



Effects of Dietary *Cordyceps militaris* on Sperm Quality and Reproductive Performance in Rabbits

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ABSTRACT

Cordyceps militaris is a medicinal mushroom rich in bioactive compounds and has been investigated for its potential antioxidant properties. The present study aimed to evaluate the effects of dietary supplementation with *Cordyceps militaris* extract on semen quality and reproductive performance in male rabbits. A total of 15 adult male rabbits, with an average body weight of 3.56 ± 0.34 kg, were randomly assigned to five treatments receiving 0 (control), 20, 40, 80 and 160 mg of *Cordyceps militaris* extract daily for 14 weeks. Semen was collected at weeks 6, 8, 10, 12, and 14, with an average volume of 0.4 ± 0.1 mL per collection, and evaluated for sperm viability, motility, acrosome and membrane integrity, DNA fragmentation, and antioxidant capacity. A total of 15 adult female rabbits, with an average body weight of 3.37 ± 0.26 kg, were used for mating and were managed under the same experimental conditions. After eight weeks of supplementation, treated males were naturally mated with female rabbits to evaluate fertility and litter performance. Compared with the control group, *Cordyceps militaris* supplementation improved sperm motility, viability, membrane integrity, acrosome integrity, and antioxidant capacity, while reducing DNA fragmentation. The 40 mg/day treatment showed the most favourable overall effects on semen quality across the evaluation period. At week 8, rabbits receiving 40 mg/day showed higher sperm motility (74.34%), viability (90.85%), acrosome integrity (99.92%), membrane integrity (39.79%), and seminal antioxidant capacity (35.04%), together with lower DNA fragmentation (35.76%), compared with the control group. The 40 mg/day group showed a significantly higher pregnancy rate at first mating than the control group, reaching 100%. Rabbits that received 40 mg/day achieved a 100% first-mating pregnancy rate, while litter traits and offspring growth did not differ significantly among treatments. Overall, dietary supplementation with *Cordyceps militaris* improved semen quality and reproductive performance in male rabbits, with 40 mg/day providing the most favourable overall response among the tested supplementation levels.

Keywords: Antioxidant capacity, Dietary supplementation, DNA fragmentation, Sperm, Reproductive performance

INTRODUCTION

Rabbit farming is an important component of small-scale farming systems in many countries due to the nutritional value of rabbit meat, which is characterized by a high protein content and relatively low levels of fat and cholesterol (Gašperlin et al., 2006). Furthermore, rabbit farming is recognized as a potentially sustainable livestock production system due to their efficient feed utilization and high reproductive productivity (Siddiqui et al., 2024). Improving reproductive performance, particularly male fertility, is a key factor contributing to productivity and sustainability in rabbit farming systems (Fadl et al., 2024). Semen quality in breeding males is of particular importance, as reproductive efficiency directly influences conception rates, litter performance, and overall productivity in rabbit production (Brun et al., 2016). Nevertheless, male reproductive performance is susceptible to nutritional and environmental conditions, particularly oxidative stress (Agarwal et al., 2014). An imbalance between ROS production and antioxidant defenses can adversely affect sperm membrane function, motility, and DNA integrity, thereby compromising fertility (Agarwal et al., 2014; Sies, 2017).

Natural plant-based feed additives with antioxidant properties have received increasing attention as potential alternatives to synthetic antioxidant vitamins in livestock nutrition (Manuelian et al., 2021). Bioactive compounds derived from medicinal plants, herbs, and other natural sources have been studied for their potential to enhance animal health and reproductive performance through regulating oxidative status (Manuelian et al., 2021). Dietary antioxidant supplementation with vitamin C, vitamin E, and selenium has been reported to improve semen quality and reproductive performance in male rabbits, potentially through enhanced antioxidant status and attenuation of oxidative damage (Yousef et al., 2003; Fadl et al., 2024).

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Cordyceps militaris (*C. militaris*) is a medicinal fungus containing a variety of bioactive compounds, including cordycepin, adenosine, polysaccharides, and phenolic compounds (Jędrejko et al., 2021; Wang et al., 2022). Previous studies have reported antioxidant, anti-inflammatory, and immunomodulatory activities of *C. militaris* and its bioactive components, particularly polysaccharides and cordycepin (Miao et al., 2022; Phull et al., 2022). Recent studies in livestock have shown that supplementation with *C. militaris* or its spent mushroom substrate can enhance antioxidant capacity and reduce oxidative stress markers in pigs (Boontiam et al., 2020). In addition, dietary *C. militaris* supplementation has been reported to improve intestinal barrier function and modulate gut microbiota in pigs (Zheng et al., 2022). Furthermore, supplementation with fermented products from *C. militaris* has been reported to enhance reproductive development and support testicular function in male rats, suggesting a potential beneficial role in male reproduction (Lin et al., 2022). Thus, the findings suggest that the bioactive components of *C. militaris* may contribute to improved reproductive performance through their antioxidant and cytoprotective effects.

Although the biological properties of *C. militaris* have been investigated in several species, information regarding its effects on semen quality and reproductive performance in rabbits remains limited. Therefore, the present study aimed to evaluate the effects of different dietary supplementation levels of *C. militaris* extract on semen quality, antioxidant capacity, and reproductive performance in rabbits.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Can Tho University Animal Ethics Committee (Approval No. CTU-AEC24036), Can Tho City, Vietnam.

Animals

Rabbits were housed individually in elevated cages under natural environmental conditions. Throughout the experimental period, all rabbits were maintained under the same feeding conditions and were provided with fresh elephant grass (*Pennisetum purpureum*), fresh clean vegetables, and commercial pelleted rabbit feed (De Heus 5640, De Heus Vietnam, Vietnam). Fresh drinking water was provided *ad libitum*. The same types of feed were provided to all experimental animals throughout the study to maintain consistent nutritional conditions among experimental groups. Animals were monitored daily throughout the experiment.

Cordyceps militaris extract

Cordyceps militaris extract used in the current study was provided by the Institute of Pharmaceutical and Health Food, Thu Dau Mot University, Ho Chi Minh City, Vietnam (Nguyen et al., 2024). The extract was administered orally at doses of 0, 20, 40, 80, and 160 mg/day per rabbit according to the experimental treatments. The extract quality and composition were analyzed and certified in accordance with ISO/IEC 17025:2017 standards. The cordycepin content in the extract was determined to be 0.15% (w/w).

Study design

The study was conducted using 15 adult male rabbits (3.56 ± 0.34 kg) and 15 adult female rabbits (3.37 ± 0.26 kg) aged 9–12 months at the experimental farm of Can Tho University, Vietnam. A total of 15 adult male rabbits were randomly allocated to five dietary treatments (three rabbits in each treatment), receiving 0 (control), 20, 40, 80, and 160 mg of *C. militaris* extract daily for 14 weeks. The *C. militaris* extract was mixed into pelleted feed (De Heus 5640, De Heus Vietnam, Vietnam) with the daily diet throughout the experimental period. Semen was obtained by artificial vagina collection once per week. Assessments were performed at weeks 6, 8, 10, 12, and 14 to characterize sperm quality, including motility, viability, plasma membrane, and acrosomal integrity. Antioxidant status and sperm DNA fragmentation were also examined to further evaluate sperm functional and cellular integrity. After eight weeks of supplementation, treated males were naturally mated with female rabbits to evaluate reproductive performance. Pregnancy rate, gestation length, litter size, and offspring growth were recorded.

Assessment of sperm quality

Motility

Sperm motility was observed under an Eclipse Si microscope (Nikon, Japan) at 40× magnification. The percentage of total motile sperm was determined by the proportion of total motile sperm to the total number of sperm counted (Tran et al., 2023).

Viability

Sperm viability was assessed using the Eosin–Nigrosin vital staining method and examined under a light microscope (Nikon, Japan; Figure 1A). Sperm viability was determined following the method of Tran et al. (2023). Dead sperm cells were identified by their purple staining, while viable cells remained unstained. Viability was expressed as the proportion of live spermatozoa among the total number of sperm cells evaluated.

Membrane integrity

Sperm plasma membrane integrity was assessed using the hypo-osmotic swelling test (HOST) following the procedure described by [Tran et al. \(2025\)](#). Briefly, semen (20 μ L) was diluted with 80 μ L of hypo-osmotic solution and maintained at 37°C for 30 min. Following incubation, an aliquot of the suspension was transferred onto a glass slide and examined microscopically (400 $\times$ magnification) to determine sperm membrane integrity. Spermatozoa showing coiled or swollen tails were considered to have intact plasma membranes, whereas spermatozoa without tail swelling were considered membrane-damaged (Figure 1B). At least 200 spermatozoa were evaluated per sample.

Acrosome integrity

Acrosome integrity was assessed using the Giemsa staining method described by [Nguyen et al. \(2025\)](#). Spermatozoa were fixed on glass slides and air-dried at room temperature for 10 minutes. The samples were then immersed in 96% ethanol for 15 seconds for fixation, followed by air-drying at room temperature (25°C) for an additional five minutes. Subsequently, the slides were stained with 10% Giemsa solution for 10 minutes. After staining, the slides were gently rinsed with water, air-dried at room temperature, and examined under the microscope (Figure 1C). Spermatozoa with intact acrosomes exhibited purple staining at the head, whereas spermatozoa with damaged or incomplete acrosomes did not.

Antioxidant capacity

Seminal antioxidant activity was determined by measuring the ABTS radical-scavenging capacity based on the method of [Nenadis et al. \(2004\)](#), with slight modifications. Fresh semen (10 μ L) was added to 990 μ L of ABTS⁺ working solution and allowed to react for 6 minutes at room temperature (28–30°C). The absorbance of the reaction mixture was recorded at 734 nm using a UV–Vis spectrophotometer (Thermo Fisher Scientific, USA). Each sample was analyzed in three independent measurements, and antioxidant activity was reported as the percentage of ABTS⁺ radical scavenging.

DNA fragmentation

DNA fragmentation was assessed using the Sperm Chromatin Dispersion (SCD) method. Sperm samples were stained with Giemsa and observed under the microscope (Figure 1D). Spermatozoa with intact DNA displayed a halo around the nucleus, whereas those with fragmented DNA did not, as described by [Absalan et al. \(2012\)](#).

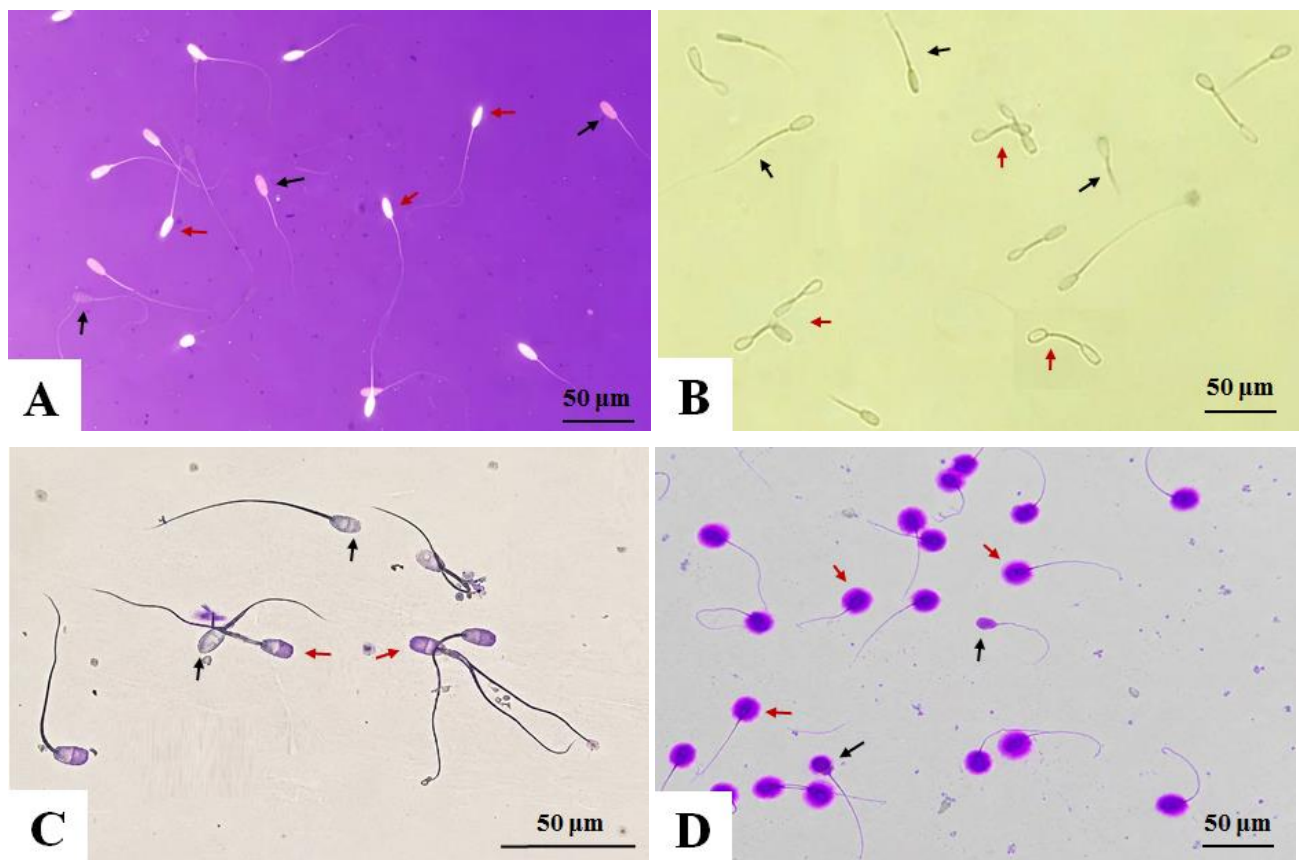


Figure 1. Representative photomicrographs of rabbit sperm used for semen quality evaluation. **A:** Viable spermatozoa (red arrow) and dead spermatozoa (black arrow), **B:** Spermatozoa with intact plasma membranes (red arrow) and damaged membranes (black arrow), **C:** Spermatozoa with intact acrosomes (red arrow) and damaged acrosomes (black arrow), **D:** Spermatozoa with non-fragmented DNA (red arrow) and fragmented DNA (black arrow).

Assessment of *in vivo* reproductive performance of female rabbits

The *in vivo* reproductive performance of female rabbits was evaluated based on pregnancy rate, gestation length, litter size, and offspring body weight. Mating was conducted in up to three consecutive rounds, and pregnancy was diagnosed 10 days after mating. Non-pregnant or pseudo-pregnant females were remated after a resting period of at least one month. Gestation length was calculated as the interval from mating to parturition and typically ranged from 28 to 32 days. Litter size was determined by counting the number of live-born kits at birth, while dead kits were recorded separately. Neonatal body weight was measured using an electronic balance before suckling, and total litter weight as well as average kit weight were calculated at birth and again at eye opening (10-12 days of age; [Morshedy et al., 2022](#)).

Statistical analysis

Data were analyzed using Minitab 16 software. Semen quality parameters measured at different sampling times were analyzed using repeated measures analysis of variance (ANOVA), with nutritional supplementation level as the inter-subject factor and sampling time as the intra-subject factor. When significant differences were detected, the mean values of the treatment groups were compared using Tukey's post hoc test. Reproductive performance and offspring growth parameters were analyzed using one-way ANOVA, with nutritional supplementation level as the primary factor, followed by Tukey's post hoc test. Normality of residuals and homogeneity of variance were assessed before statistical analysis. Data are presented as mean $\pm$ standard deviation (SD), and differences were considered statistically significant at p-value less than 0.05.

RESULTS

Semen quality

Semen quality parameters of male rabbits supplemented with different levels of *C. militaris* extract are presented in Table 1. Dietary supplementation affected several semen quality traits during the experimental period. In general, supplemented groups showed higher sperm motility, viability, membrane integrity, acrosome integrity, and antioxidant capacity, together with lower DNA fragmentation than the control group during weeks 6-12 ($p < 0.05$). Among the treatment groups, the 40 mg/day group generally produced the most favorable response across the measured parameters ($p < 0.05$). Subsequently, from week 14 onwards, several semen quality parameters declined significantly.

Overall sperm motility

Differences in sperm motility among dietary treatments became evident during the later stages of the experiment (Table 1). Between weeks 6 and 12, rabbits receiving *C. militaris* supplementation consistently maintained higher motility than those in the control group, with significant differences observed among all supplemented treatments ($p < 0.05$). Among the supplemented treatments, the 40 mg/day group generally showed the highest motility values, reaching 74.34% at week 8 and remaining above 70% until week 12 ($p < 0.05$). In comparison, sperm motility in the control group remained relatively stable at approximately 54-55% during the same period. Although supplementation levels of 80 and 160 mg/day also improved sperm motility compared with the control treatment, their values were not significantly higher than those observed in the 40 mg/day group ($p > 0.05$). Sperm motility changed significantly throughout the experimental period across all treatment groups from week 6 to week 14. Sperm motility increased from week 6 to week 8 and remained relatively stable through week 12, followed by a decline at week 14 in all treatment groups. Compared with the control group, sperm motility was significantly higher in the supplemented groups during weeks 6-12 ($p < 0.05$), whereas no significant difference was observed at week 14 ($p > 0.05$).

Sperm viability

Dietary supplementation with *C. militaris* increased sperm viability compared with the control group during weeks 6-12 (Table 1; $p < 0.05$). By week 14, no significant differences in sperm viability were detected among any of the treatment groups, including the control group ($p > 0.05$). The maximum viability was recorded at week 8 in rabbits receiving 40 mg/day. No significant differences in sperm viability were observed among the supplemented groups, suggesting that increasing the supplementation level beyond 20 mg/day did not provide an additional beneficial effect on sperm viability.

Membrane integrity

Supplementation with *C. militaris* extract improved sperm membrane integrity compared to the control group during weeks 6-12 ($p < 0.05$). Specifically, at week 8, the 40 mg group achieved the highest value (39.79%) compared to the control group (30.00%), and maintained high levels at week 10 (39.27% with 29.97%) and week 12 (38.53% with

29.61%). Doses of 20, 80, and 160 mg also showed higher values than the control group (approximately 36.4-37.7%), but there were no significant differences between these doses ($p > 0.05$). Overall, the 40 mg dose provided the best and most stable results across all time points. By week 14, sperm membrane integrity had decreased across all groups, with values ranging from approximately 28.39% to 30.01%. No significant differences were detected among the groups at this time point ($p > 0.05$).

Table 1. Effects of *Cordyceps militaris* extract supplementation on sperm quality parameters in 9-month-old male rabbits over 14 weeks

Parameter (%)	Dose (mg)	Week 6	Week 8	Week 10	Week 12	Week 14
Overall sperm motility	0	55.08 ^b ± 1.31	55.12 ^c ± 1.19	55.06 ^c ± 1.33	54.41 ^c ± 1.22	54.19 ^{ab} ± 1.35
	20	68.80 ^a ± 1.23	70.05 ^b ± 1.25	69.14 ^b ± 1.23	67.83 ^b ± 1.21	52.83 ^b ± 0.94
	40	70.24 ^a ± 1.15	74.34 ^a ± 1.22	73.38 ^a ± 1.20	71.99 ^a ± 1.18	56.06 ^a ± 0.92
	80	69.82 ^a ± 1.50	71.08 ^{ab} ± 1.53	70.16 ^{ab} ± 1.51	68.83 ^{ab} ± 1.44	53.60 ^{ab} ± 1.15
	160	69.07 ^a ± 0.53	69.97 ^b ± 0.57	69.06 ^b ± 0.49	67.76 ^b ± 0.52	52.77 ^b ± 0.40
Membrane integrity	0	29.97 ^b ± 1.55	30.00 ^b ± 1.67	29.97 ^b ± 1.61	29.61 ^b ± 1.59	29.49 ^a ± 1.21
	20	37.01 ^a ± 0.73	37.68 ^a ± 0.93	37.19 ^a ± 0.98	36.48 ^a ± 0.97	28.41 ^a ± 0.75
	40	37.59 ^a ± 1.00	39.79 ^a ± 1.05	39.27 ^a ± 1.04	38.53 ^a ± 1.02	30.01 ^a ± 0.79
	80	36.12 ^a ± 0.53	36.94 ^a ± 1.14	37.44 ^a ± 0.87	37.02 ^a ± 0.81	27.82 ^a ± 0.68
	160	36.97 ^a ± 0.98	37.64 ^a ± 1.07	37.15 ^a ± 0.66	36.45 ^a ± 0.76	28.39 ^a ± 0.73
Viability	0	64.15 ^b ± 2.31	64.21 ^b ± 2.27	64.14 ^b ± 2.12	63.37 ^b ± 2.28	63.12 ^a ± 2.35
	20	85.43 ^a ± 2.89	86.98 ^a ± 2.95	85.85 ^a ± 2.83	84.23 ^a ± 2.81	65.60 ^a ± 2.20
	40	85.84 ^a ± 4.54	90.85 ^a ± 2.80	89.67 ^a ± 2.74	87.98 ^a ± 2.60	68.52 ^a ± 2.62
	80	84.27 ^a ± 2.14	87.70 ^a ± 2.64	85.12 ^a ± 2.67	84.59 ^a ± 2.77	65.78 ^a ± 2.32
	160	85.35 ^a ± 2.62	86.89 ^a ± 2.87	85.76 ^a ± 2.45	84.14 ^a ± 2.64	65.53 ^a ± 2.29
Acrosome integrity	0	85.50 ^b ± 3.08	85.57 ^c ± 3.09	85.48 ^c ± 3.01	84.46 ^c ± 3.14	84.12 ^a ± 3.03
	20	92.93 ^a ± 1.39	94.61 ^b ± 1.44	93.38 ^b ± 1.40	91.62 ^b ± 1.35	71.35 ^b ± 1.06
	40	94.41 ^a ± 1.41	99.92 ^a ± 1.49	98.62 ^a ± 1.38	96.76 ^a ± 1.45	75.36 ^b ± 1.13
	80	92.93 ^a ± 1.33	94.61 ^b ± 1.53	93.38 ^b ± 1.46	91.62 ^b ± 1.47	71.35 ^b ± 1.10
	160	92.84 ^a ± 1.25	94.52 ^b ± 1.21	93.29 ^b ± 1.33	91.53 ^b ± 1.26	71.28 ^b ± 1.23
Antioxidant capacity	0	22.60 ^b ± 0.77	22.62 ^b ± 0.83	22.60 ^b ± 0.82	22.33 ^b ± 0.76	22.24 ^b ± 0.80
	20	33.01 ^a ± 1.19	33.61 ^a ± 1.22	33.18 ^a ± 1.28	32.55 ^a ± 1.12	25.35 ^a ± 0.95
	40	33.54 ^a ± 1.21	35.50 ^a ± 1.18	35.04 ^a ± 1.16	34.37 ^a ± 1.24	26.77 ^a ± 0.97
	80	33.01 ^a ± 1.11	33.61 ^a ± 1.27	33.18 ^a ± 1.30	32.55 ^a ± 1.37	25.35 ^a ± 0.88
	160	32.98 ^a ± 1.07	33.58 ^a ± 1.11	33.14 ^a ± 1.25	32.52 ^a ± 1.33	25.32 ^a ± 0.91
DNA fragmentation	0	45.38 ^a ± 1.08	45.42 ^a ± 1.12	45.37 ^a ± 1.23	44.83 ^a ± 1.09	44.65 ^a ± 1.16
	20	38.24 ^b ± 1.38	37.48 ^{bc} ± 1.35	37.86 ^{bc} ± 1.37	38.28 ^{bc} ± 1.38	38.78 ^{bc} ± 1.40
	40	37.62 ^b ± 1.36	35.76 ^c ± 1.29	35.98 ^c ± 1.30	36.23 ^c ± 1.31	36.55 ^c ± 1.32
	80	38.24 ^b ± 1.31	37.48 ^{bc} ± 1.24	38.01 ^{bc} ± 1.28	38.58 ^{bc} ± 1.44	39.14 ^{bc} ± 1.41
	160	39.06 ^b ± 1.41	39.84 ^b ± 1.44	40.56 ^b ± 1.46	41.34 ^{ab} ± 1.49	42.02 ^{ab} ± 1.53

^{a,b,c,d} Means within a column of each parameter with different superscript letters differ significantly ($p < 0.05$)

Acrosome integrity

The results showed that supplementation with *C. militaris* increased acrosome integrity compared to the control group at all time points ($p < 0.05$). Specifically, the 40 mg group achieved the highest level at week 8 (99.92%) compared to 85.57% in the control group, and maintained high levels at week 10 (98.62%) and week 12 (96.76%; $p < 0.05$). The 20, 80, and 160 mg/day supplementation groups also exhibited significantly higher values than the control group, ranging from approximately 91% to 94% ($p < 0.05$). By week 14, acrosome integrity values decreased to approximately 71.28-75.36%; however, they remained statistically significant compared to the control group ($p < 0.05$).

Antioxidant capacity

Antioxidant capacity in semen samples was significantly higher in supplemented groups than in the control group throughout the experimental period (Table 1; $p < 0.05$). The control group-maintained values of approximately 22%

during weeks 6-14, whereas supplemented groups showed values ranging from approximately 32% to 35% during weeks 6-12. The 40 mg/day treatment showed the highest antioxidant capacity, particularly at weeks 8 and 10. No significant differences in antioxidant capacity were observed among the supplemented groups ($p > 0.05$). At week 14, antioxidant capacity decreased compared with week 12 across all supplemented groups.

DNA fragmentation index

A significant reduction in sperm DNA fragmentation was observed in rabbits receiving *C. militaris* supplementation compared with the control group (Table 1; $p < 0.05$). The difference in DNA fragmentation index was evident from weeks 6 to 12, during which all supplemented groups exhibited significantly lower DNA fragmentation levels than the control group ($p < 0.05$). During weeks 8-12, the 40 mg/day group showed the lowest numerical sperm DNA fragmentation among the supplemented groups, although the differences among supplementation levels were not statistically significant ($p > 0.05$). Sperm DNA fragmentation in the 160 mg/day group increased progressively over the experimental period and was no longer significantly different from the control group by week 14 ($p > 0.05$).

In vivo fertility and offspring

Pregnancy rate

Pregnancy rate following mating differed among treatments during the first mating period (Table 2). Female rabbits mated with males receiving *C. militaris* supplementation generally showed significantly higher pregnancy rates than those mated with control males ($p < 0.05$). The control group recorded the lowest first-mating pregnancy rate (33.33%), whereas the 40 mg/day treatment achieved 100% pregnancy. Intermediate values (66.67%) were observed in the 20, 80, and 160 mg/day treatments. Following the second mating, pregnancy rate reached 100% in all remaining groups, and no significant differences among treatments were detected ($p > 0.05$).

Table 2. Pregnancy rates of female rabbits after mating with males supplemented with *Cordyceps militaris*

Treatment group	First mating (%)	Second mating (%)
0 mg	33.33 ^c	100
20 mg	66.67 ^b	100
40 mg	100.00 ^a	Not applicable (all females pregnant after first mating)
80 mg	66.67 ^b	100
160 mg	66.67 ^b	100

^{a,b,c,d} Means within a column with different superscript letters differ significantly ($p < 0.05$)

Reproductive and offspring parameters

Table 3 summarizes the reproductive performance and early growth characteristics of the offspring. No significant differences were detected among the treatment groups for gestation length, litter size, litter birth weight, litter weight at two weeks, individual pup birth weight, or pup weight at two weeks ($p > 0.05$). Gestation length remained relatively stable across treatments, ranging from 30.1 to 31.3 days.

Although the differences were not statistically significant, numerical variations were observed among the supplemented groups in several reproductive and offspring growth parameters, including litter size, litter weight, and pup body weight ($p > 0.05$). Litter size increased from 3.67 kits in the control group to 6.67 kits in the 160 mg/day treatment. Similarly, supplemented groups showed a tendency toward higher litter birth weight and litter weight at two weeks of age, particularly at the higher supplementation levels. However, the increase in total litter weight was not consistently associated with higher individual pup weight. Pup weight at birth and at two weeks remained relatively similar among the control, 20 and 40 mg/day treatments, whereas lower values were observed in the 80 mg/day group.

Table 3. Reproductive performance and offspring development parameters in female rabbits mated with males treated with different doses of *Cordyceps militaris*

Parameter	Treatment				
	0 mg	20 mg	40 mg	80 mg	160 mg
Gestation length (days)	30.7 ± 0.6	30.7 ± 0.6	30.7 ± 0.6	31.3 ± 0.6	30.0 ± 1.0
Litter size (kits)	3.7 ± 0.6	4.0 ± 1.0	5.0 ± 2.6	6.0 ± 1.0	6.7 ± 2.5
Litter birth weight (g)	186 ± 43	207 ± 61	227 ± 101	260 ± 78	323 ± 121
Litter weight at 2 weeks (g)	367 ± 96	409 ± 121	474 ± 176	473 ± 128	590 ± 219
Pup weight at birth (g)	50.5 ± 5.9	51.3 ± 2.5	46.5 ± 5.6	42.9 ± 8.2	48.4 ± 5.4
Pup weight at 2 weeks (g)	100.2 ± 18.5	101.6 ± 4.9	99.7 ± 17.8	77.9 ± 10.9	88.6 ± 1.0

There were no significant differences between the treatments in reproductive performance and offspring development parameters ($p > 0.05$)

DISCUSSION

The improvement in semen quality observed following *C. militaris* supplementation is consistent with previous evidence that dietary antioxidants can enhance reproductive function in male rabbits. Vitamin E and selenium, for example, have been reported to improve sperm quality, antioxidant status, and fertility in rabbit bucks. Fadl et al. (2024) found that supplementation with vitamin E and selenium significantly improved fresh and post-thaw semen characteristics and increased conception and kindling rates in sub-fertile rabbits. Similarly, dietary organic selenium at 0.3 mg/kg dry matter improved sperm functional characteristics, total antioxidant capacity, DNA integrity, and fertility in rabbit bucks exposed to natural heat stress (Hosny et al., 2020). These findings are in agreement with the present results, in which *C. militaris*-supplemented rabbits showed improved sperm motility, viability, membrane and acrosome integrity, antioxidant capacity, and reduced DNA fragmentation compared with the control group during weeks 6–12.

Other natural antioxidant sources, including vitamin E and propolis, have also demonstrated beneficial effects on rabbit semen quality (Hashem et al., 2013). Dietary supplementation with vitamin E or propolis at 150 mg/kg diet for 10 consecutive weeks improved semen quality and oxidative status in rabbit bucks during the hot season (Hashem et al., 2013). In another study, dietary supplementation with vitamin E at 200 mg/kg diet, combined with vitamin C at 0.5 g/L drinking water, was provided for 120 days. The combination of these vitamins at supranutritional levels inhibited oxidative processes and improved the characteristics of fresh and stored rabbit semen (Castellini et al., 2003). More recently, *Origanum vulgare* essential oil, applied *in vitro* at a 10^{-2} dilution, was reported to improve sperm motility in rabbits and increase the proportions of sperm subpopulations characterized by high velocity and progressivity (Zarhouti et al., 2024). Taken together, these findings support the view that enhancement of antioxidant protection is a common mechanism through which nutritional and phytogetic additives may help preserve sperm function. The present study provides further evidence that *C. militaris* supplementation was associated with improvements in multiple indicators of sperm quality and a higher first-mating pregnancy rate.

However, the magnitude and nature of the response vary depending on the antioxidant interventions and experimental conditions (Fadl et al., 2024). Previous studies have demonstrated the beneficial effects of common antioxidants in rabbits, particularly under conditions inducing oxidative stress. For example, Hosny et al. (2020) reported that dietary supplementation with organic selenium at a dosage of 0.3 mg/kg dry matter for 12 consecutive weeks improved sperm functional parameters, plasma membrane and DNA integrity, antioxidant status, and reproductive performance in male rabbits exposed to natural heat stress. Similarly, a recent study on male rabbits with impaired fertility showed that combined supplementation with vitamin E (100 or 200 IU/head) and selenium (0.1 or 0.2 mg/kg body weight), respectively, once weekly for 8 consecutive weeks improved fresh and post-thaw semen quality and increased conception and kidding rates, with the higher supplementation level producing the most favorable responses (Fadl et al., 2024). The results reinforce the protective role of antioxidant supplementation in maintaining male reproductive function under conditions of oxidative stress or fertility impairment (Hosny et al., 2020; Fadl et al., 2024). In contrast, the current study was conducted on healthy male rabbits under standard experimental conditions, which may have resulted in a less pronounced response to nutrient supplementation. Furthermore, differences in supplementation strategies, dosages, and the physiological status of the animals among the cited studies make direct comparisons of efficacy difficult. In the present study, supplementation with 40 mg/day of *C. militaris* yielded positive responses (in terms of data) regarding certain semen parameters, although the differences between supplementation levels were not always statistically significant. Therefore, the current results should be viewed as evidence of the potential beneficial effects of *C. militaris* supplementation on semen quality, rather than as conclusive proof that this additive outperforms traditional antioxidants such as vitamin E or selenium.

The beneficial effects observed in the present study may be related to the bioactive compounds present in *C. militaris*, including cordycepin, adenosine, and polysaccharides. Among these constituents, *C. militaris* polysaccharides have been reported to possess antioxidant activity, while cordycepin and adenosine have also been identified as important bioactive constituents of this fungus (Jiang et al., 2011; Zhang et al., 2019; Gu et al., 2022). The favorable changes in sperm function observed in the present study may be linked to the biological effects of cordycepin. In experimental rats, cordycepin supplementation was associated with enhanced antioxidant defense and attenuation of age-related testicular dysfunction (Kopalli et al., 2019). Previous studies indicated that oral administration of cordycepin isolated from *C. militaris* at 5, 10, and 20 mg/kg body weight/day for six months attenuated testicular inflammation in naturally aged rats, with the effects associated with modulation of the NF- κ B/MAPK signaling pathway (Kopalli et al., 2022). Therefore, the increased antioxidant capacity and reduced DNA fragmentation observed in supplemented rabbits in the present study may be associated with the protective effects of *C. militaris*-derived bioactive compounds against oxidative damage in sperm cells (Jiang et al., 2011; Zhang et al., 2019; Gu et al., 2022). Previous studies have also reported beneficial effects of *C. militaris* on antioxidant status and male reproductive function in several animal species.

In male Sprague–Dawley rats, dietary supplementation with *C. militaris* mycelium at 1% or 5% for 6 weeks increased epididymal sperm count and sperm motility, with the effects becoming evident after 5 weeks of supplementation (Chang et al., 2008). Similarly, in juvenile male mice, dietary administration of different *C. militaris* fermentation products at 5% of the diet for 28 days increased sperm concentration and the proportion of motile sperm in several treatment groups, while some fermentation products also promoted testicular development and testosterone synthesis (Lin et al., 2022). These findings were broadly consistent with the present study in male rabbits, in which dietary supplementation with *C. militaris* extract was associated with improvements in sperm motility, viability, plasma membrane and acrosome integrity, antioxidant capacity, and DNA integrity. However, the present study differs from previous studies in both animal species and supplementation strategy. Whereas Chang et al. (2008) and Lin et al. (2022) evaluated *C. militaris* mycelium or fermentation products in rats and mice, respectively, the present study evaluated a standardized *C. militaris* extract in adult male rabbits at doses of 20, 40, 80, and 160 mg/day over a longer 14-week experimental period. In addition, the present study assessed a broader range of sperm functional parameters and reproductive outcomes, including pregnancy rate following natural mating. These differences suggest that the reproductive effects of *C. militaris* may depend on the animal species, form of supplementation, dosage, and duration of administration.

The higher first-mating pregnancy rate observed in the present study is consistent with previous evidence that improving semen quality through nutritional supplementation can enhance reproductive performance in male rabbits. Fadl et al. (2024) reported that repeated intramuscular administration of vitamin E (100 or 200 IU/head) combined with selenium (0.1 or 0.2 mg/kg body weight, respectively) once weekly for 8 consecutive weeks improved sperm characteristics and increased conception and kindling rates in sub-fertile rabbit bucks. Similarly, Hosny et al. (2020) found that dietary supplementation with organic selenium at 0.3 mg/kg dry matter for 12 consecutive weeks (84 days) improved semen quality, DNA integrity, antioxidant status, and fertility in rabbit bucks exposed to natural heat stress. The reproductive response observed following *C. militaris* supplementation may therefore be associated with the improvement in sperm functional characteristics and antioxidant status observed in the present study. In contrast, the absence of significant effects on litter size and early offspring growth differs from the fertility-related improvements reported in some antioxidant supplementation studies. Such differences may be related to variation in animal physiological status, supplementation strategy, experimental duration, and sample size among studies (Hosny et al., 2020; Fadl et al., 2024).

CONCLUSION

The findings of the current study indicated that dietary *C. militaris* supplementation, particularly at 40 mg/day, has the potential to improve semen quality and reproductive performance in rabbit bucks. The relatively low sample size and limited physiological measurements represent important limitations of the present study. Further studies should determine the optimal supplementation dose, duration and clarify the underlying physiological mechanisms.

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Authors' contributions

Bui Le Khanh Ngoc, Cao Quoc Huy, and Le Hoang Kim Lan contributed to the conception, design, and methodology. Le Hoang Phuong Anh, Nguyen Luong Hao, and Nguyen Lam Khanh Duy have participated in the investigation, acquisition, analysis, and interpretation of data. Nguyen Thi Lien Thuong has contributed to methodology. Nguyen Trong Hong Phuc and Tran Thi Thanh Khuong conducted the funding acquisition, writing-review, editing, supervision, and project administration. All authors have read and approved the final edition of the manuscript before publication in the present journal.

Availability of data and materials

All data supporting the findings of the present study are available within the manuscript upon reasonable request from the corresponding author.

Ethical considerations

All authors confirmed that the present study is an original article, has not been published previously, and is not under consideration for publication elsewhere. ChatGPT (OpenAI) was used as an artificial intelligence (AI) assistance tool during the preparation and revision of the manuscript, mainly for language editing, grammar checking, and

improving the clarity and readability of the text. No AI tools were used to generate, analyze, or interpret the experimental data. All AI-assisted revisions were carefully reviewed and approved by the authors, who take full responsibility for the accuracy, originality, scientific content, and integrity of the final manuscript.

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Competing interests

The authors declare no conflict of interest.

REFERENCES

- Absalan F, Ghannadi A, Kazerooni M, Parifar R, Jamalzadeh F, and Amiri S (2012). Value of sperm chromatin dispersion test in couples with unexplained recurrent abortion. *Journal of Assisted Reproduction and Genetics*, 29(1): 11-14. DOI: <https://www.doi.org/10.1007/s10815-011-9647-0>
- Agarwal A, Virk G, Ong C, and du Plessis SS (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health*, 32(1): 1-17. DOI: <https://www.doi.org/10.5534/wjmh.2014.32.1.1>
- Boontiam W, Wachirapakorn C, and Wattanachai S (2020). Growth performance and hematological changes in growing pigs treated with *Cordyceps militaris* spent mushroom substrate. *Veterinary World*, 13(4): 768-773. DOI: <https://www.doi.org/10.14202/vetworld.2020.768-773>
- Brun JM, Theau-Clément M, Bolet G, Esparbié J, and Falières J (2016). Genetic parameters of rabbit semen traits and male fertilising ability. *Animal Reproduction Science*, 166: 15-21. DOI: <https://www.doi.org/10.1016/j.anireprosci.2015.12.008>
- Castellini C, Lattaioli P, Dal Bosco A, Minelli A, and Mugnai C (2003). Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Reproduction, Nutrition, Development*, 43(1): 91-103. DOI: <https://www.doi.org/10.1051/rnd:2003008>
- Chang Y, Jeng KC, Huang KF, Lee YC, Hou CW, Chen KH, Cheng FY, Liao JW, and Chen YS (2008). Effect of *Cordyceps militaris* supplementation on sperm production, sperm motility and hormones in Sprague-Dawley rats. *The American Journal of Chinese Medicine*, 36(5): 849-859. DOI: <https://www.doi.org/10.1142/S0192415X08006296>
- Fadl AM, Samir H, and Shahat AM (2024). Repeated injections of vitamin E and Se improves testicular morphology, testosterone and *in vitro* and *in vivo* sperm fertility in subfertile rabbits. *Veterinary Research Communications*, 48(5): 3157-3166. DOI: <https://www.doi.org/10.1007/s11259-024-10439-4>
- Gašperlin L, Polak T, Rajar A, Skvarča M, and Zlender B (2006). Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. *World Rabbit Science*, 14(3): 157-166. DOI: <https://www.doi.org/10.4995/wrs.2006.558>
- Gu CM, Zhang DB, Zhai WJ, Zhang HP, Wang S, Lv SY, Bao YX, Zhu DZ, Feng SC, and Guo SF (2022). Research progress on *Cordyceps militaris* polysaccharides. *Food Bioscience*, 45: 101503. DOI: <https://www.doi.org/10.1016/j.fbio.2021.101503>
- Hashem NM, Abd El-Hady A, and Hassan O (2013). Effect of vitamin E or propolis supplementation on semen quality, oxidative status and hemato-biochemical changes of rabbit bucks during hot season. *Livestock Science*, 157(2-3): 520-526. DOI: <https://www.doi.org/10.1016/j.livsci.2013.09.003>
- Hosny NS, Hashem NM, Morsy AS, and Abo-Elezz ZR (2020). Effects of organic selenium on the physiological response, blood metabolites, redox status, semen quality, and fertility of rabbit bucks kept under natural heat stress conditions. *Frontiers in Veterinary Science*, 7: 290. DOI: <https://www.doi.org/10.3389/fvets.2020.00290>
- Jędręko KJ, Lazur J, and Muszyńska B (2021). *Cordyceps militaris*: An overview of its chemical constituents in relation to biological activity. *Foods*, 10(11): 2634. DOI: <https://www.doi.org/10.3390/foods10112634>
- Jiang Y, Wong JH, Fu M, Ng TB, Liu ZK, Wang CR, Li N, Qiao WT, Wen TY, and Liu F (2011). Isolation of adenosine, iso-sinensetin and dimethylguanosine with antioxidant and HIV-1 protease inhibiting activities from fruiting bodies of *Cordyceps militaris*. *Phytomedicine*, 18(2-3): 189-193. DOI: <https://www.doi.org/10.1016/j.phymed.2010.04.010>
- Kopalli SR, Cha KM, Lee SH, Hwang SY, Lee YJ, Koppula S, and Kim SK (2019). Cordycepin, an active constituent of nutrient powerhouse and potential medicinal mushroom *Cordyceps militaris* Linn., ameliorates age-related testicular dysfunction in rats. *Nutrients*, 11(4): 906. DOI: <https://www.doi.org/10.3390/nu11040906>
- Kopalli SR, Cha KM, Cho JY, Kim SK, and Koppula S (2022). Cordycepin from medicinal fungi *Cordyceps militaris* mitigates inflammaging-associated testicular damage via regulating NF-κB/MAPKs signaling in naturally aged rats. *Mycobiology*, 50(1): 86-95. DOI: <https://www.doi.org/10.1080/12298093.2022.2035515>
- Lin S, Hsu WK, Tsai MS, Hsu TH, Lin TC, Su HL, and Jin D (2022). Effects of *Cordyceps militaris* fermentation products on reproductive development in juvenile male mice. *Scientific Reports*, 12: 13720. DOI: <https://www.doi.org/10.1038/s41598-022-18066-2>
- Manuelian CL, Pitino R, Simoni M, Mavrommatis A, De Marchi M, Righi F, and Tsiplakou E (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on livestock mammals' performances, health, and oxidative status: A review of the literature in the last 20 years. *Antioxidants*, 10(9): 1461. DOI: <https://www.doi.org/10.3390/antiox10091461>
- Miao M, Yu WQ, Li Y, Sun YL, and Guo SD (2022). Structural elucidation and activities of *Cordyceps militaris*-derived polysaccharides: A review. *Frontiers in Nutrition*, 9: 898674. DOI: <https://www.doi.org/10.3389/fnut.2022.898674>
- Morshedy SA, Zahran SM, Sabir SA, and El-Gindy YM (2022). Effects of increasing levels of orange peel extract on kit growth, feed utilization, and some blood metabolites in the doe rabbits under heat stress conditions. *Animal Biotechnology*, 34(4): 1532-1543. DOI: <https://www.doi.org/10.1080/10495398.2022.2038615>
- Nenadis N, Wang LF, Tsimidou M, and Zhang HY (2004). Estimation of scavenging activity of phenolic compounds using the ABTS assay. *Journal of Agricultural and Food Chemistry*, 52(15): 4669-4674. DOI: <https://www.doi.org/10.1021/jf0400056>
- Nguyen KD, Nguyen CM, Le DA, Huynh HT, Tran MT, Truong ATN, Nguyen THD, Vu DC, and Nguyen LTT (2024). The mixture of *Ganoderma lucidum* and *Cordyceps militaris*: Chemical composition and protective effect against oxidative stress. *Journal of Agriculture and Food Research*, 15: 101045. DOI: <https://www.doi.org/10.1016/j.jafr.2024.101045>
- Nguyen LKD, Nguyen TDH, and Tran TTK (2025). Optimization of glycerol and egg yolk concentrations for cryopreservation of Saanen buck sperm under tropical conditions in the Mekong Delta, Vietnam. *Veterinary World*, 18(11): 3631-3639. DOI:

<https://www.doi.org/10.14202/vetworld.2025.3631-3639>

- Phull AR, Ahmed M, and Park HJ (2022). *Cordyceps militaris* as a bio functional food source: Pharmacological potential, anti-inflammatory actions and related molecular mechanisms. *Microorganisms*, 10(2): 405. DOI: <https://www.doi.org/10.3390/microorganisms10020405>
- Siddiqui SA, Adli DN, Nugraha WS, Yudhistira B, Lavrentev FV, Shityakov S, Feng X, Nagdalian A, and Ibrahim SA (2024). Social, ethical, environmental, economic and technological aspects of rabbit meat production: A critical review. *Heliyon*, 10(8): e29635. DOI: <https://www.doi.org/10.1016/j.heliyon.2024.e29635>
- Sies H (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology*, 11: 613-619. DOI: <https://www.doi.org/10.1016/j.redox.2016.12.035>
- Tran TTK, Chiem TN, Tran VBN, and Duong NDT (2023). Cryopreservation of local black rabbit spermatozoa in the Mekong delta region of Vietnam: Evaluating cryoprotectant effectiveness. *Reproduction in Domestic Animals*, 58(8): 1139-1145. DOI: <https://www.doi.org/10.1111/rda.14415>
- Tran KTT, Nguyen TN, and Nguyen DLK (2025). Developing a simple universal hypo-osmotic swelling test (HOST) for assessing sperm membrane integrity in pigs, rabbits, and goats. *Journal of Advanced Veterinary and Animal Research*, 12(4): 477-486. DOI: <https://www.doi.org/10.5455/javar.2025.1913>
- Wang L, Yan H, Zeng B, and Hu Z (2022). Research progress on cordycepin synthesis and methods for enhancement of cordycepin production in *Cordyceps militaris*. *Bioengineering*, 9(2): 69. DOI: <https://www.doi.org/10.3390/bioengineering9020069>
- Yousef MI, Abdallah GA, and Kamel KI (2003). Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Science*, 76(1-2): 99-111. DOI: [https://www.doi.org/10.1016/S0378-4320\(02\)00226-9](https://www.doi.org/10.1016/S0378-4320(02)00226-9)
- Zarhouti A, Sakoui S, El Khalfi B, Mbaye MM, Yañez Ramil U, Quintela Arias LA, Becerra González JJ, García Herradón P, and Soukri A (2024). The impact of *Origanum vulgare* essential oil supplementation on sperm motility and subpopulation alterations in bulls, dogs, and rabbits. *Research in Veterinary Science*, 170: 105200. DOI: <https://www.doi.org/10.1016/j.rvsc.2024.105200>
- Zhang J, Wen C, Duan Y, Zhang H, and Ma H (2019). Advance in *Cordyceps militaris* (Linn) Link polysaccharides: Isolation, structure, and bioactivities: A review. *International Journal of Biological Macromolecules*, 132: 906-914. DOI: <https://www.doi.org/10.1016/j.ijbiomac.2019.04.020>
- Zheng H, Cao H, Zhang D, Huang J, Li J, Wang S, Lu J, Li X, Yang G, and Shi X (2022). *Cordyceps militaris* modulates intestinal barrier function and gut microbiota in a pig model. *Frontiers in Microbiology*, 13: 810230. DOI: <https://www.doi.org/10.3389/fmicb.2022.810230>

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