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Effects of Black Seed (*Nigella sativa*) Powder on Semen Parameters in Japanese quail (*Coturnix japonica*)

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Dedication

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For my perseverance, patience, and strength.

This work is the result of my dedication.

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Abstract

The aim of this study was to assess the positive impact of dietary supplementation with *Nigella sativa* powder on semen parameters in quails. A total of 200 Japanese quails (*Coturnix japonica*) at one week of age, the subjects were randomly assigned to two groups: a control group (C) contains 100 subjects, which received a standard commercial chicken diet, and an experimental group (E) contains 100 subjects, which received the same diet supplemented with 2% *Nigella sativa* powder. At 13 weeks old, 12 quails from each group were sacrificed, and an incision was made on the left side to extract the testis-epididymis complex along with the vas deferens for semen collection. Semen analysis focused on evaluating sperm vitality, morphology, and concentration. The results showed that group E had a higher percentage of sperm vitality compared to group C, although the difference was not statistically significant (6.46%; $P > 0.05$). No significant difference was observed between the two groups regarding total sperm abnormalities ($P > 0.05$), although a non-significant reduction ($P > 0.05$) in head (47.88%) and tail abnormalities (8.29%) was found in group E. Sperm concentration was significantly higher (12.24%; $P < 0.05$) in group C than in group E. In conclusion, dietary supplementation with *Nigella sativa* seed powder has a beneficial effect on certain semen quality parameters, particularly an increase in vitality and a reduction in abnormal spermatozoa in quail.

Keywords : Japanese quail, semen parameters, vas deferens, *Nigella sativa*

Résumé

L'objectif de cette étude était d'évaluer l'impact positive de la supplémentation alimentaire en poudre de graines *Nigella sativa* sur les paramètres du sperme chez les cailles. Un total de 200 cailles japonaises (*Coturnix japonica*) âgées d'une semaine ont été réparties aléatoirement en deux groupes : un groupe contrôle (C) composé de 100 sujets recevant un aliment commercial standard pour poulet et un groupe expérimental (E) composé de 100 sujets recevant le même aliment supplémenté avec 2 % de poudre de graines *Nigella sativa*. À l'âge de 13 semaines, 12 cailles de chaque lots ont été sacrifiées et une incision a été pratiquée du côté gauche pour extraire le complexe testicule–épididyme ainsi que le canal déférent en vue du prélèvement de sperme. L'analyse du sperme a porté sur l'évaluation de la vitalité, de la morphologie et de la concentration des spermatozoïdes. Les résultats ont montré que le E présente un pourcentage de vitalité des spermatozoides plus élevé que C, avec une différence non significative (6,46% ; $P > 0,05$). Aucune différence significative n'a été observée entre les deux groupes en ce qui concerne les anomalies spermatiques totales ($P > 0,05$), bien qu'une réduction non significative ($P > 0,05$) des anomalies de la tête (47.88%) et du flagelle (8.29%) a été retrouvé dans le E. La concentration spermatique est significativement plus élevée (12,24 % ; $P < 0,05$) dans groupe C que le E. En conclusion, la supplémentation alimentaire en poudre de garines *Nigella sativa* a un effet bénéfique sur certains paramètres de la qualité du sperme, en particulier une augmentation de la vitalité et une réduction des spermatozoïdes anormaux chez la caille.

Mot-clé : Caille japonaise, paramètres spermatiques, poudre des graines de Nigelle (*Nigella sativa),* canal dèferent.

الملخّص

يهدف هذا البحث الى تقيم تأثيرالاجابي لمسحوق بذور الحبة السوداء الى الغذاء على خصائص السائل المنوي لدى السمان. تم استخدام 200 طائر من السمان الياباني بعمر أسبوع واحد حيث تم توزيعها عشوائيا الى مجموعتين: مجموعة شاهدة: تحتوي على 100 طائر، تلقت علف تجاري من نوع الدواجن.

مجموعة تجريبية: تحتوي أيضا على 100 طائر تلقت نفس العلف مضافا اليه 2 % من مسحوق بذور الحبة السوداء

عند عمر 13 أسبوع تم ذبح **12**طير حيث قمنا بإجراء شق في الجانب الايسر من اجل استخراج الخصية والبربخ بإضافة الى استخراج قناة نقل السائل المنوي وتم إجراء شق عميق في الجزء الذّيلي من القناة الناقلة باستخدام مشرط لتحرير الحيوانات المنوية، لتحليل السائل المنوي من اجل تقييم الحيوية و الشكل و تركيز الحيوانات المنوية

أظهرت النتائج أن المجموعة **E** سجلت نسبة اعلى من حيوية الحيوانات المنوية مقارنة بالمجموعة **C** لكن الفرق لم يكن معنويًا إحصائيًا ($P>0.05$; 6.46%) حيث لم نلاحظ أي فرق معنوي في نسبة التشوهات الحيوانات المنوية بين

المجموعتين ($P>0.05$) رغم ذلك تم تسجيل انخفاض في تشوهات الرأس (47.88%) والذيل (8.29%) في

الحيوانات المنوية لدى المجموعة التجريبية مقارنة بالمجموعة الشاهدة ومن ناحية أخرى سجلت المجموعة الشاهدة تركيزًا أعلى من الحيوانات المنوية بشكل معنوي(12.24% ; $P<0.05$). وبالنتيجة، فإن المكمل الغذائي من مسحوق بذور الحبة السوداء أظهر تأثيرًا إيجابيًا على بعض معايير جودة السائل المنوي، خاصةً من خلال زيادة الحيوية وتقليل نسبة الحيوانات المنوية غير الطبيعية لدى طيور السمان.

الكلمات المفتاحية: السمان الياباني، خصائص السائل المنوي، مسحوق بذور الحبة السوداء، قناة نقل السائل المنوي

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Introduction

Introduction

People first domesticated the Japanese quail around the 11th century as a pet, primarily for its song (Kayang et al., 2004). Over time, it gained economic importance in farming as a species valued for its meat and egg production, both appreciated for their flavour (Kayang et al., 2004). In addition to its economic value, the Japanese quail (*Coturnix japonica*) has been widely used as an animal model in scientific research, including studies in genetics, nutrition, toxicology, embryology, physiology, and pathology (Huss et al., 2008; Baer et al., 2015).

This bird exhibits rapid growth, early sexual maturity, a high egg-laying rate, a short generation interval, and a shorter egg incubation period compared to chickens (Kaur et al., 2008). In Algeria, quail farming has experienced significant growth in recent years (Touahri et al., 2024), due in part to its less demanding husbandry requirements and significantly lower investment costs compared to other types of poultry farming. This activity has especially expanded in response to the increasing demand from restaurants and hotels for quail meat (Berrama et al., 2011).

In recent decades, there has been renewed interest in the use of traditional medicinal plants as alternatives to antibiotics or as natural feed additives to enhance animal productivity (Hashemi and Davoodi, 2011). These plants, used to base on cultural and religious traditions have attracted attention due to factors such as their low cost, easy accessibility, and fewer side effects compared to synthetic drugs. Scientific experiments conducted since the late 19th century have highlighted the antimicrobial properties of certain plants and their bioactive components (Thakur et al., 2021).

Various cultures have used black seed *(Nigella sativa)* for centuries, particularly in Muslim communities, to treat a wide range of ailments (Hosseinzadeh et al., 2007). Researchers attribute much of the plant's biological activity to thymoquinone (TQ) , its key compound (Sadeghi et al., 2023). Studies have shown that this substance possesses antioxidant, hepatoprotective, antibacterial, antidiabetic properties, and enhances immunity (Kooti et al., 2016; Hannan et al., 2021; Thakur et al., 2021).

The present study aims to investigate the effect of incorporating 2% black seed (*Nigella sativa*) powder into the basic diet on sperm parameters of vas defrence.

This work is divided into two main parts :

- **A bibliographic section :** the first chapter presents the morphological, biological, and husbandry characteristics of the Japanese quail, and the second chapter presents current knowledge on the morphological and phytochemical characteristics as well as the pharmacological properties of *Nigella sativa* and the third chapter presents the parametrs of semen analysis.
- **An experimental section :** which focuses on evaluating the effect of dietary supplementation with *Nigella sativa* seed powder on sperm parameters.

Bibliographic Section

Chapter I: The Japanese Quail (*Coturnix japonica*)

I. Domestique quail

I.1. Historical context

The earliest known representation of the quail appears in Egyptian hieroglyphics dating back to 2000 BC, where the small bird symbolizes the letter "W" in the alphabet (Shanaway, 1994). In general, the Japanese quail was first domesticated in Japan around the 11th century as a companion animal, particularly valued for its song (Tsudzuki, 2008). By the 2000s, commercial quail production varied by country : Asia (e.g., China and Japan) and Brazil focused primarily on egg production, while Europe (e.g., Spain and France) and the United States prioritized meat production. Although the quail industry is a relatively small segment of overall animal production, quail farming significantly contributes to the supply of eggs and meat in several countries (Minivielle, 2004).

I.2. Taxonomic classification

The taxonomic classification of the Japanese quail *(Coturnix japonica)* is presented in the table 1.

Table 1 : Taxonomic classification of quails (Shanaway, 1994)

Kingdome :	Animal
Phylum :	Chordate
Sub-phylum :	vertebrata
Class :	Avis
Order :	Galliformes
Sub-order :	Galli
Family :	Phasianidae
Sub- family :	Perdicinae
Genus :	*Coturnix*

Based on the classification system established by the International Ornithological Congress (Version 3.01.2012), the most recognized species of quail within the Coturnix genus include :

• Japanese quail *(Coturnix japonica*)
• Harlequin quail *(Coturnix delegorguei)* (Figure 1)
• Common quail *(Coturnix coturnix)* (Figure 2)
• Stable quail *(Coturnix pectoralis)* (Figure 3)
• Blue-breasted quail (king quail) *(Coturnix coromandelica)* (Figure 4)
• Tasmanien quail *(Coturnix ypsilophora)* (Figure 5)
• New Zealand quail *(Coturnix novaezelandiae)*, which has been extinct since 1875.

The classification of the Japanese quail has been the subject of significant debate and confusion. It was once regarded by many researchers as a subspecies of the common quail *(Coturnix coturnix)* and was referred to as *Coturnix coturnix japonica*. However, later taxonomic research provided strong evidence that the Japanese quail and the common quail are in fact separate and distinct species (Mills et al.,1997).

Figure 1 : Harlequin quail (Philip et al., 2021)

Figure 2 : Common quail (Varesvuo, 2016)

Figure 3 : Stable quail (Roderick, 2023)

Figure 4 : Blue-breasted (Philip et al., 2021)

Figure 5 : Tasmanian quail (Murray, 2018)

II. Description of the Japanese quail

II.1. Morphology

The Japanese quail *(Coturnix japonica)* (Figure 6) exhibits sexual dimorphism in both size and weight. On average, individuals reach approximately 17 cm in length, with males being slightly smaller than females. In the wild, adult females typically weigh around 100 g, while males average approximately 90 g (Kawahara and Saito, 1967). However, domestication and selective breeding for production purposes have led to increased body mass, with domesticated females reaching about 140 g and males around 130 g (Gerken and Mills, 1993) . It exhibits a short, robust beak with the lower mandibles presenting slight serrations, which may assist in food manipulation. The wings are relatively short but well-muscled, adapted for brief and low-altitude flight. The tail is markedly short and exhibits minimal sexual dimorphism (Menassé et al., 1986). The pelvic limbs are well-developed and display a greyish-orange pigmentation. Each foot bears three anterior digits, interconnected at the base by a fine interdigital membrane, and a reduced posterior digit (hallux) that remains free and non-functional in perching (Laroche et al., 1990).

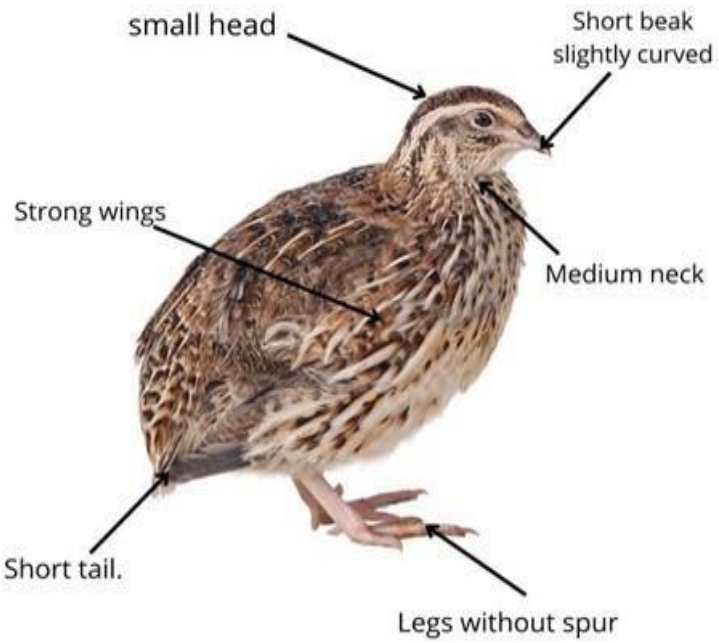


Figure 6 : External morphology of the Japanese quail (Modified personal photo)

II.2. Sexing

Sexing of Japanese quail can start within one day of hatching through cloacal examination, although accuracy in performing this technique may take years to develop. By 4-6 weeks post-hatch, males and females are sexually dimorphic and can be differentiated based on plumage coloration (Figure 7), with females having light tan feathers and black speckling on their chest and throat and males characterized by a rusty brown throat and breast feathers (Baer et al., 2015).

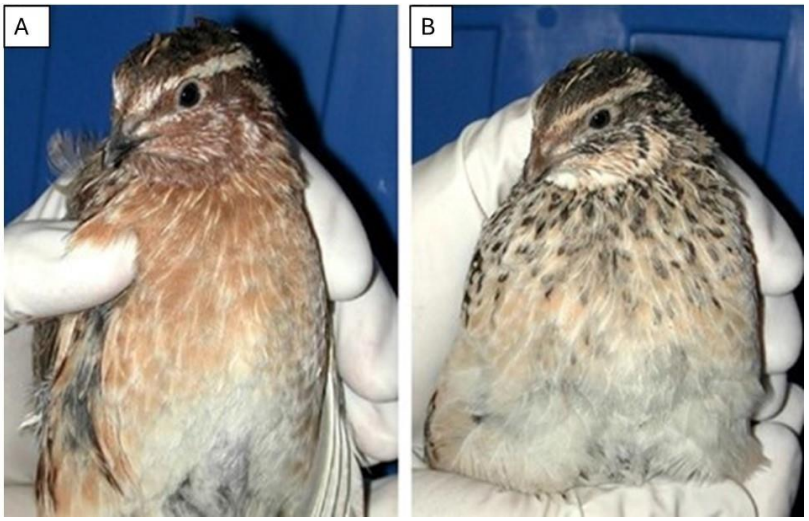


Figure 7 : Plumage of an adult Japanese quail (*Coturnix japonica)*, male (A) and female (B) (Baer et al., 2015)

II.3. Morphology of the male reproductive system

The male reproductive system of the quail is composed of the testes, vas deferens, and the cloaca (Figure 8) (Sasanami et al., 2015).

- **Testes :** These are located inside the body. Their function is to produce sperm as well as sex hormones, such as testosterone.
- **Epididymis :** In the Japanese quail (*Coturnix japonica*), the epididymis is located on the dorsomedial surface of the testis. It is continuous with the ductus deferens, which runs ventral to the epididymis (Shahad et al., 2018). its main role is to increase sperm motility (Brett et al., 2014).
- **Deferent duct or Vas deferens :** These ducts transport sperm from the testes to the cloaca in preparation for ejaculation during mating.
- **Cloaca** : The avian cloaca is a common cavity of the digestive, urinary, and genital system having complex structure which consisting of three compartments : coprodeum, urodeum and proctodeum (Joshi et al., 2019).

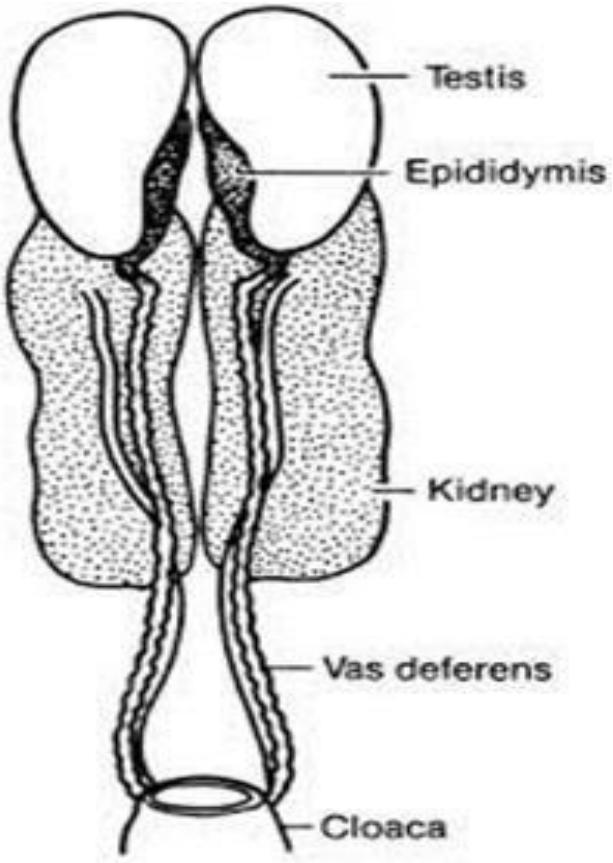


Figure 8 : Morphology of male reproductive system in bird (Parkhurst et al., 1988)

III. Reproductive physiology

The Japanese quail *(Coturnix japonica)*, serve as valuable models for studying avian reproductive behavior due to their rapid maturation, high fertility, and well-documented mating patterns (Padgett and Ivey, 1959). Their reproductive strategies encompass intricate hormonal regulation, social interactions, and environmental responsiveness (Egbuniwe and oguejifor, 2024). The Japanese quails reach sexual maturity between 6 to 8 weeks of age (Huss et al., 2008). In natural settings, breeding is influenced by photoperiod and temperature, with peak activity during spring and summer (Sharp and Peter, 2005).

III.1. Sexual dimorphism

Male and female Japanese quails show clear sexual dimorphism in behavior. Males are more active in courtship and copulatory behaviors (Mills et al., 1997).

III.2. Male copulatory behaviors

Sexual behavior in Japanese quail has been extensively studied and is characterized by a stereotyped sequence of actions performed by the male. This sequence typically includes a neck grab, mounting, a cloacal contact movement during which the male arches his back and positions his cloaca in alignment with that of the female and finally, cloacal contact itself (Noble, 1973). Successful insemination can often be identified by the presence of a distinctive foam in the female's cloaca, which is transferred along with the semen during copulation (Adkins-Regan, 1974). Male quails also produce vocalizations, which are generally emitted in the absence of females (Potash, 1975). Interestingly, these vocal signals have been shown to exert physiological effects on females, such as increasing oviduct weight, suggesting a potential role in reproductive priming (Guyomarc'h et al., 1984).

III.3. Female responses

The role of the female in quail mating behavior is crucial, though less overt than that of the male. Upon a male's neck grab and mounting attempt, the female may adopt a receptive posture by squatting and maintaining a horizontal back, thereby facilitating copulation. Alternatively, she may resist by standing upright and moving away, which destabilizes the male and limits his access to her cloaca (Adkins-Regan, 1999). Additionally, post-copulatory female control over fertilization has been proposed. Fertilization success appears to decline

when the female initially avoids the male's approach (Adkins-Regan, 1995) or when the male displays aggressive behavior during mating (Persaud and Galef, 2005).

III.4. Motivation and condition

The frequency and intensity of these behaviors are influenced by the body condition and motivation of the birds, especially in males. Well-conditioned males tend to show more frequent and vigorous copulatory behaviors (Correa et al., 2011).

III.5. Hormonal factor

Testosterone plays a critical role in driving male sexual behaviors. The presence of females and environmental cues can stimulate hormonal changes and increase sexual activity (Correa et al., 2011).

III.6. Reproductive success

Successful copulation typically involves all stages of the male's behavioral sequence and a cooperative response from the female. Mating success is linked to reproductive fitness and body condition *(*Correa et al., 2011).

IV. Breeding conditions

The parameters of the breeding condition are the following :

IV.1. Temperature

Temperature is critical for one-day-old quail chicks due to their sensitivity to cold. The thermal neutral zone ranges from 35°C to 37°C. Brooding should start at 35°C for the first three days, then decrease by about 1°C every two days until reaching 21–23°C by three weeks of age. Chick behavior is the best indicator for adjusting temperature. Inadequate heat early on can significantly raise mortality (Shanaway, 1994).

IV.2. Humidity

Maintaining appropriate humidity levels is crucial for the health of quail chicks. Both insufficient and excessive humidity, as well as abrupt changes, can disrupt feather development and increase the risk of respiratory problems as the chicks grow. To ensure optimal rearing conditions, it is recommended to keep the relative humidity around 70% (Shanaway, 1994).

IV.3. Light

Lighting is essential for quail growth and productivity. Chicks need continuous light (24h/day) for the first two weeks. Meat quail benefit from 23 hours of light and 1 hour of darkness, or intermittent lighting, to boost weight gain. Layers require 14–16 hours of light daily for optimal egg production. Light intensity should be about 20 lux for chicks and reduced to 5 lux for adult layers to support reproduction and minimize stress (Shanaway, 1994).

IV.3. Nutrition

Proper nutrition is vital for quail growth, reproduction, and egg production. Layers and breeders need 3.2% calcium for eggshells and 0.42% phosphorus for metabolism. Energy requirements increase with age: 12.1 MJ ME/kg in the first 2 weeks, 12.5 MJ from weeks 2–4, and 12.9 MJ from weeks 4–6. Layers need 10.9–12.1 MJ ME/kg, while breeders require 12.2–12.7 MJ. Protein intake should start at 270 g/kg feed (0–2 weeks), drop to 230 g/kg (2–4 weeks), and reach 200 g/kg (4–6 weeks). Adults need 18–21% crude protein for layers and 23–24% for breeders (Shanaway, 1994).

Chapter II:

Nigella sativa

I. History of black seeds

An asian middle eastern native angiosperm belonging to the Ranunculaceae family (Ahmad et al.,2013). Even before the advent of modern medicine, this miracle herb was being used in folkloric traditional medicine across the globe for treatments of all kinds of ailments and diseases (Alberts et al., 2024). Ancient medicinal traditions, unlike modern allopathic medicine observed health of an individual in a holistic manner as a culmination of mind, body, soul, and nature (Alberts et al., 2024). Black cumin and black seed or habatus sauda and حبة البركة are other alternate names of Nigella seeds and have been extensively used as medicines in all Abrahamic cultures. It has been extensively utilized for the treatment of liver, lung, kidney, gastric, and psychological disorders (Selon and Blunden, 2003).

The Prophet Muhammad ﷺ said: "In the black seed, there is a cure for every disease, except death." (Abu Huraira AR. Hadith 592. Sahih Bukhari Book 71. 850;7.)

Ibn Sina recommended black seed for treating fever, wounds, skin diseases, and bites or stings from venomous animals (Thakur et al., 2021).

II. Taxonomic classification

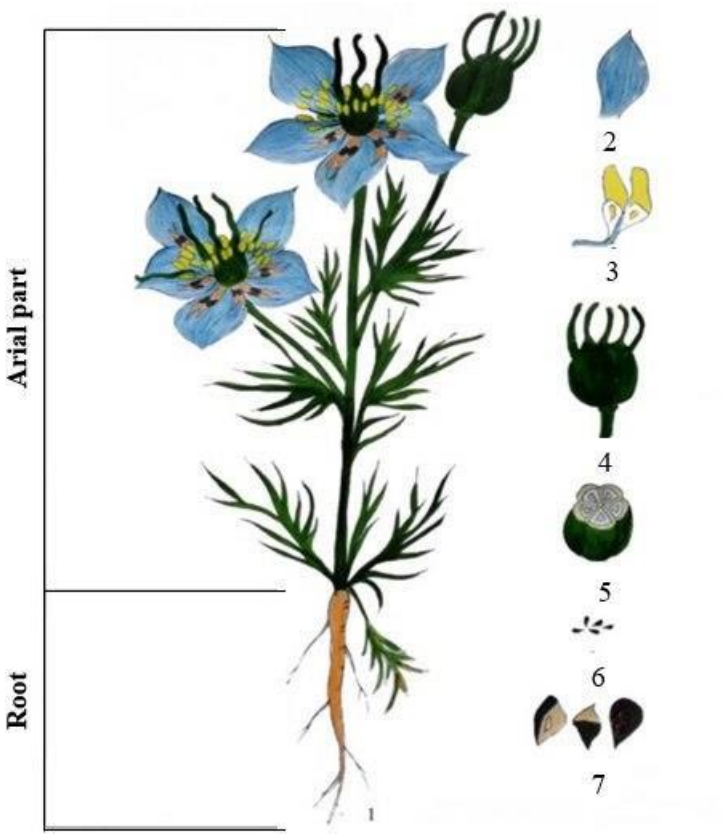
The taxonomic classification of the *Nigella Sativa* is presented in the table 2.

Table 2 : Taxonomic classification of *Nigella sativa* (Ijaz et al., 2017)

Kingdom :	Plantae
Subkingdom :	Tracheobionata
Supervision :	Spermatophyte
Order :	Ranunculales
Family :	Ranunculaceae Butter cup family
Genera :	*Nigella*
Specie :	*sativa*

III. Plant morphology

The *Nigella sativa* is a self-pollinating annual herb that typically reaches a height of about 60 cm (Figure 9). It has upright, branched stems that become hollow as the plant matures, with a colour ranging from light to dark green (Dalli et al., 2021). The flowers are solitary and may appear bluish-white, pale yellow, or whitish. The terminal fruit is a capsule composed of multiple compartments (locules), with a pocket-like epicalyx present beneath the floral structure (Ijaz et al., 2016). The seeds are small, black, dicotyledonous, and trigonous in shape, featuring a rough, tuberculate (rugulose) surface (Jabbar et al., 2006).



1- Whole plant 2- Petal 3- Stamens 4- capsule 5- Transverse section of capsule 6-Seeds (top view) 7- Seeds (cut view)

Figure 9 : Morphology of *Nigella sativa* (Abou-Zeid et al., 2022)

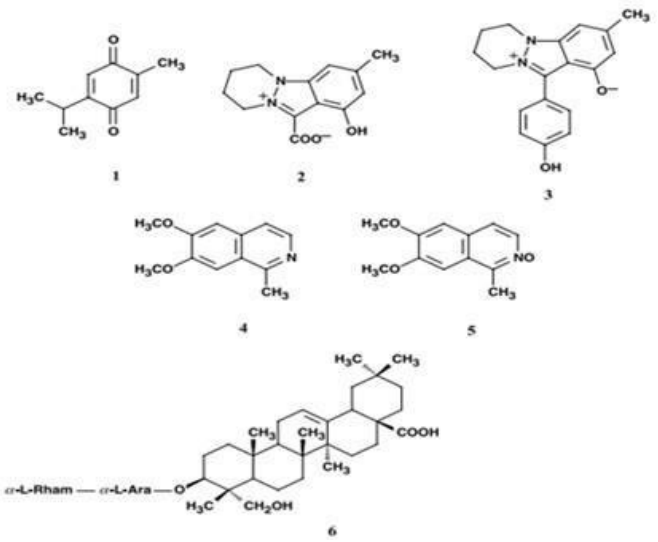
IV. Chemical composition

Nigella sativa seeds contain a wide range of bioactive compounds as shown in Table 3 (Tembhurne et al., 2014).

The most active constituents of *N. sativa* (Figure 10) are thymohydroquinone, pcymene, dithymoquinone, thymoquinone, carvacrol, sesquiterpene longifolene. Black seed of *N. sativa* contains alkaloids like nigellicimine, isoquinoline, pyrazol and nigellicimine-N-oxide (Tiwari et al., 2019).

Table 3 : Chemical composition of *Nigella sativa* (Tembhurne et al., 2014)

Type	Concentration (%)	Subtype	Components
Fixed Oil	32-40	Saturated fatty acids Palmitic acid	Stearic and Myristic acid (30%).
		Unsaturated Fatty Acids	Arachidonic, Eicosadienoic acid (3%), Linoleic (50-60%), Linolenic, Oleic Acid (20%), Almitoleic acid, β-Sitosterol, αSitosterols (44-54%), Cycloeucalenol, Cycloartenol, Sterol Esters and Sterol Glucosides
Volatile Oil	0.4-0.45	-	Nigellone, Thymoquinone (30-48%), Thymohydroquinone, Dithymoquinone, Thymol, Carvacrol, α & β-Pinene, Dlimonene, D-citronellol, P-cymene (7-15%) and 2-(2-Methoxypropyl)-5-Methyl-1,4-Benzenediol
Proteins	16-19.9	Amino Acids	Arginine, Glutamic acid, Leucine, Lysine, Methionine, Tyrosine, Proline and Threonine
Minerals	1.79-3.74	-	Cu, P, Zn, Fe Etc.
Carbohydrates	33.9	-	-
Fiber	5.5	-	-



1) Thymoquinone
2) Nigellicine
3) Nigellimine
4) N-oxide
5) Monodesmosidic triterpene saponin
6) α Hederin

Figure 10 : Chemical structures of some major constituents of *Nigella sativa* seeds (Ali and Blunden, 2003)

The diverse biological activities attributed to *Nigella sativa* are largely due to its various extracts and bioactive compounds, particularly thymoquinone (TQ), the seed's major active constituent. These include anti-oxidant, anti-inflammatory, hepatoprotective, analgesic, anti-neoplastic, anti-mutagenic, nephroprotective, immunostimulatory, hypoglycemic, anti-ulcer, anti-microbial, and anti-parasitic effects (Dalli et al., 2021).

V. Pharmacological activity

Black cumin and its primary bioactive compound TQ, exhibit a wide range of pharmacological effects (Figure 11). These include reducing oxidative stress and inflammation, enhancing immune function, supporting cell survival, and regulating energy metabolism. Together, these actions contribute to their numerous health benefits, offering protection against a variety of conditions such as metabolic, cardiovascular, digestive, hepatic, renal, respiratory, reproductive, and neurological disorders, as well as cancer (Hannan et al ., 2021).

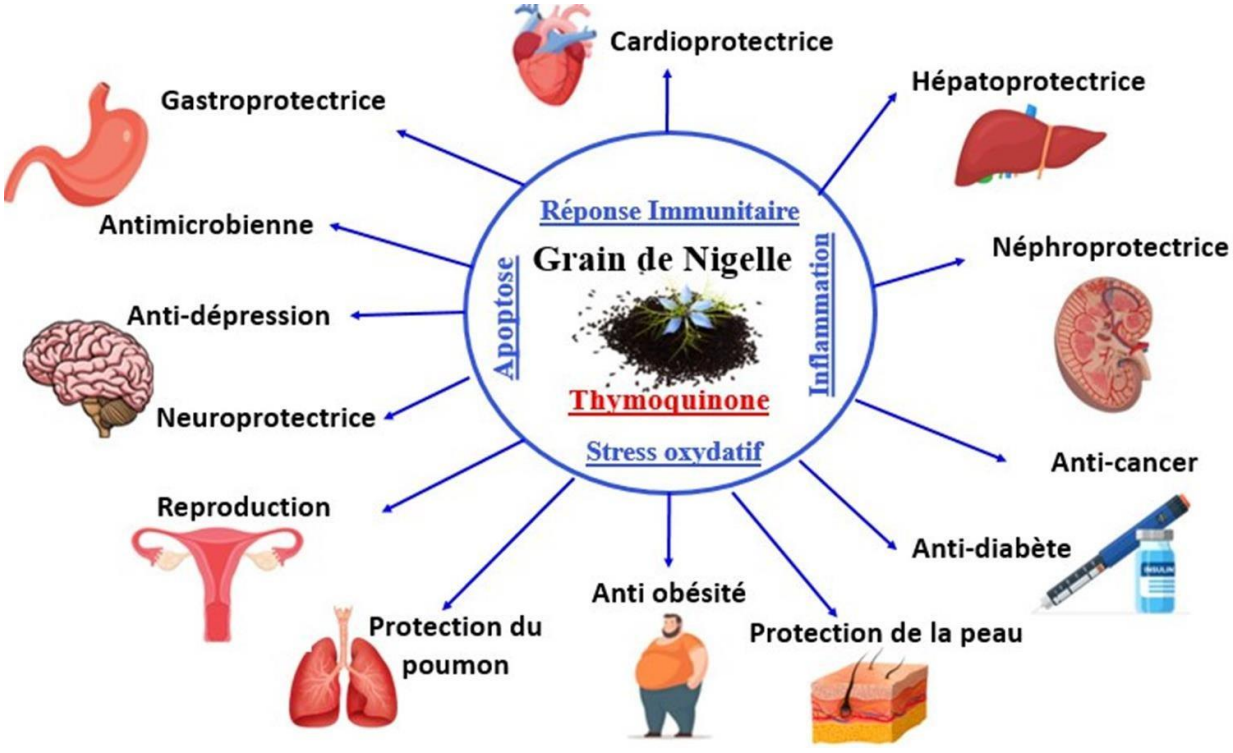


Figure 11 : *Detailed pharmacological profile of* N. sativa *and summary of its protective mechanisms in various organ-based disease*

Chapter III: Semen analysis

Semen analysis

The semen analysis consists in assessing the characteristics of the semen collected, especially those related to the fertilizing power and packaging technologies. Semen analysis is divided into two parts: macroscopic evaluation (phenotypic/visual) and microscopic analysis (Krausz and Farnetani, 2023).

I. Macroscopic examinations

The visual evaluation allows us to assess the volume of ejaculate, colour, and viscosity. It is quickly realized after the collection of sperm (Amann and Graham, 1993).

I .1. Volume

The volume of semen collected varies by species, and within a given species, it is influenced by multiple factors including the physiological state, age, season, breed, method of collection, health status, and nutritional conditions. Additionally, semen volume may also be affected by psychological and environmental factors (Amann and Graham, 1993).

I.2. pH

The pH at ejaculation depends on the relative contribution of acidic prostatic secretion and alkaline seminal vesicular secretion. In the ejaculate, there is no efficient control of the pH of the fluid. In vitro, there will be a continuous loss of CO 2 that causes a gradual increase in pH. The clinical interest of ejaculate pH is a low value. If pH is to be assessed, it should be done at a uniform time, preferably 30 minutes after collection, but in any case, within 1 hour of ejaculation. For normal samples, pH test strips in the range 6.0–10.0 should be used.

1. Mix the semen sample well.
2. Spread a drop of semen evenly onto the pH strip.
3. Wait for the colour of the impregnated zone to become uniform (< 30 seconds).
4. Compare the colour with the calibration strip to read the pH (World Health Organization, 2021).

II. Microscopic examinations

They consist of assessing sperm motility, vitality, sperm concentration of the

ejaculate, sperm morphology and oxidative status.

Motility is a key parameter in assessing ejaculate quality. It is a routine, standardized, and essential test used to eliminate ejaculates deemed unsuitable for cryopreservation. An additional motility test is often performed on a separate sample after thawing, prior to final storage. This final evaluation helps discard semen samples with inadequate post-thaw motility (Amann and Graham, 1993).

II.1. Motility

There are two types of motilities typically assessed: mass motility and individual motility.

II.1.1. Mass motility

It should be examined immediately after semen collection while maintaining the sample at approximately 38 °C, as motility declines rapidly with temperature fluctuation. Spermatozoa in healthy samples usually exhibit a forward, progressive movement. Mass motility is primarily influenced by sperm concentration, the percentage of motile cells, and the velocity of sperm movement (Bearden and Fuquay, 2000).

II.1.2. Individual motility

It is evaluated using an optical microscope at 200× magnification between a slide and coverslip. It reflects the percentage of spermatozoa displaying progressive, linear motion across the microscope field (Amann and Graham, 1993; Salisbury et al., 1978).

II.2. Vitality

The percentage of living sperm is evaluated under an optical microscope on a semen smear stained with eosin-nigrosine. Spermatozoa with damaged membranes (dead) absorb the dye and appear pink (eosin) against a dark background (nigrosine), while live spermatozoa with intact membranes remain colourless (Bearden and Fuquay, 2000).

II.3. Morphology

The study of sperm morphology enables the detection of various abnormalities affecting different parts of the sperm cell. Morphological examination is commonly performed using staining techniques. Several staining methods have been described in the

literature: spermatozoa can be visualized using dyes such as Giemsa, Bengal, pink, Opal blue, and various fluorochromes. However, eosin-nigrosine staining remains the most widely used method due to its simplicity and effectiveness (Bearden and Fuquay, 2010).

Three major sperm morphology classification systems are commonly referenced in the literature (World Health Organization, 2021).The first is based on the origin of the abnormality and distinguish between primary and secondary anomalies:

- Primary anomalies refer to defects that occur during spermatogenesis within the seminiferous tubules.
- Secondary anomalies arise after spermatogenesis, particularly during sperm maturation in the epididymis or during ejaculation (Salisbury et al., 1978; Bearden and Fuquay, 2000).

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Experimental Section

Materials and Methods

This study was conducted to evaluate the impact of dietary supplementation with *Nigella sativa* seed powder on semen parameters in quails. All experiments were carried out at the Cynegetic Centre Laboratory of Zeralda, during the period from February to May 2025. The experimental protocol was established in accordance with current ethical standards, and the conditions of housing, treatment, and sampling were strictly controlled to ensure the reliability of the results. This section outlines the different steps of the study, including the selection of animals, the preparation of the supplemented diet, and the various stages of semen analysis.

I. Materiel

I.1. Biological Material

The study involved 200 Japanese quails (*Coturnix japonica*), one week old at the beginning of the experiment. The birds were divided into two groups:

→ Control group: received a standard diet.

→ Experimental group: received the same diet supplemented with 2% *Nigella sativa* seed powder.

At 13 weeks of age, the male and the females were sperated and only 12 males from each group (control and experimental) were used for the analysis of sperm parameters.

I.2. Experimental location

Japanese quail they were studied and reared at Cynegetic Centre of Zeralda (Figure 12) during the experimental period from February to May 2025.

Figure 12 : Entrance of the Cynegetic Centre of Zeralda

It is a public administrative establishment, created in 1983 by executive order to improve and produce game species for the purpose of repopulating wildlife of natural environments. The Zeralda Cynegetic Centre is located 30 km west of Algiers, 50 km east of Tipaza and 2 km from the Mediterranean (Figure 13).

Figure 13 : Geographical location of the Cynegetic Center of Zeralda

I.3. Breeding duct

Before setting up the quail chicks in rearing, the rearing room was prepared by performing the following operations:

- Cleaning and disinfection of the livestock room by spraying with a disinfectant and bleaching the floor and walls with lime.
- Place a litter consisting of chopped straw with a thickness of about 5 cm to isolate the slats from contact with the ground.
- Installation of livestock equipment (gas radian and drinkers).

After hatching (Figure 14), 200 quail chicks were divided into two groups (control and experimental) and transferred to a brooder, which had been preheated to a temperature between 37 and 38°C during the first week of rearing using a gas radiator suspended one meter above the ground. Starting from the first week, the chicks were placed in individual isolated cages (Figure 15).

Figure 14 : Quail chicks at hatching

Figure 15: Quail housing

From the second week, the temperature was lowered to 34–35°C and then maintained between 20 and 30°C until the end of the experiment. To ensure continuous lighting, two 18-watt neon lights were installed on the ceiling.

During the first days after hatching, the chicks received a prophylactic treatment via drinking water consisting of erythromycin combined with a vitamin complex (Vigal 2X®).

Figure 16 : Powder vigal « Vigal 2 X ®

The water supply was renewed every morning and evening, and even more frequently during the first few days of rearing. Drinking water was provided using drinkers with a capacity of 0.5 L (Figure 17) and distributed in ad libitum.

Figure 17 : Water reservoir with a volume of 0.5 L

I.4. Feed

I.4.1. Feed composition and distribution

The feed (Figure 18) distribuetd is provided from livestock feed ONAB (Figure 18A) composed of corn, soy meal and of mineral and vitamin complex (Figure 18B), in ad libitum.

Figure 18 : Poultry feed. Feed bag (A) ; Feed composition (B)

For E, an additional 2% of Nigella seed powder was added to the base feed of the control group.

I.4.2. Preparation steps of the supplemented ration

The same supplier provided all the Nigella seeds (Figure 19) used in the experiment, which were then ground before use (Figure 20).

Figure 19 : Nigella seeds

Figure 20 : Grinding of Nigella seeds

At the laboratory, the powder of the seeds of Nigella (*Nigella sativa*) was weighed with a balance of maximum capacity of 500g (Figure 21) an amount of 2% was added and mixed into the basic ration (Figure 22).

Figure 21 : Weighing of *Nigella sativa* seed powder

Figure 22 : Mixing the feed with *Nigella sativa* seed powder

I.4.3. Food distribution

The feed was distributed in plastic plate-type feeders (Figure 23A) on an ad libitum during the first week. The first feeding was carried out only after the quail chicks had sufficiently rehydrated, 24 hours after placement.

Starting from the third week, second-age plastic hopper-type feeders were progressively introduced (Figure 23B).

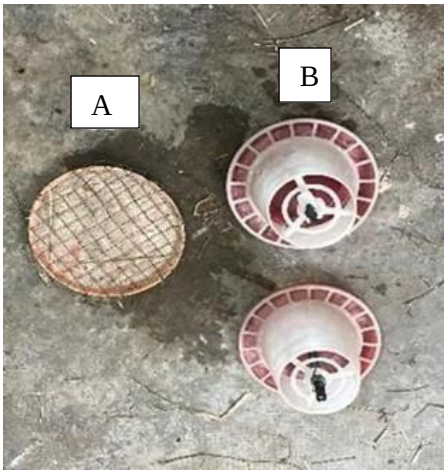

Figure 23: Type of feeder: (A) plate-type and (B) hopper-type

At the age of 13 weeks, 12 quails of each group were slaughtered by bleeding according to the Islamic method in the Cynegetic Centre Laboratory of Zeralda (Figure 24).

Figure 24: Slaughter method by bleeding

II.1. Semen collection

The steps for sperm collection from the vas deferens in quail are represented in figure 25. After the sacrifice of the quails, an incision was made on the left side to extract the testis-epididymis complex along with the vas deferens for semen collection (Figure 25A). A deep incision was then made in the caudal region of the vas deferens using a scalpel to release the sperm (Figure 25B), which was subsequently collected and transferred into an Eppendorf tube (Figures 25 C, D) and maintained it in a water bath (NUVE; Model: NB 20 (unstirred water bath) at 37°C until analysis.

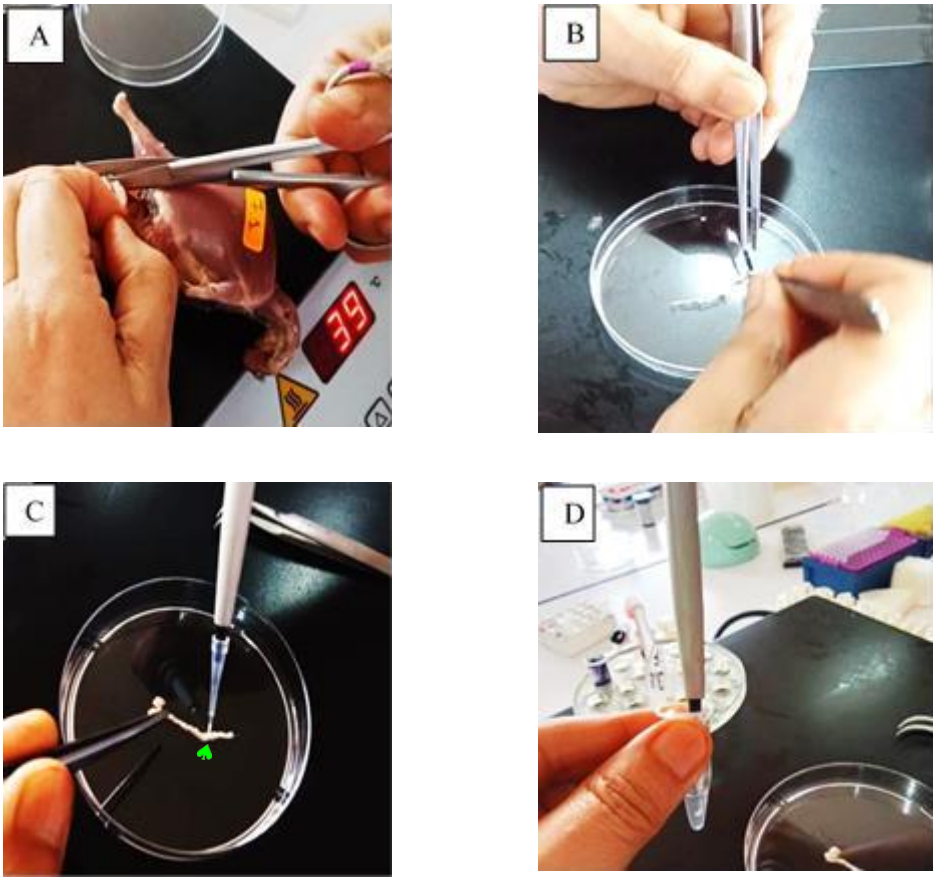

Figure 25 : Steps for sperm collection from the vas deferens in quail. A : Extraction of left vas deferens ; B : Mincing the vas deferens to release the sperm ; C : Extracting the sperm (♠) with micropipette ; D : Extracting the sperm with micropipette

II.2.Microscopic analysis

The Eppendorf tube maintained in a water bath at 37°C (Figure 26) are used for microscopic examination.

Figure 26 : Eppendorf tube maintained in a water bath

Microscopic parameters (vitality and morphology) are analysed using the CASA system (Computer-Assisted Sperm Analysis).

II.2.1.Description of CASA system

A computer system connected to an HP monitor and Nikon digital camera (Figure 27) enables real-time display of both live and digitized microscope images. The setup includes a Nikon microscope (Figure 27) equipped with negative phase contrast objectives (x10, x20, x40, and x60). Sperm analysis is performed using SCA (Sperm Class Analyzer) software, which quantifies various motility patterns such as slow, medium, fast, and progressive movement and calculates essential kinematic parameters including velocity, trajectory angle, and linearity. The system also allows for accurate sperm counting.

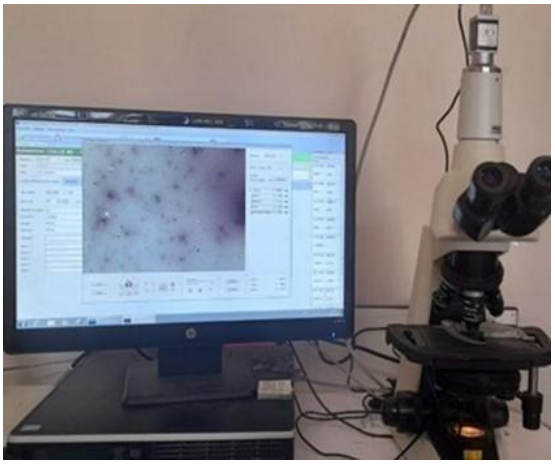

Figure 27 : CASA system (personal photo)

II.2.2. Determination of sperm concentration

This procedure determines the sperm concentration per millilitres of undiluted semen using a Thoma-type hemocytometer (Figure 28A), which has two grids, each subdivided into 16 large squares, further divided into 16 smaller squares.

A 10 µL semen sample is mixed with 1990 µL of 10% physiological saline. The diluted semen is homogenized using a vortex mixer and loaded into the hemocytometer (Figure 28C).

To prepare the chamber, it is placed on a level surface, and a coverslip is affixed by moistening the edges and gently sliding it into place (Figure 28B). A drop of the diluted semen is then placed at the edge of the coverslip to fill both grids by capillary action (Figures 28D–E). The slide is left undisturbed for 10 minutes to allow spermatozoa to settle. It is then observed under a phase-contrast microscope at 40× magnification (Figure 28F). Sperm are counted in the two central columns (4 squares each) of both grids. Sperm touching the top and right borders are excluded from the count.

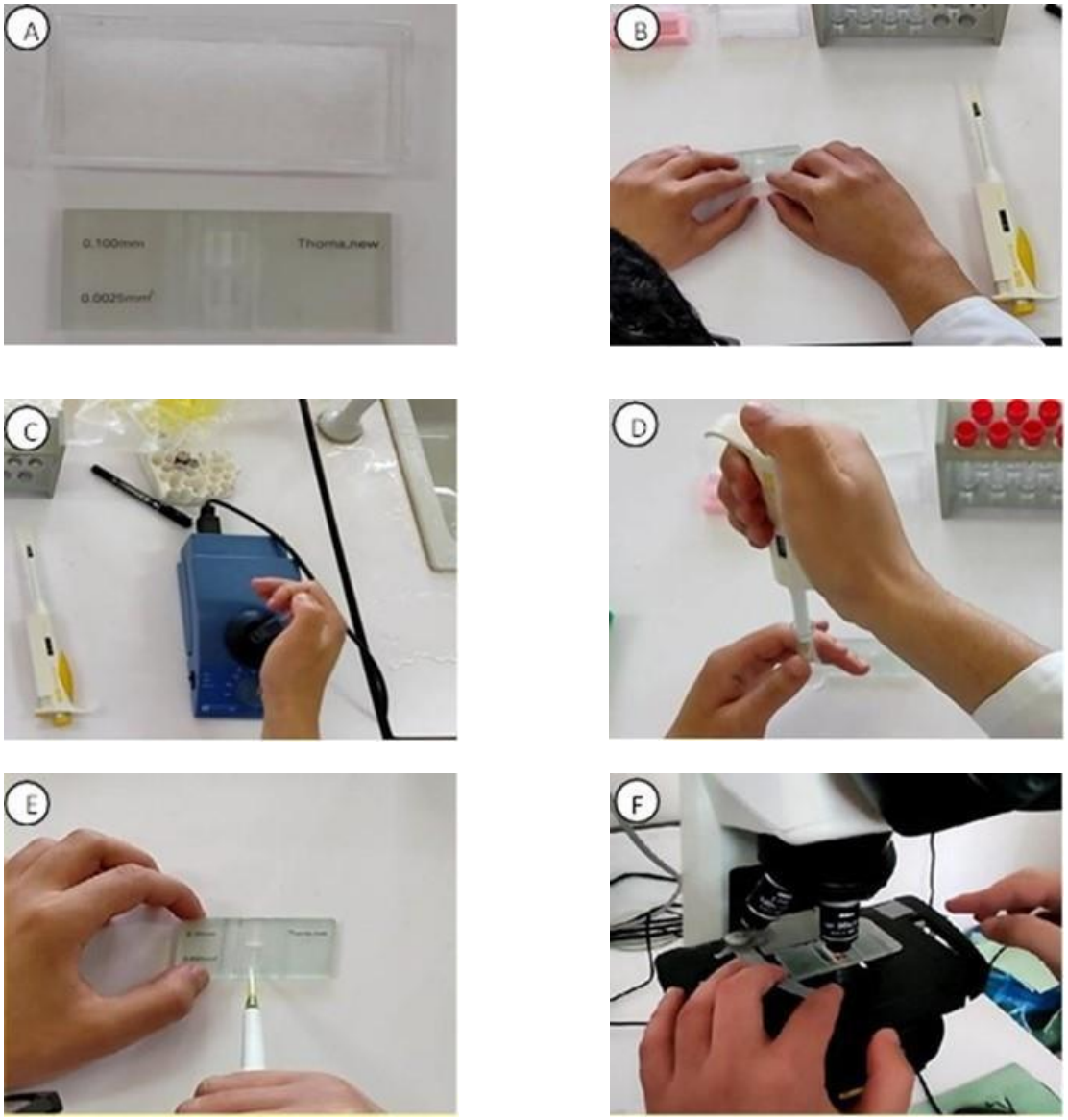

Figure 28 : Steps for assessing sperm concentration. A : Thoma-type hemocytometer , B : perpetration of the Thoma- type hemocytometer ; C : Homogenization of the solution using a vortex mixer ; D : Collection of seminal fluid using a micropipette; E : Filling of the two grids of the Thoma chamber by capillarity ; F : Observation of the Thoma chamber under a phase-contrast microscope (x40)

The calculation of the concentration is performed according to Jacky (2007) as follows:

The two central columns of one grid contain 8 x 16 = 128 small squares. Given that the volume of one small square is 1/4000 mm^3, the total volume of the 8 large squares is 0.032 mm^3.

To determine the concentration of spermatozoa (Cn) per millilitre of diluent, considering both the upper and lower grids, the formula used is:

$$\mathbf{Cn} = \frac{X \times D \times \mathbf{1000}}{\mathbf{Vo}lume\ \mathbf{counted} \times \mathbf{2}}$$

Where:

- X = total number of spermatozoa counted in the 8 large squares of both the upper and lower grids
- D = dilution factor of the semen = 200
- Volume counted = 0.032 mm^3 per grid (hence multiplied by 2 for both grids)

II.3.1. Sperm vitality

We assessed sperm viability by determining the percentage of live and dead spermatozoa using the CASA system at x20 magnification. Before observation, we prepared a smear by placing 10 µL of diluted in physiological salin (1/200) semen on a microscope slide (Figure 30A). We then added 10 µL of eosin staine (Figure 30B) and 10 µL of nigrosine stain using a micropipette (Figure 30C). We mixed the components and spread the mixture evenly across the slide using the edge of another slide held at a 45° (Figure 30D). Finally, we allowed the smear to air-dry.

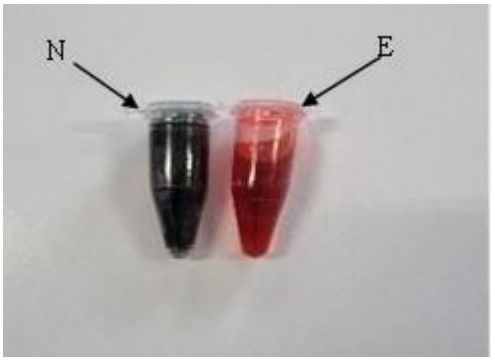

Figure 29 : Eppendorf tubes containing eosin (E) and nigrosine (N)

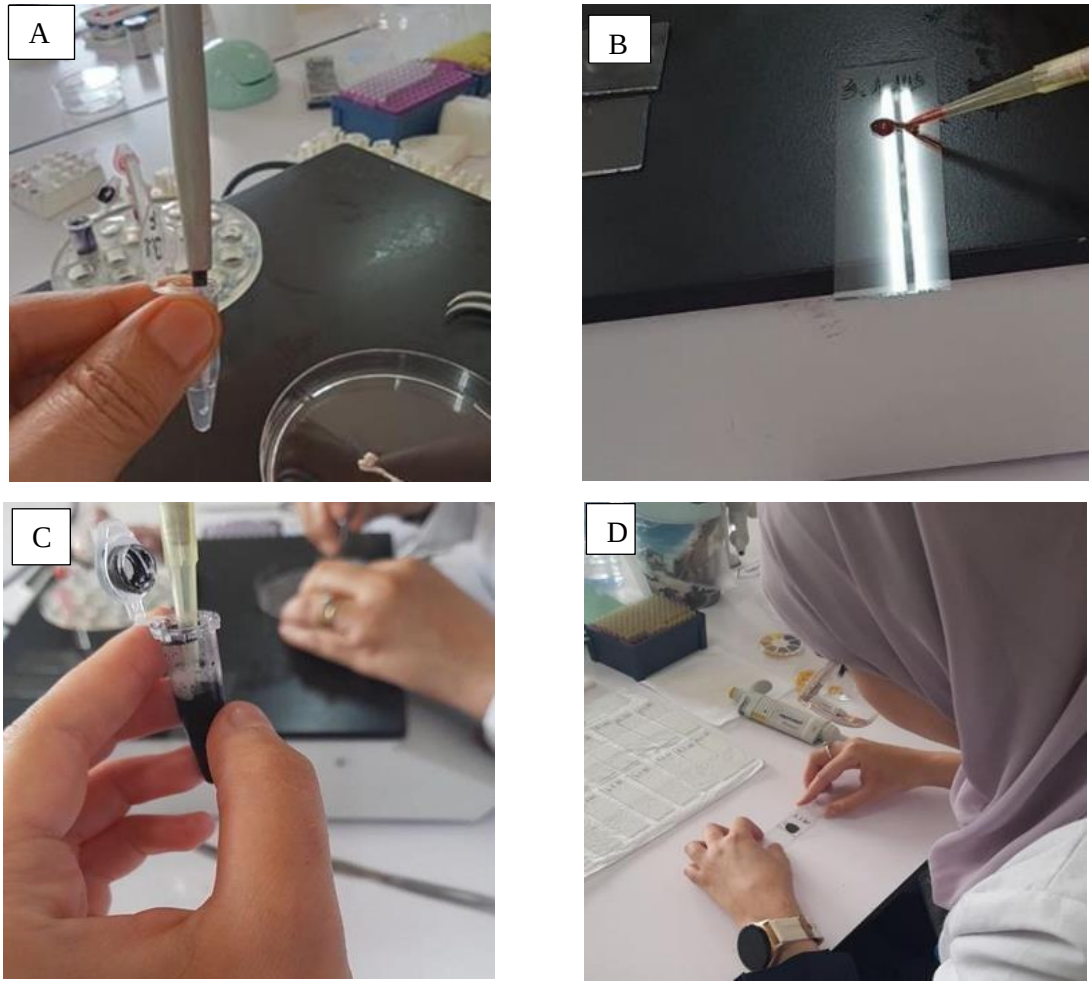

Figure 30 : Preparation of slides for vitality assessment. A : Semen smear prepared by applying 10 µL of fresh ejaculate on a microscope slide ; B : the addition of 10 µL of eosin stain on a microscope slide ; C : the addition of 10 µL of nigrosin stain on a microscope slide ; D : Semen–stain mixture evenly spread across the slide using the edge of a second slide held at a 45° angle

II.3.2. Morphology

We stained sperm smears from the epididymis with eosin-nigrosin to assess sperm abnormalities. Using phase-contrast microscopy, we identified spermatozoa with abnormalities in the head, midpiece, or tail. We evaluated a total of 200 spermatozoa and calculated the percentage of abnormal forms.

II.4. Statistical analysis

The numerical results are presented as arithmetic means accompanied by the standard

error of the mean (SEM), using Microsoft Excel version 2013.

- Arithmetic mean: $X = \frac{\sum xi}{n}$

SEM = $\frac{\sigma}{\sqrt{n}}$ with $\alpha = \sqrt{\frac{\sum (xi - x)^2}{n-1}}$

- xi: individual values
- n: number of values
- σ: standard deviation

The statistical validity of the differences between the means of two experimental series is calculated using the ANOVA test, performed with the SPSS:

$t = \frac{\bar{X}_1 - \bar{x}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$ with $S^2 = \sqrt{\frac{\sum (x_1 - x_1)^2 + \sum (x_2 - x_2)^2}{n_1 + n_2 - 2}}$

Where:

$\bar{x}_1$ and $\bar{x}_2$ arithmetic mean values of each series

- $\bar{x}_1$: individual values of the first series
- $\bar{x}_2$: individual values of the second series
- n_1 and n_2: number of values in each series

The probability "p" is determined from the t-distribution table based on the degrees of freedom ($n_1 + n_2 - 2$) : if

P > 0.05 : result not significant

P < 0.05 : significant result (*)

P < 0.01 : very significant result (**)

P < 0.001 : highly significant result (***)

Percentage difference (%)

Percentage difference = ((Final value - Initial value) / Initial value) × 100

Conclusion

In the present study, dietary supplementation with *Nigella sativa* in male Japanese quails involves a slight improvement in sperm viability and morphology. This finding not statistically significant effect on reproductive parameters. This effect may be attributed to the antioxidant and hormone-modulating properties of its bioactive compounds, particularly thymoquinone and essential fatty acids. However, the effectiveness of Nigella seed powder depends on several factors, such as the dose used, the duration of administration, the type of ration, the age of the poultry and the rearing conditions. This means that further research is needed to understand the mechanisms of action of this plant, not only on sperm parameters, but also on production performance in quails.

In the perspective, further experiments on a larger number of birds with a range of different doses of *Nigella sativa*. These experiments should take into account the main hormonal profiles involved in the control of reproductive activity.

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Biologie et physiologie de la reproduction

Effects of Black Seed (*Nigella sativa*) Powder on Semen Parameters in Japanese quail (*Coturnix japonica*)

Saidi Imane and **Rahali Khadidja**

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