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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: Ginger rhizome essential oil has many properties (antimicrobial, stimulating animal digestive system, antioxidants, antifungal, antiparasitic, anti-inflammatory) used in animal production to improve their growth and reproductive performances. The present study was designed to investigate the effect of ginger (*Zingiber officinale*, Rosc.) roots essential oil on some reproductive characteristics of male Japanese quail. The total of 96, 3 weeks old males Japanese quail (*Coturnixcoturnix 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 group 1 (control) received by orally distilled water (100 μL kg^{-1} body weight) while the other three test groups during the same period, received respectively by the same method 50, 100 and 150 μL kg^{-1} 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. The result revealed that the testicular and vas deferens weights increased significantly ($p<0.05$) in quails treated with 100 and 150 μL kg^{-1} 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 μL kg^{-1} b.w. In conclusion, the ginger root essential oil can be used to boost the male reproductive performances by improving spermatozoa characteristics and fertility rate.

INTRODUCTION

Increase in global population has led to an increase in demand for proteins. Cameroon, like all developing countries, is experiencing exponential population growth of 2.6% per year[1] which creates an imbalance between the demand and supply of animal protein. The shortage of protein, particularly, those of animal origin prevail in most developing countries and Cameroon in particular, estimated at ~13 g/day/head as against 35 g/day/head as prescribed by FAO in 2001. The deficit in animal proteins affects >70% of the Cameroonian population due to the high cost of meat and primary ingredient such as maize. Thus making meat consumption reserved for the privileged minority[2]. To remedy the problem of shortage of animal protein intake, intensive production of short cycle animals such as poultry, should be encouraged. Poultry meat consumption accounts for 9.81 g/day/adult equivalent and eggs accounts for 7.15 g cooked/day in the Cameroonian population[3]. Despite the contribution of the poultry sector, it is still facing managerial and technical problems including poor qualitative and quantitative diet and various diseases that affects growth induce high mortality rates and important economic losses[4]. Among the solutions considered, growth promoters were developed and used to improve feed efficiency and poultry health[5]. The above solution led to the use of synthetic antibiotics and growth hormones in animal feed which resulted to the development of antibiotic resistance in these animals and equally negative impact of these antibiotics on human health. This has in turn led to the prohibition of antibiotic based feed additives for more than one decade. This prohibition has resulted to poor performances and opened new research parts on alternatives to these antibiotics, principally plant base products such as essential oil which have seen their utilization being developed these past few years[6]. The use of plant and plant based products is 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].

Essential oil is the concentrate and hydrophobic liquid of volatile aromatic compounds such as phenolics and polyphenols, terpenoids, saponins, quinine, esters, flavone, flavonoids, tannins, alkaloids and non-voltiles residues. These molecules have many properties (antimicrobial, stimulating animal digestive system, antioxidants, antifungal, antiparasitic, anti-inflammatory) 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, 9]. Based on these activities, Ginger essential oil protects cell DNA and other important molecules from the oxidation responsible for cell death, improves sperm characteristics (mobility and viability) and the fertility of animals. 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 March-June 2020 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’East and at an altitude of 1420 m above sea level. Annual temperature varies between 10 and 25°C. Rainfall ranges from 1500-2000 mm per annum over a 9 months rainy season (March-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[10].

Animal and experimental design: About 96, 3 week’s old male quails weighing between 120 and 130 g, produced from a parents 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 μL kg^{-1} 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 μL kg^{-1} 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. The 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 equipments.

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.

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[11].

Blood sampling and organ weights: At the end of the experiment, 12 quails per treatment were randomly selected and fasted for 24 h, weighed and slaughtered as described by Jourdain[12]. 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^{-3} 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 40× magnification using the scale describe by Tarif *et al*.[13]. Sperm viability was analyzed using hypo-osmotic swelling test[14]. 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.

Table 1: Composition and proximate analysis of the experimental diet

Constituents	Quantity (kg/100 kg)
Corn	60
Bran wheat	4.5
Soybean meal	22
Fishmeal	4.5
Oeister shell	2
Bone meal	2
Premix 5%*	5
Total	100
Chemical composition	
Crude protein (%)	20.15
Metabolizable energy (kcal kg^{-1})	2906.80
Calcium (%)	2.03
Phosphorus (%)	1.27
Lysine (%)	0.44
Methionine (%)	0.14
Natrium (%)	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

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 Ducan post hoc test with the significance level set at 0.05. The p-value was done using the student t-test. A $p<0.05$ 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 AND DISCUSSION

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^{-1} b.w. compared to the control and quails treated with the smallest dose of essential oil (50 μL kg^{-1} b.w.) (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; $p<0.01$).

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^{-1} b.w.

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^{-1} b.w. 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^{-1} b.w. 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^{-1} b.w. was comparable to that of birds receiving 150 μL kg^{-1} b.w. but significantly higher ($p<0.05$) than the value recorded in quails treated with 50 μL kg^{-1} b.w. of essential oil.

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^{-1} b.w. were comparable but significantly ($p<0.05$) higher than the surface recorded in quails treated with 50 μL kg^{-1} b.w. (Table 3). The surface of the cloacal gland is positively and significantly correlated with serum content in testosterone ($\rho = +0.99$, $p<0.01$) and FSH ($\rho = +0.99$; $p<0.01$) (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^{-1} b.w. 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^{-1} of b.w.) (Table 3). The LH content was positively and not significantly correlated 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 μL kg^{-1} b.w. than the value obtained with 50 μL kg^{-1} b.w. (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).

Effects of ginger roots essential oil on the male Japanese quail fertility: The fertility rate increased significantly ($p<0.05$) with 100 (90.71±4.90%) and 150 μL kg^{-1} b.w. of essential oil (88.54±2.02%), compared to that of control quails (81.89±4.91%) and quails treated with essential oil at 50 μL kg^{-1} b.w. (82.14±3.11%) (Fig. 1). The relative weight of the testes was positively and significantly correlated with the

Table 2: Effects of ginger roots essential oil on relative weight of the reproductive organ and sperm characteristics in Japanese quail

		Essential oil doses (μL kg^{-1} body weight)			
Parameters	Control (n = 12)	50 (n = 12)	100 (n = 12)	150 (n = 12)	p-values
Organs weight (g/100 g b.w.)					
Testes	2.01±0.48^{b}	2.08±0.16^{b}	2.63±0.21^{a}	2.54±0.32^{a}	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^{b}	0.05±0.01^{b}	0.07±0.01^{a}	0.07±0.01^{a}	0.00
Sperm characteristics					
Sperm density per gram of tissue (×10^{6})	21.97±0.73	20.54±0.16	23.10±0.60	23.71±0.94	0.88
Motility (%)	61.25±5.20^{c}	65.00±4.56^{bc}	85.00±5.40^{a}	77.63±11.15^{ab}	0.00
Viability (%)	86.33±4.89^{c}	89.95±5.52^{b}	92.17±5.24^{a}	91.08±4.63^{ab}	0.00

$^{a-d}$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

		Essential oil doses (μL kg^{-1} body weight)			
Parameters	Control (n = 12)	50 (n = 12)	100 (n = 12)	150 (n = 12)	p-values
Surface of the cloacal gland (mm^{2})	151.71±20.69^{c}	189.48±20.95^{b}	276.71±25.85^{a}	285.12±28.91^{a}	0.00
LH (mIU mL^{-1})	2.63±0.69^{b}	2.44±0.78^{b}	4.00±0.88^{a}	4.31±0.13^{a}	0.00
FSH (mIU mL^{-1})	18.33±3.84^{b}	20.78±4.79^{b}	26.13±2.75^{a}	27.44±3.41^{a}	0.00
Testosterone (ng mL^{-1})	0.44±0.09^{c}	0.61±0.16^{b}	0.97±0.08^{a}	0.98±0.18^{a}	0.00

$^{a-c}$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

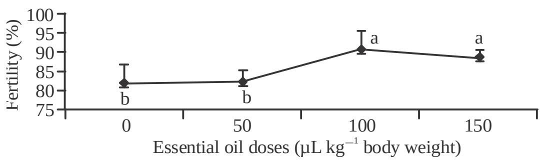

Fig. 1: Effects of ginger roots essential oil on male Japanese quail fertility

fertility rate (ρ = +0.98; p<0.05). The same observation was recorded between sperm motility and fertility rate (ρ = +0.99; p<0.01). A positive and non-significant correlations were recorded between the relative weight of the vas deferens and the fertility rate (ρ = +0.94, p>0.05) and between the surface of the cloacal gland and the fertility rate (ρ = +0.95; p>0.05) (Table 4).

The treatment of male Japanese quail with the ginger rhizomes essential oil at doses of 100 and 150 mg/kg/day for 64 days induced an increase in the relative weight of the sexual organs in treated quails. These results are consistent with those of Khaki *et al*.[15] 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*.[16] who observed an increase in sex organs weight of male rats treated with 600 mg kg^{-1} b.w. 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[17]. 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[16]. Testicular proteins are one of the constituents that ensure the maturation of spermatozoa and it concentration is correlated to testicular growth[16].

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[17]. The testosterone is the major male gonad hormone produced by Leydig cells in the testes under the control of the hypothalamic-pituitary axis[18]. 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[19]. It has been reported that increases in both sperm count and sexual organs weight are an indicator of improved male fertility[17]. Ferrouk *et al*.[20] 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 is 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, Biswas and Arora[21] 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 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 Shanoon *et al*.[24] in ROSS 308 breeders treated with ginger rhizomes essential oil at doses of 5 and 10 kg ton^{-1} of feed for 20 weeks.

The ginger rhizomes 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 Shanoon[24] in broilers fed on 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 μL kg^{-1} b.w. for 64 consecutive days to quails significantly increased their fertility as compared to the control. The present results are in agreement with the findings of Simek *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.

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.

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