serratia marcescens	R	R	R	S	R	S	S	R	R	S	S	S	S	S	R	R	S	S	S	S	S
Staphylococcus delphini	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus simulans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus warneri	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	S
Bacterial resistancy (%)	100	83.3	83.3	75	83.3	75	75	100	75	66.6	66.6	50	50	33.3	100	50	33.3	25	25	25	16.6
Bacterial sensitivity (%)	0	16.6	16.6	25	16.6	25	25	0	25	33.3	33.3	50	50	66.6	0	50	66.6	75	75	75	83.3

a: Un-encapsulated, b: Encapsulated

can be transmitted into female causing considerable decline in their fertility and production performance (Roberts *et al.,* 2011; Moyle *et al.,* 2016). Moreover, contamination with bacterial families including *Salmonella*, *Clostridium* or *Enterobacteriaceae* can affect human through products contamination (Roberts *et al.,* 2011; Moyle *et al.,* 2016). Bacterial resistance is becoming a serious concern and going to restrict antibiotics utilization in poultry (Al Azad *et al.*, 2019; Roth *et al.,* 2019). ROEO has been reported to have comprehensive antimicrobial activities against wide range of bacterial strains (Chaftar *et al.,* 2015; Outaleb *et al.,* 2015). The sensitivity of *Escherichia coli* to ROEO in current study is promising as it showed considerable degree of resistance to a broad scope of antibiotics including tetracycline, ampicillin, streptomycin, ciprofloxacin and erythromycin (Roth *et al.,* 2019). The *E. faecium* and *E. coli* has been shown resistance respectively to imipenem and ertapenem in a previous experiment in our lab (Lenický *et al.*, 2021), whereas in current work, both of them showed sensitivity to ROEO supplementation. *Escherichia coli* showed the capability to adhere to receptors on sperm acrosome and flagellum, leading to an impaired motility and cell death (Schulz *et al.,* 2010). The semen contamination with *E. coli*, and *Enterococcus faecalis* was highly associated with sperm DNA fragmentation and cell death accompanied by impaired motility and membrane disintegration (Zeyad *et al.,* 2018). The lowered concentration of ROS in current study (12.72, 10.32, 9.47 and 15.09 RLU/sec/106 of ROS respectively in 0, 5, 10 and 25 µg/ml of ROEO) can be related to inhibitory effects of ROEO on bacterial load and diversity as they has been reported as the main inducer of ROS overproduction through either direct or indirect stimulation of inflammatory response (Fraczek *et al.*, 2007; Aitken & Drevet, 2020). The sensitivity of *Enterococcus faecalis* to ROEO in current work is in agreement with Kačániová *et al.* (2023) who reported inhibitory effect of ROEO on *S. enterica*, *E. faecalis*, *Y. enterocolitica* and *S. aureus*. The antibacterial property of ROEO originate from active compounds including rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol and also the combined actions of different minority components (Nieto *et al.,* 2018). These antibacterial properties are excreted through their ability to influence genetic material and nutrients, transport of electrons, leakage of cellular components and production changes in fatty acid and also change in permeability of bacterial membranes through binding to the amine and hydroxylamine groups of the membrane proteins (FUNG *et al.,* 1977; Gallucci *et al.*, 2009). Moreover, ROEO has been reported to enhance the susceptibility of some bacteria to standard antibiotics (Marinas *et al.,* 2012).

Plant extract and essential oils are highly active, volatile and sensitive to environmental conditions such as oxidants, light, pH and temperature and also hydrophobic, which make their incorporation in aqueous products challenging. Encapsulation technique was acquired to enhance essential oils water solubility, reduced their volatility and scape from the media solution and hence, prolonging their presence in media and affectivity (Hcini *et al.,* 2021; Sundar & Parikh, 2023). Encapsulated rosemary extract has been reported to enhanced the oxidative and microbiological stability of beef meat during refrigerated storage (Rashidaie Abandansarie *et al.,* 2019). To the best of our knowledge, this is the first experiment evaluating the encapsulated ROEO utilization in semen extender for short term storage. The overall positive effects of encapsulated ROEO on inhibition of bacterial growth can be related to its improved solubility in semen aqueous medium. Moreover, the higher beneficial effect of encapsulated ROEO at 25 µg/ml of supplementation compared to un-encapsulated form can be related to preventing the likely detrimental effects of active compounds on sperm cell immediately after administration and the slow, continuous and prolonged release of these active compounds from encapsulated form might be the reason for more inhibition of bacterial growth in response to prolongation of semen storage time.

CONCLUSION

In summary, rosemary essential oil (ROEO), particularly in its encapsulated form, demonstrated promising antimicrobial properties which can lead to reduced bacterial load in turkey semen during short-term low temperature storage. Lower concentrations (5 and 10 µg/mL) proved to be most effective, while the encapsulation technique enhanced the safety and efficacy of ROEO, especially at higher doses and extended storage durations. These findings highlight the potential of encapsulated plant-based bioactives such as ROEO as a practical supplement in poultry semen extenders.

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Writing-original draft preparation: ATAEI-NAZARI, S.

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All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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