



# Spermatozoa Characteristics, Reproductive Hormones and Fertility Rate in Male Japanese Quail Fed on Ginger Rhizome (*Zingiber Officinale*, Roscoe) Essential Oil

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## Abstract

**Background and Aim:** Domestic animals face many environmental factors including poor quality of feed and water, temperature fluctuations, high densities in farms that reduce reproductive performance and lead to economic losses in the farmer. The ginger rhizome (*Zingiber officinale*, Roscoe) essential oil due to its bioactive molecules with various pharmacological properties could mitigate the effects of these factors and boost the performance of animals.

**Materials and Methods:** From November 2022 to January 2023, ninety-six (96) 3 weeks old males Japanese quail (*Coturnix coturnix japonica*) weighing between 120 and 130 g were randomly assigned to 4 dietary treatment groups in a completely randomized design replicated 4 times each of them. Each group was separated into 4 subgroups of 6 quails. For 64 days, animals in group1 (control) received by orally distilled water (100 µl/kg body weight), while the other three test groups during the same period, received respectively by the same method 50, 100 and 150 µl per kg body weight (b.w) of ginger roots essential oil. At 12 weeks old, 4 male quail per treatment, with hypertrophied cloacal gland were chosen at random and individually kept in cages, with four untreated females for fertility assessment. Throughout the experiment, semen quality, reproductive hormones and fertility rate were evaluated.

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**Keywords:** Fertility, ginger essential oil, male reproduction, quail, sperm quality.



**Results:** The result revealed that the testicular and vas deferens weights increased significantly ( $P < 0.05$ ) in quails treated with 100 and 150  $\mu\text{l}/\text{kg}$  b.w. of ginger roots essential oil. The sperm motility and viability significantly increased ( $p < 0.05$ ) in quails treated with essential oil compared to the control. The serum content in LH, FSH and testosterone significantly increased ( $p < 0.05$ ) in a dose-dependent manner. Fertility rate was respectively 82.14 %, 90.71% and 88.54 % with 50, 100 and 150  $\mu\text{l}/\text{kg}$  b.w.

**Conclusion:** The ginger root essential oil can be used to boost the male reproductive performances by improving spermatozoa characteristics and fertility rate.

## Introduction

The Japanese quail is a poultry with very good reproduction performances. It lays 300 to 400 eggs/year with a laying period of 5 to 6 months. The laying reaches its peak very quickly and remains very intense during the 4th and 5th month. This bird is very prolific with the first eggs laid between the 6th and 7th week of age. The incubating eggs with 8g at the beginning of laying at 7th week of age should weigh between 10 to 12g at the 10th week of age [1]. The quail has an extremely rapid embryonic development and the total duration of incubation is 16 days, of which 14 days of incubation itself, and 2 days of hatching. The young quails grow very quickly, their weight increases from 10g to 125g, from hatching to 35 days, and to 165g at 42 days. Quails show few pathological symptoms as long as they are kept away from other poultry farms [2].

In spite of these multiple assets, the productivity remains low and does not able to satisfy the demand of the population, especially in developing country. This weakness is justified by the lack of mastery of breeding techniques, the unavailability of raw materials for feed, which leads to underfeeding, the poor quality of the ingredients for the ration, the poor quality of water for watering, and the fluctuations in the values of the climate characteristics [2]. The combination of the effects of these factors would be responsible for the imbalance between the production of reactive oxygen species and antioxidant enzymes. This effect affects several systems of the animal organism including the hormonal system and consequently acts on fertility and reproductive performance [3].

Among the solutions considered, the use of plant and plant-based products linked to their availability, accessibility and diverse bioactive molecules with various pharmacological activities with benefit effects on animal health without prejudice to the environment [4], is obvious. Among plant-based products used is classified Essential oil.

Essential oil is the concentrate and hydrophobic liquid of volatile aromatic compounds [5], such as phenolics and polyphenols, terpenoids, saponins, quinine, esters, flavone, flavonoids, tannins, alkaloids and non-volatile residues. These molecules have many properties (antimicrobial, stimulating animal digestive system, antioxidants, antifungal, antiparasitic, anti-inflammatory) [6] which can reduce loss of energies and improved nutrients absorption in benefit of growth and reproductive performances in animals. Among the aromatic plants containing essential oil is classified the Ginger (*Zingiber officinale*) which is used worldwide as a culinary spice and traditional medicine to preserve and cure various diseases ranging from infections to infertility [7]. It has long been considered that ginger has several

beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, antiviral, antifungal and androgenic properties [8]. The studies carried out by Mostafa et al. [9] have shown that the inclusion of ginger powder at dose of 15 g in quail feed improves ejaculate volume, sperm concentration, mobility, viability and sperm-egg penetration. In the same direction. Majid et al. [10] have mentioned that Ginger improves semen quality and increases fertility of sperm by regulating the liberation of the reactive oxygen species responsible of oxidative stress that disrupts the levels of gonadotropin hormones (LH, FSH) and sex hormones (such as testosterone) in the blood. These few results are on the whole ginger, others research on substances near to active molecules such as essential oil. The objective of this study was to evaluate the effect of ginger root essential oil on male quail reproductive parameters.

## Materials and Methods

### Study area

This study was carried out from November 2022 to January 2023 at the poultry unit in the Teaching and Research Farm of the University of Dschang, Cameroon. This farm is located at 5°26' North and 10°26' EST and at an altitude of 1420 m above sea level. Annual temperature varies between 10°C and 25°C. Rainfall ranges from 1500-2000 mm per annum over a 9 months rainy season (March to November).

### Plant material and essential oil extraction

Fresh Ginger roots were harvested from the agricultural zone of Santchou (LN 5° 16' 55", LE 9° 58' 27") in the Menoua division, West Region of Cameroon, washed and then ground in a mortar and pounded in order to liberate the tissues. Oil extraction was done by hydrodistillation following the protocol described by Wang and Weller [11].

### Animal and experimental design

Ninety-six (96) 3 weeks old male quails weighing between 120 and 130 g, produced from a parent's flock in the Teaching and Research Farm of University of Dschang were used. Each bird was identified by a ring bearing his number in one of its paws.

At the beginning of the experiment, all the quails were weighed and then assigned randomly to 4 dietary treatment groups in completely randomized design. Each group was divided into 4 subgroups of 6 quails each. For 64 days, birds in group1 (control) received orally distilled water (100  $\mu\text{l}/\text{kg}$  body weight), while birds in the other three test groups, during the same period, received respectively by gastric intubation and daily 50, 100 and 150  $\mu\text{l}$  per kg body weight of ginger rhizomes essential oil. At 12 weeks old, twelve birds per treatment were randomly selected and slaughtered for organs and spermatozoa characteristics assessment. Blood samples were also collected for reproductive hormone level evaluation. 4 male quails per treatment with hypertrophy of cloacal gland were also chosen at 12 weeks old and individually kept in cages with four untreated females reared under the same conditions, for fertility assessment. During the experimental period, feed (**Table 1**) and water were given *ad libitum* to quails in adapted equipment.

This study was carried out in strict accordance with recommendations of institutional guidelines for the care and use of laboratory animals. Quails were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

**Table 1:** Composition and proximate analysis of the experimental diet

Constituents	Quantity (kg/100kg)
Corn	60
Bran wheat	4.5
Soybean meal	22
Fishmeal	4.5
Oeister shell	2
Bone meal	2
Premix 5%*	5
Total	100
<b>Chemical composition</b>	
Crude protein (%)	20.15
Metabolizable energy (Kcal/Kg)	2906.80
Calcium (%)	2.03
Phosphorus (%)	1.27
Lysine (%)	0.44
Methionine (%)	0.14
Sodium (%)	0.22

\*Premix 5%: mixture of vitamins A, B complex, D, K and E plus Iron, Cu, Zn, Se, Mn, Methionine, Lysine principally and incorporated at 5% in diet.

### Cloacal gland

Before the sacrifice of each quail, the diameter and height of the cloacal gland were measured using an INSIZE digital caliper (0-150 mm /0-1.52 cm). The surface of cloacal gland was determined by multiplying the diameter by the height [12].

### Blood sampling and organ weights

At the end of the experiment, 12 quails per treatment were randomly selected and fasted for 24 hours, weighed and slaughtered as described by Jourdain [13]. Blood samples were collected from the jugular vein in non-heparinized tubes, the serum isolated were stored at -20°C for biochemical analysis.

Organs of sacrificed quails including testes, epididymis and vas deferens were carefully removed, rid of adipose tissue, blotted dry and weighed separately using a scale of 160 g capacity and 10<sup>-3</sup> g precision. The relative organ weight was calculated as follow:

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight (mg)}}{\text{Live body weight (g)}} \times 100$$

### Sperm characteristics

Immediately after weighting, vas deferens of each quail was minced in 10 ml of warm (36°C) NaCl solution for sperm motility, viability and density. Sperm motility was direct estimated with 20 µl of this solution at 40X magnification using the scale describe by Mamun *et al.* [14]. Sperm viability was analyzed using hypo-osmotic swelling test [15]. The sperm density was determined using Thoma hemocytometer.

### Biochemical analysis

Serum content in LH, FSH and testosterone were determined by ELISA method using a commercial kits Omega DIAGNOSTICS; Ref: OD497, Omega DIAGNOSTICS; Ref: OD357 and Omega DIAGNOSTICS; Ref: OD337 respectively.

### Fertility

A number of 56 eggs per group were collected during 8 days and incubated. After artificial incubation for 19 days, the unhatched eggs were broken-out to verify whether they were truly unfertilized or if they presented embryonic mortalities. The fertility rate was calculated by dividing the number of fertilized eggs with the total number of eggs incubated.

### Statistical Analysis

The statistical analysis was carried out using the SPSS 20.0 software. Results were expressed as mean ± standard deviation. Differences between groups were assessed using one way ANOVA followed by Duncan post hoc test with the significance level set at 0.05. P-value was done using the student t- test. A p value of less than 0.05 was considered as significant. The normality of data was tested by the Shapiro-Wilk Test and the relationships between different parameters highlighted by the correlation coefficient of Bravais Pearson.

### Results

#### Effects of ginger roots essential oil on relative weight of the reproductive organs and sperm characteristics in Japanese quail

The cumulative relative weight of the two testes as well as the vas deferens weight increased significantly (P <0.05) in quail treated with essential oil at doses of 100 and 150 µl/kg bw compared to the control and quails treated with the smallest dose of essential oil (50 µl/kg bw) (Table 2). The relative weight of the testes was positively and significantly correlated with the surface of the cloacal gland (ρ = +0.97; P <0.05), and serum content in testosterone (ρ = +0.98; P <0.01). The same observations were made between the surface of the cloacal gland and the relative weight of the vas deferens (ρ = +0.99; P <0.01), and between the serum content in testosterone and the relative weight of the vas deferens (ρ = +1.00; P <0.01) (Table 4). The relative weights of the epididymis recorded in quails treated with essential oil at all tested doses were comparable (P > 0.05) to that of quails in the control group. However, the epididymis relative weight tends to increase with essential oil at doses of 100 and 150 µl/kg bw.

It can be seen from table 2 that, the ginger rhizomes essential oil whatever the dose had no significant effects (P <0.05) on the sperm density per gram of tissue. The sperm motility increased significantly (P <0.05) in quails treated with 100 and 150 µl/kg bw of essential oil compared to that of quails in the control group. When considering only the quails treated with increasing doses of essential oil, the increase in sperm motility was significant only in birds treated with 100 µl/kg bw. The viability of spermatozoa increased significantly (P <0.05) in quails treated with essential oil whatever the dose compared to that of quail in the control group. However, the value of this spermatozoa characteristic obtained in quail treated with 100 µl/kg bw was comparable to that of birds receiving 150 µl/kg bw, but significantly higher (P <0.05) than the value recorded in quails treated with 50 µl/kg bw of essential oil.

**Table 2:** Effects of ginger roots essential oil on sperm characteristics and biochemical parameters in male Japanese quail.

Parameters	Control (n=12)	Essential oil doses (µl /kg body weight)			P value
		50 (n=12)	100 (n=12)	150 (n=12)	
<b>Organ weights (g/100 g bw)</b>					
Testes	2.01 ± 0.48 <sup>b</sup>	2.08 ± 0.16 <sup>b</sup>	2.63 ± 0.21 <sup>a</sup>	2.54 ± 0.32 <sup>a</sup>	0.01
Epididymis	0.03 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.09
vas deferens	0.04 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>b</sup>	0.07 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>a</sup>	0
<b>Sperm characteristics</b>					
Sperm density per gram of tissue (x 10 <sup>6</sup> )	21.97 ± 0.73	20.54 ± 0.16	23.10 ± 0.60	23.71 ± 0.94	0.88
Motility (%)	61.25 ± 5.20 <sup>c</sup>	65.00 ± 4.56 <sup>bc</sup>	85.00 ± 5.40 <sup>a</sup>	77.63 ± 11.15 <sup>ab</sup>	0
Viability (%)	86.33 ± 4.89 <sup>c</sup>	89.95 ± 5.52 <sup>b</sup>	92.17 ± 5.24 <sup>a</sup>	91.08 ± 4.63 <sup>ab</sup>	0

a, b, c, d: On the same line, means with the same letter were not significantly different (p > 0.05). n = number of quails.

**Table 3:** Effects of ginger roots essential oil on surface of the cloacal gland and the reproductive hormones in male Japanese quail.

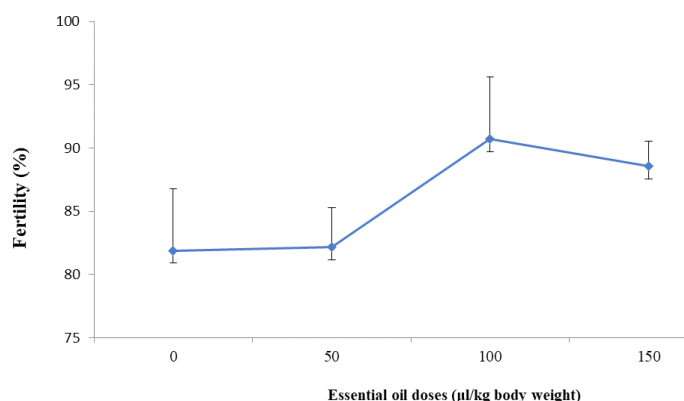
Parameters	Control (n=12)	Essential oil doses (µl /kg body weight)			P value
		50 (n=12)	100 (n=12)	150 (n=12)	
Surface of the cloacal gland (mm <sup>2</sup> )	151.71 ± 20.69 <sup>c</sup>	189.48 ± 20.95 <sup>b</sup>	276.71 ± 25.85 <sup>a</sup>	285.12 ± 28.91 <sup>a</sup>	0.00
LH (mIU/ml)	2.63 ± 0.69 <sup>b</sup>	2.44 ± 0.78 <sup>b</sup>	4.00 ± 0.88 <sup>a</sup>	4.31 ± 0.13 <sup>a</sup>	0.00
FSH (mIU/ml)	18.33 ± 3.84 <sup>b</sup>	20.78 ± 4.79 <sup>b</sup>	26.13 ± 2.75 <sup>a</sup>	27.44 ± 3.41 <sup>a</sup>	0.00
Testosterone (ng/ml)	0.44 ± 0.09 <sup>c</sup>	0.61 ± 0.16 <sup>b</sup>	0.97 ± 0.08 <sup>a</sup>	0.98 ± 0.18 <sup>a</sup>	0.00

a, b, c : On the same line, means with the same letter were not significantly different (p > 0.05). LH: luteinizing hormone; FSH: follicle stimulating hormone; n = number of quails.

**Table 4:** Correlations between reproductive parameters in male Japanese quail.

Parameters	Testes relative weight	Fertility rate	surface of the cloacal gland	Vas deferens relative weight
Sperm motility	0.99*	0.99**	0.95	0.95*
Testosterone	0.98**	0.95	0.99**	1.00**
Vas deferens relative weight	0.98*	0.94	0.99	-
Testes relative weight	-	0.98*	0.97*	-
LH	-	0.94	0.99**	-
FSH	-	0.93	-	-
Fertility rate	-	-	0.95	-

(\*) The correlation is significant at the 0.05. (\*\*) The correlation is significant at the 0.01.



**Figure 1:** Effects of ginger roots essential oil on male Japanese quail fertility.

**Effects of ginger roots essential oil on the cloacal gland surface and the reproductive hormones in male Japanese quail**

Whatever the dose of essential oil administered, the surface of the cloacal gland increased significantly (P < 0.05) in exposed quails compared to controls. When considering only the quails treated with the essential oil, the surface of the cloacal gland recorded with 100 and 150 µl/kg bw were comparable, but significantly (P < 0.05) higher than the surface recorded in quails treated with 50 µl/kg bw (Table 3). The surface of the cloacal gland is positively and significantly correlated with serum content in testosterone (ρ = +0.99, P < 0.01), and FSH (ρ = +0.99; P < 0.01) (Table 4).

The serum content in luteinizing hormone (LH) and follicle stimulating hormone (FSH) increased significantly (P < 0.05) in quails treated with 100 and 150 µl/kg bw of essential oil compared to those of the quails in the control group and the group received the smallest dose of essential oil (50 µl/ kg of bw) (Table 3). The LH content was positively and not significantly corre-



lated with the motility of spermatozoa ( $\rho = +0.92$ ;  $P > 0.05$ ), and fertility rate ( $\rho = +0.94$ ;  $P > 0.05$ ). The same observations were made between FSH content and sperm motility ( $\rho = +0.92$ ;  $P > 0.05$ ), and between FSH and fertility rate ( $\rho = +0.93$ ;  $P > 0.05$ ) (Table 4).

The ginger rhizomes essential oil whatever the dose significantly increased ( $P < 0.05$ ) the serum content in testosterone in the treated quails compared to the controls. Between quails exposed to the essential oil, the testosterone level was significantly higher ( $P < 0.05$ ) with 100 and 150  $\mu\text{l}/\text{kg}$  bw than the value obtained with 50  $\mu\text{l}/\text{kg}$  bw (Table 3). A positive and non-significant correlation was recorded between the testosterone level and the fertility rate ( $\rho = +0.95$ ;  $P > 0.05$ ) (Table 4).

## Discussion

The treatment of male Japanese quail with the ginger rhizomes essential oil at doses of 100 and 150 mg/kg/day for 64 days has induced an increase in the relative weight of the sexual organs in treated quails. These results are consistent with those of Arash *et al.* [16] in male rats treated with ginger rhizomes powder at doses of 50 and 100 mg/kg/day. The present results are also in agreement with those of Kamchouing *et al.* [17] who observed an increase in sex organs weight of male rats treated with 600 mg/kg bw of aqueous extract of ginger rhizomes for 8 consecutive days. In male animals, the weight, the size and the secretory functions of the testes and epididymis are regulated by androgens [18]. The increase in sex organs weight in this study would be attributed to the androgenic properties of the ginger rhizomes essential oil. Androgens and substances with androgenic activities have anabolic action that results in an increase in protein synthesis and therefore muscle mass [17]. Testicular proteins are one of the constituents that ensure the maturation of spermatozoa and its concentration is correlated to testicular growth [17].

The results obtained in this study showed that the *Z. officinale* essential oil induced a significant increase in serum content of testosterone relative to the control. This result suggests a possible steroidogenic action of this essential oil which results in the synthesis of testosterone by Leydig cells. In general, any increase in serum content of testosterone or androgen is accompanied by an increase in the secretory activity of the organs [18]. The testosterone is the major male gonad hormone produced by Leydig cells in the testes under the control of the hypothalamic-pituitary axis [19]. This hormone is necessary for the initiation and maintenance of spermatogenesis as well as for the stimulation of growth and function of the sexual organs [20]. It has been reported that increases in both sperm count and sexual organs weight are an indicator of improved male fertility [18]. Ferrouk *et al.* [21] reported that testicular size is the primary endpoint for spermatogenesis since seminiferous tubules and germinal elements constitute about 98% of the total testis mass. Thus, the significant increase in testicular weight in quails treated with essential oil could be the consequence of the spermatogenesis efficiency improvement. The surface of the cloacal gland was positively and significantly correlated to the serum content in testosterone. This effect suggests an increase in testosterone level with development of cloacal gland. In accordance to the present result, Mostafa *et al.* [9] mentioned that the serum testosterone level in quails is led to the development of cloacal gland. The quails with big cloacal gland would produce high quantity of testosterone. The same author also reported that the incorporation of ginger powder at the concentration of 10 and 15 g/Kg feed induces an increase

of cloacal gland area and cloacal gland volume relative to the control.

The gonad maturation is under the pituitary gland control by gonadotropic hormones and close communication between Leydig cell - Sertoli cell - germ cells in the testes. The Sertoli cell possesses the receptors of FSH, a pituitary hormone involved in the onset of spermatogenesis at puberty and its maintenance during adulthood [22]. Leydig's cell is under the control of LH and is able to synthesize testosterone from plasma cholesterol but also from cell membranes [23]. The present study revealed an increase in serum LH and FSH levels in male Japanese quail treated with *Z. officinale* essential oil compared to controls. The increase in LH and FSH content could be related to the ability of this essential oil to act on the hypothalamic-pituitary-testicular axis. These results are in agreement with those of Shanon *et al.* [24] in ROSS 308 breeders treated with ginger rhizomes essential oil at doses of 5 and 10 kg/ton of feed for 20 weeks. In the same direction, Majid *et al.* [10] have observed that the administration of aqueous extract of *Z. officinale* in drinking water at 5% and 10% daily for 20 weeks increase FSH, testosterone and LH levels in broiler chicken.

The ginger rhizome essential oil induced an increase in sperm motility and viability in the treated quails compared to the control. These results are similar to those obtained by Shanon [24] in broilers fed on ginger rhizome powder and that of Mostafa *et al.* [9] in Japanese quails submitted to 10 and 15 g/Kg feed of ginger rhizome powder. This increase in sperm motility and viability can be attributed to the antioxidant properties of the ginger rhizomes essential oil on male reproductive functions. This activity would allow it to protect spermatozoa membrane from reactive oxygen species attacks and consequently make them more active. In this study, essential oil administration at the doses of 100 and 150  $\mu\text{l}/\text{kg}$  bw for 64 consecutive days to quails significantly increased their fertility as compared to the controls. The present results are in agreement with the findings of Şimşek *et al.* [25] in Japanese quail treated with cinnamon and rosemary oils. The increase in fertility rate in this study can be attributed to the improvement in the characteristics of the spermatozoa which consequently, make them more active in the female genital tract. According to Froman [26], when higher proportion of high motile spermatozoa enters the sperm storage tubules of female birds, it resulting in a high proportion of fertile eggs. The present results revealed a positive correlation between the spermatozoa motility and the fertility rate suggesting an improvement in quail fertility rate with the increasing of spermatozoa motility. The antioxidant and androgenic properties of ginger significantly improves the biological parameters of sperm (number, total motility, survival rate and normal morphology) associated to high fertility rate [10].

## Conclusion

The present study revealed that, ginger rhizomes essential oil due to their diverse properties improve spermatozoa characteristics, increase reproductive hormone concentration and subsequently fertility rate.

## Author Contributions

HT, JRK and FN conceived, designed the research and reviewed the manuscript. CMMM, HVN, SND and EFB collected the data, carried out data analysis and wrote the manuscript. All authors read and approved the final manuscript.

### Availability of data and materials

The data sets used during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors certify that no competing interests exist.

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