Scientific Research Publishing

Open Journal of Veterinary Medicine, 2025, 15(4), 51-66
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

# Effects of *Curcuma longa* (*Tumeric*) Rhizomes Essential Oil on Some Reproductive and Biochemical Parameters in Laying Japanese Quail (*Coturnix coturnix* Japonica)

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**How to cite this paper:** Ngoumtsop, H.V., Tchoffo, H., Deutcheu, S.N., Kana, D.A., Tchouhon, S.N. and Ngoula, F. (2025) Effects of *Curcuma longa* (*Tumeric*) Rhizomes Essential Oil on Some Reproductive and Biochemical Parameters in Laying Japanese Quail (*Coturnix coturnix* Japonica). *Open Journal of Veterinary Medicine*, **15**, 51-66.
https://doi.org/10.4236/ojvm.2025.154004

**Received:** December 30, 2024
**Accepted:** March 29, 2025
**Published:** April 1, 2025



## Abstract

Domestics animals face many environmental factors including temperature fluctuation, high densities in farms that reduce reproductive performance and lead to economic losses in the farmer. The *Curcuma longa* rhizomes essential oil due to its bioactive molecules with various pharmacological properties could mitigate the effects of these factors and boost the performance of animals. This study was conducted to evaluate the effects of *Curcuma longa* rhizomes essential oil on the reproductive parameters in laying Japanese quails and study the effect on egg yolk oxidative stability during storage. A total of 96 female's Japanese quails (*Coturnix coturnix* Japonica) aged 3 weeks and weighing between 122 - 134 g were randomly assigned into 4 dietary treatment groups in a completely randomized design. In 10 weeks, birds in group 1 (control) received orally distilled water (75 µl/kg body weight), while the other three (group tests) during the same period, received respectively by gavage 75, 150 and 225 µl of *Curcuma longa* rhizomes essential oil per kg body weight. At 13 weeks old, eight birds per group in each treatment were randomly selected and slaughtered, and blood samples were collected for analysis. In addition, 12 eggs (4/unit or cage) were randomly picked out from each treatment for three times and the yolk of each egg was manually isolated and homogenized, and egg yolk samples were collected for oxidative parameters analysis.

On the other hand, the remaining females were maintained 4 with one untreated male in identical boxes per treatment, for fertility assessment, eggs characteristics and hatchability traits. Throughout the experiment, biochemical parameters, eggs characteristics, fertility and hatchability traits were analyzed. The serum level of LH, FSH and estradiol; the total hatchability and hatchability of fertile eggs; the egg level of SOD, CAT, and GPx significantly increased ($p < 0.05$) in birds received 150 and 225 µl/kg b.w compared to the control. On the other hand, the embryonic mortality rates decreased significantly ($p < 0.05$) in female quail treated with 225 µl/kg b.w compared to those of birds in control group and those received 75 and 150 µl/kg b.w. The egg level of MDA was significantly decreased ($p < 0.05$) in birds received 225 µl/kg b.w compared to the control group. It was concluded that administration of 225 µl/kg b.w of *Curcuma rhizomes* essential oil enhances bird reproductive hormone levels, hatchability traits and oxidative yolk parameters in female quails. Nonetheless a comprehensive study needs to be performed in order to evaluate the impact of quail dietary *Curcuma longa* rhizome essential oil supplementation on eggs biochemical quality as well as its mechanism of action.

## Keywords

*Curcuma rhizome* Essential Oil, Eggs Characteristics, Females Japanese Quail, Herbal Medicine, Biochemical Parameters and Laying Birds

## 1. Introduction

Essential oils are aromatic, volatile liquids obtained from plant material through steam distillation and named after the plant from which they are derived [1]. They are volatile and highly concentrated in active molecules having diverse properties including antiparasitic, antifungal, antibacterial, anti-inflammatory and antioxidant activities [2]. In animal production, these properties are utilized to limit the loss of energy in favor of growth and reproduction [3].

*Curcuma longa* (turmeric) is a perennial medicinal plant that belongs to the ginger (Zingiberaceae) family and is a major source of prominent polyphenol curcumin [4]. Kamari *et al.* [5] and Tozo *et al.* [6] reported that Curcumin may act as an anti-infectious, anticarcinogenic, antifungal, anti-inflammatory, antiparasitic, antiviral, antioxidant, and antimutagenic agent [7] [8]. Diferuloylmethane is the main phenolic compound of turmeric rhizome powder that has an antioxidant effect [9] [10]. It inhibits lipid peroxidation, scavenges the superoxide anion and hydroxyl radicals [11], and enhances the activities of detoxifying enzymes, such as glutathione-S-transferase and Superoxide dimustase [12]. It also increased the level of antioxidatives enzymes [13]. In addition, Curcumin is a phytoestrogen [14] and can, therefore, interact with the endocrine system, affect the hypothalamo-hypophysial-ovarian axis [15]. Inano *et al.* [16] and Thakur *et al.* [17] reported that this interaction of Curcumin with the endocrine system may be increased respectively the levels of LH and Estrogen in mousse irradiate. Moctar *et* *al*. [18] indicated that supplementation of laying hen diet with *Curcuma longa* root at the rate of 3% increased hatchability traits and eggs weight.

It is widely known that during the later stages of egg incubation, chick embryos may experience stress caused by excessive heat production [19]. The development of the chick embryo is associated with the accumulation of highly polyunsaturated fatty acids within the lipids of several embryonic tissues [20] [21]. This makes embryonic tissues highly susceptible to lipid peroxidation and free radicals through hatching period [22]. Tissue specific features in the susceptibility to lipid peroxidation were found with the brain being the most susceptible to lipid peroxidation at day 25 and in day-old poults of Turkey [22] and at day 15 of incubation period of chicken eggs [20]. Therefore, the integrated antioxidant systems in the chicken tissues are responsible for protection of polyunsaturated fatty acids, protein and DNA from damaging effect of free radicals and toxic products of their metabolism [22]. In such condition, oxidative stress may be a problem during the last days of prenatal and 1st days of postnatal chick life. This leads to decrease hatchability and increase mortality post hatch and consequently economic impact on the poultry industry [23]. These necessitate the development of effective antioxidant capacities in the tissues to prevent lipid peroxidation. The antioxidant system of the embryo and newly hatched chick is based on antioxidant enzymes such as superoxide dismutase, glutathione reoxidase and catalase [24], ascorbic acid [25], reduced glutathione [26] and carotenoids [27].

Several studies have been carried out on the use of *Curcuma longa* rhizomes powder in the diets of broiler chickens to improve growth [28] [29] and reproductive parameters [30]-[32]. These studies showed some positive effects of *Curcuma longa* root powder on reproductive performances but, the effects of *Curcuma longa* rhizomes essential oil which is highly concentrated in active molecules, show limited studies on animal reproduction, especially in birds. The objective of this study is to investigate the possible effects of *Curcuma longa* rhizomes essential oil on some reproductive parameters in laying Japanese quails and study the effect on egg yolk oxidative stability during storage.

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out at Teaching and Research Farm of the Higher Institute of Agriculture and Management of Obala (LN 04˚10', LE 11˚31'). Obala is located about 1420 m above sea level. Climate is Guinean temperate by altitude and about 2157 mm of rainfall spread over a two season from mid-March to mid-Jun and mid-August and mid-October. The temperature is 20˚C - 32˚C with an average to 26˚C and relative humidity generally exceeds 55%.

### 2.2. Plant Material and Essential Oil Extraction

Fresh Curcuma longa 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. It was washed and then ground in a mortar in order to liberate the tissues. Oil extraction was done by hydrodistillation in Laboratory, monastery of Mbabeté, Mbouda Cameroon following the protocol described by [33]. After extraction, the phytochemical screening done according to the methods described by [34] [35] showed the following results (**Table 1**).

**Table 1.** Phytochemical constituents of *Curcuma longa* essential oil.

| Constituents | (+) present; (−) absent |
|---|---|
| Alkaloids | + |
| Triterpenoid | + |
| Steroid | + |
| Flavonoid | + |
| Phenol | + |

## 2.3. Experimental Animal

Ninety-six (96) female Japanese quails (3 weeks old, weight: 122 - 134 g) hatched in the Teaching and Research Farm of the Higher Institute of Agriculture and Management of Obala were used for the experiment. Each bird was identified by a ring bearing his number in one of its paws.

## 2.4. Experimental Design

At the beginning of the experiment, birds were weighed and then assigned randomly into 4 dietary treatment groups in completely randomized design and with 3 replicates of 8 birds each. In 10 weeks, quails in group 1 (control) received orally distilled water (100 µl/kg body weight), while birds of the three test groups during the same period, received respectively by gavage 75, 150 and 225 µl per kg body weight of *Curcuma longa* roots essential oil. The doses used in this study were selected from a pilot study and represent 1/266, 1/133 and 1/89 of LD50 value obtained in quails (20,000 µl per kg b.w) (Personal communication). During the treatment, body weight was weekly measured. At 13 weeks old, eight female quails per treatment were randomly selected and fasted for 24 hours, weighed and slaughtered as indicated by [36], blood samples were collected for biochemical analysis. On the other hand, the remaining females per treatment were maintained 4 with one untreated male in identical boxes under the same environmental conditions, for fertility assessment, egg characteristics and hatchability traits. The birds were raised at a natural nycthemeral rhythm (12h/12h) and 26˚C throughout the treatment period.

Experimental protocols used in this study were approved by the Ethical committee of the Department of Animal Science of the University of Dschang (EC-DAS-UDs 23/02/2015/UDs/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [37].

# 3. Data Collection

## 3.1. Blood and Egg Sampling Preparation

At the slaughter moment, Blood samples were collected from the jugular vein in non-heparinized tubes and then centrifuged at 3000 rpm for 15 min. The resultant serum (supernatant) was stored in 1.5 ml Eppendorf tubes at −20˚C for protein analysis. In addition, 12 eggs (4/unit or sub-groups) were picked out from each treatment on the same day of blood sampling [3]. The yolk of each egg was manually isolated and homogenized with ice-cold isotonic physiological saline (0.154 mol/l; pH = 7.4) in the ratio of 1:9 for 5 min. The homogenate was then centrifuged at 3000 rpm for 30 min and the resultant supernatants were kept at −20˚C for Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) analysis.

## 3.2. Organ Weight

After killing birds, organs including ovary and uterus were carefully removed, rid of adipose tissue, blotted dry and weighed separately. The relative organ weight was calculated using the above formula:

$$\text{Relatif ồrgan wight}(\%) = \frac{\text{Organ weight }(\text{mg})}{\text{Live body weight }(\text{g})} \times 100$$

Egg production and egg weight were recorded daily, during the last 4 consecutive weeks at the end of the experiment [38].

## 3.3. Biochemical Analysis

The egg yolk total protein contents were determined using Biuret methods [39]. The yolk content in malondialdehyde was measured by the thiobarbituric acid method [40]. Catalase (CAT), Superoxide Dismutase (SOD) and peroxidase glutathione (GPx) activities were carried out according to the method of [41]-[43] respectively. Serum LH, FSH and estradiol were determined using a commercial kit (ELISA) (Diagnosis Automation, Inc., Calabasas, USA).

## 3.4. Egg Characteristics, Hatchability Traits and Chick Weight

### 3.4.1. Egg Characteristics

At the start of laying, the eggs were collected every morning per treatment for one week, the weighed using an electronic balance of 500 g capacity and of 0.01g precision to evaluate the weight of eggs using the following formula [44].

The internal and external quality of eggs were evaluated [45]. To achieve this purpose, when the birds were 12 weeks, 12 eggs per treatment were collected for three times. The weight of the egg yolk and Shape were evaluated using respectively an electronic balance of 500 g capacity and 0.01 g precision and digital caliper (Mitutoyo) of 0.01 mm.

#### 3.4.2. Hatchability Traits

A total of 34 healthy eggs per treatment were randomly collected on three separate occasions, each spanning six consecutive days, at 11-,12- and 13-weeks-old. The eggs were weighed individually and then incubated. After 19 days of incubation, the eggs hatched. Any unhatched eggs were the examined and cracked and were classified as either infertile or having experienced embryonic mortality.

At hatching after incubation, the following indices were calculated [44] for each replicate according to the following formulas:

$$\text{Fertility rate }(\%) = \frac{\text{Number of fertile eggs}}{\text{Total number of eggs incubated}} \times 100$$

$$\text{Hatchability rate of fertile eggs }(\%) = \frac{\text{Number of chicks hatched}}{\text{number of fertile eggs}} \times 100$$

$$\text{Total hatchability rate }(\%) = \frac{\text{Number of chicks hatched}}{\text{number of eggs incubated}} \times 100$$

$$\text{Embryonic mortality} = \frac{\text{Number of eggs with dead embryos}}{\text{Total number of eggs incubated}} \times 100$$

### 3.5. Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) at $p < 0.05$ [46]. When differences were significant, Duncan multiple range test was used to separate means [47]. All statistical analyses were performed using SPSS 21.0.

## 4. Results

### 4.1. Effects of *Curcuma longa* Rhizomes Essential Oil on Body and Reproductive Organs Weight in Female Japanese Quails

The effects of *Curcuma longa* rhizomes essential oil on the body weight and the relative reproductive organ weights are shown in **Table 2**. *Curcuma longa* roots essential oil had no significant effect ($p > 0.05$) on relative uterus weight. Furthermore, the final body weight and the body weight gain increases significantly ($p < 0.05$) in female quail treated with 150 and 225 µl/kg b.w compared to those of birds in control group and those received 75 µl/kg b.w. The relative ovary weight increases significantly ($p < 0.05$) in female quail treated with 75 and 225 µl/kg b.w compared to that of birds in control group.

#### 4.1.1. Egg Characteristics

Data in **Table 3** showed that, egg weight increased significantly ($p < 0.05$) in Japanese quail received *Curcuma longa* rhizomes essential oil at doses 150 and 225 µl/kg b.w compared to those of birds of control group and those received 75 µl/kg b.w. On the other hand, oral administration of *Curcuma longa* rhizomes essential

Table 2. Effects of *Curcuma longa* rhizomes essential oil on the final body weight, body weight gain and the relative weight of the reproductive organs in female Japanese quail.

| Parameters | Control Essential oil doses (µl/kg body weight) | | | | |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | p |
| Initial body weight (g) | 136.63 ± 23.68 | 138.88 ± 30.21 | 136.13 ± 19.83 | 135.88 ± 19.93 | 0.96 |
| Final body weight (g) | 207.50 ± 28.77$^{c}$ | 216.88 ± 31.35$^{c}$ | 250.25 ± 28.17$^{b}$ | 289.13 ± 16.99$^{a}$ | 0.00 |
| Body weight gain (g) | 70.88 ± 39.07$^{c}$ | 78.00 ± 26.20$^{c}$ | 114.13 ± 30.89$^{b}$ | 153.25 ± 29.25$^{a}$ | 0.01 |
| **Organ weight (g/100g b.w)** | | | | | |
| Ovary | 4.50 ± 1.92$^{c}$ | 6.62 ± 1.06$^{ab}$ | 5.75 ± 0.88$^{bc}$ | 7.83 ± 1.09$^{a}$ | 0.00 |
| Uterus | 0.98 ± 0.06 | 1.00 ± 0.08 | 1.00 ± 0.86 | 1.00 ± 0.07 | 0.27 |

a, b, and c: on the same line, means with the same letter are not significantly different (p > 0.05).

Table 3. Effects of *Curcuma longa* rhizomes essential oil on egg characteristics, hatchability traits and chick weight in female Japanese quail.

| Parameters | Control Essential oil doses (µl/kg body weight) | | | | |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | p |
| **Egg characteristics** | | | | | |
| Egg weight (g) | 7.63 ± 3.73$^{c}$ | 10.38 ± 3.54$^{bc}$ | 10.88 ± 1.64$^{ab}$ | 11.63 ± 0.51$^{a}$ | 0.01 |
| Yolk egg (g) | 3.75 ± 1.38$^{c}$ | 5.13 ± 1.12$^{b}$ | 5.25 ± 1.48$^{b}$ | 6.75 ± 1.03$^{a}$ | 0.00 |
| Shape index | 0.75 ± 0.04 | 0.77 ± 0.02 | 0.75 ± 0.05 | 0.79 ± 0.04 | 0.32 |
| **Hatchability traits (%)** | | | | | |
| Fertility | 78.67 ± 5.65$^{b}$ | 85.33 ± 5.75$^{ab}$ | 87.00 ± 5.77$^{ab}$ | 95.37 ± 5.60$^{a}$ | 0.02 |
| Total hatchability | 71.65 ± 4.79$^{b}$ | 71.67 ± 5.75$^{b}$ | 85.01 ± 1.77$^{a}$ | 88.33 ± 5.70$^{a}$ | 0.03 |
| Hatchability of fertile eggs | 72.66 ± 4.22$^{b}$ | 75.23 ± 6.23$^{b}$ | 92.49 ± 4.40$^{a}$ | 96.30 ± 3.41$^{a}$ | 0.02 |
| Embryonic mortality | 8.92 ± 2.78$^{a}$ | 8.33 ± 3.21$^{a}$ | 7.40 ± 2.30$^{a}$ | 3.70 ± 1.41$^{b}$ | 0.05 |
| Chick's weight (g) | 6.38 ± 1.06$^{b}$ | 6.38 ± 0.74$^{b}$ | 7.63 ± 1.06$^{a}$ | 7.88 ± 0.83$^{a}$ | 0.04 |

a, b and c: on the same line, means with the same letter are not significantly different (p > 0.05), p = probability; Values are presented as Means ± standard deviation.

oil had no significant effects ($p < 0.05$) on egg shape index. The yolk eggs weight increased in dose-dependent manner with curcuma longa essential oil.

### 4.1.2. Hatchability Traits

As presented in Table 3, fertility increased significantly ($p < 0.05$) in Japanese quails treated with 225 µl/kg b.w of *Curcuma longa* roots essential compared to those of control group birds. In addition, total hatchability, and hatchability of fertile eggs increased significantly ($p < 0.05$) in Japanese quails treated with 150 and 225 µl/kg b.w of *Curcuma longa* roots essential compared to those of control group birds and to those which received 75 µl/kg b.w. Furthermore, the embryonic mortality rates decreased significantly ($p < 0.05$) in female quail treated with 225 µl/kg b.w compared to those of birds in control group and those received 75 and

225 µl/kg b.w.

#### 4.1.3. Chick's Weight

Chick's weight significantly ($p < 0.05$) increased in female quails which received 150 and 225 µl/kg b.w of *Curcuma longa* rhizomes essential oil compared to those of control birds and those received 75 µl/kg b.w (Table 3).

### 4.2. Effects of *Curcuma longa* Essential Oil on Biochemical Parameters in Female Japanese Quails

#### 4.2.1. Egg yolk Oxidative Stress Characteristics

The effects of Curcuma longa rhizome essential on yolk content in malondialdehyde (MDA) and antioxidant enzymes including peroxidase glutathione (GPx), catalase (CAT) and Superoxide Dismutase are shown in Table 4. The yolk MDA concentration decreased significantly ($p < 0.05$) in Japanese quails treated with 150 and 225 µl/kg b.w of Curcuma longa roots essential compared to those of control group birds. The SOD activity increased significantly ($p < 0.05$) in Japanese quails treated with 150 and 225 µl/kg b.w of Curcuma longa roots essential compared to those of birds in control group. The GPx activity increased significantly ($p < 0.05$) in Japanese quails treated with 75 and 225 µl/kg b.w of Curcuma longa roots essential compared to those of birds in control group. The CAT activity increased significantly ($p < 0.05$) in Japanese quails treated with 150 and 225 µl/kg b.w of Curcuma longa roots essential compared to those of control group and to those which received 75 µl/kg b.w. The oral administration of *Curcuma longa* rhizomes essential oil had no significant effects ($p < 0.05$) on total protein's level. A negative and significant correlation was recorded between the MDA and: the total protein's ($\rho = -0.72$; $p < 0.05$); the embryonic mortality ($\rho = -0.92$; $p < 0.05$); the hatchability of fertile eggs ($\rho = -0.75$; $p < 0.05$) and the fertility rate ($\rho = -0.95$; $p < 0.05$).

#### 4.2.2. Reproductive Hormone

The effects of *Curcuma longa* rhizomes essential oil on reproductive hormone as shown in Table 4 reveal that, the serum level of FSH, LH and Oestradiol increased significantly ($p < 0.05$) in Japanese quails treated with 150 and 225 µl/kg b.w of *Curcuma longa* roots essential compared to those of control group.

**Table 4.** Effects of *curcuma longa* rhizomes essential oil on biochemical yolk parameters in Japanese quail.

| Biochemical parameters yolk | Control Essential oil doses (µl/kg body weight) | | | | *p* |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| Proteins (g/dl) | 3.38 ± 0.51 | 3.64 ± 0.51 | 3.45 ± 0.34 | 3.74 ± 0.48 | 0.27 |
| **Oxidative Stress (µg/g of yolk)** | | | | | |
| MDA | $0.064 \pm 0.02^{a}$ | $0.053 \pm 0.02^{ab}$ | $0.048 \pm 0.02^{b}$ | $0.046 \pm 0.02^{b}$ | 0.00 |
| SOD | $375.64 \pm 51.29^{b}$ | $426.07 \pm 27.47^{ab}$ | $439.54 \pm 26.63^{a}$ | $451.79 \pm 41.01^{a}$ | 0.02 |
| GPx | $389.67 \pm 60.28^{b}$ | $446.75 \pm 47.01^{a}$ | $448.09 \pm 26.47^{ab}$ | $456.80 \pm 49.01^{a}$ | 0.03 |
| CAT | $5.30 \pm 0.53^{b}$ | $5.54 \pm 0.81^{b}$ | $6.56 \pm 0.44^{a}$ | $6.60 \pm 0.58^{a}$ | 0.04 |

**Continued**

| | | | | | |
|---|---|---|---|---|---|
| **Reproductive hormone** | | | | | |
| LH (mIU/ml) | 3.03 ± 0.28$^{c}$ | 3.30 ± 0.52$^{bc}$ | 3.90 ± 0.55$^{b}$ | 4.83 ± 0.34$^{a}$ | 0.00 |
| FSH (mIU/ml) | 17.84 ± 1.44$^{b}$ | 19.46 ± 1.47$^{b}$ | 26.85 ± 1.28$^{a}$ | 27.81 ± 1.11$^{a}$ | 0.03 |
| Oestradiol (ng/ml) | 57.43 ± 2.85$^{c}$ | 61.23 ± 7.54$^{bc}$ | 67.00 ± 5.78$^{b}$ | 81.98 ± 1.92$^{a}$ | 0.00 |

(a, b, c) On the same line, values affected by the same letter were not significantly different ($p > 0.05$); p = probability; Values are presented as Means ± standard deviation.

## 5. Discussion

The improved performance of the birds' tread with 225 µl/kg b.w of *Curcuma longa* roots essential oil in the present study agrees with the finding of Suvanated *et al.* (2003) [48] and Durrani *et al.* [49] who reported that broiler chicks fed dietary turmeric powder had a higher final body weight, and body weight gain. However, Tchoffo *et al.* [3] reported that ginger roots essential oil did not have effect on the growth characteristics in female quail. The variation could be as a result of the nature of essential oil. The current study has also shown that beyond 225 µl/kg b.w, there was no additional benefit from consumption of *curcuma longa* roots essential oil. In fact, it appears that this is the tolerable level for the birds as it could be seen that administration of female quail up to 225 µl/kg b.w enhanced the egg yolk MDA. The improved performance in the current study could be attributed to the antioxidant activities of curcumin which could have stimulated feed intake and protein synthesis by the enzymatic activities in the birds [10]. This trial has revealed that inclusion of 225 µl/kg b.w of turmeric essential oil is sufficient to improve the performance of bird. This result is an indication that turmeric at this level enhanced efficient use of nutrients in the birds. Superoxide dismutase is one of the antioxidant enzymes, which are capable to minimize oxidative stress in the organelles [50]. The higher level of SOD, GPx and CAT activities in the yolk eggs in the present study is in agreement with the observation of Tchoffo *et al.* [51] who reported that ginger essential oil increased activity of SOD, GPx and decreased MDA level in yolk eggs in female quail. The same results were observed by Zainali *et al.* [52] who reported that turmeric powder enhanced the antioxidant status of heat-stressed broilers by improving the activity of glutathione peroxidase and superoxide dismutase and reducing the concentration of MDA. The high-performance indices recorded in the bird's treated group may be explained by the inhibition of the deleterious effect of lipid peroxidation brought about by the decreased free radical generation as reflected by low yolk MDA and high SOD, GPx and CAT activities. Altan *et al.* [53] [54] reported that the elevated level of SOD, GPx and CAT may be an indication of a protective mechanism against oxidative stress and lipid peroxidation.

The degree of lipid peroxidation is usually a biomarker of ROS-mediated damage [55] and the concentration of MDA is often used as indicators of lipid peroxidation [56]. MDA is a product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage [32]. MDA of the birds received curcuma longa essential oil was lower than those of the birds in the control group. This may be due to antioxidant compounds (Curcumin) present in curcuma longa essential oil transferred to eggs and deposited into yolk. This antioxidant scavenges superoxide anion and hydroxyl radicals. Masuda *et al.* [57] stated that, an antioxidant mechanism of curcumin in polyunsaturated lipids was proposed, which consisted of an oxidative coupling reaction at the 3-position of the curcumin with the lipid and a subsequent intramolecular Diels-Alder reaction. [58] suggested that dietary turmeric lower lipid peroxidation by enhancing the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase).

Egg and yolk weights increased in quails exposed to the *curcuma longa* rhizome essential oil with reference to the control. These results are in agreement to those reported by Tchoffo *et al.* [51] with ginger essential oil in laying Japanese quail. The increase of egg and yolk weight in treated quails is related to the phenolic compounds because of their antioxidant property would reduce the peroxidation of cells or nutrients involved in the production of eggs and subsequently promote its good formation [3]. A negative and significant ($\rho = -98$; $p < 0.05$) correlation was found between the level of MDA and egg weights, while the positive and significant ($\rho = +0.92$; $p < 0.05$) correlation was found between the egg and the yolk weights. Based on these results, the level of MDA decreases while the egg weight increases and the egg yolk weight increases as the egg weight increases. The increase in egg component provides sufficient nutrients and centrally placed yolk to support embryonic growth and development [3].

Our study revealed that the *curcuma longa* rhizomes essential oil at dose of 150 µl/kg b.w caused significant ($p < 0.05$) decrease of embryonic mortality compared to those of control group. The low level of embryonic mortality recorded in the birds in the group who received 150 µl/kg b.w may be explained by the inhibition of the deleterious effect of lipid peroxidation brought about by the decreased free radical generation as reflected by low egg yolk MDA and high SOD, GPx and CAT activities.

The present study showed a significant increase in estradiol with the increase of *curcuma longa* rhizomes essential oil dose. The increased estradiol level may explain by the presence of curcumin which is a phytoestrogen [14] [15] compound content in the essential oil of *curcuma longa* and can, therefore, interact with the endocrine system, and may stimulate the synthesis of estradiol by acting on the hypothalamic-pituitary-gonad axis. Estrogens are steroid hormones which, together with other hormones, control the ovulatory cycle in the female animal [35]. This estrogen acts in a feedback mechanism, influencing the production of follicle stimulating hormones FSH and LH from the pituitary gland [59]. It is known that the FSH in turn promotes the development of the immature ovarian follicles, which increases the production of estrogen from the ovary. This is readily done if normal quantities of exogenous estrogen are administered, thus prevent ovulation by stimulating the release of the gonadotropin releasing factor from hypothalamus, that is exogenous hormones exert positive feedback on the hypothalamus in a manner similar to that by the naturally occurring hormones.

Our finding shows significant increase level of LH and FSH in female Japanese quails which received the essential oil compared to the control. The similar observation was reported by Mengjie *et al*. [32] in the Hy-Line brown hens exposed to heat stress and treated with curcuma roots powder. We suggest that the increase level of LH and FSH in the present study were caused by the action of curcumin which has a strong phyto-estrogenic properties [31] and can, therefore, interact with the endocrine system [15].

In the present study, it was observed that: fertility, total hatchability and the hatchability of fertile eggs rates increased with essential oil dose. These results are comparable to those found by Ali *et al*. [60] who reported that individually added of 0.5% to 1% curcuma to chicken diets increased fertility and hatchability. Tchoffo *et al*. [3] reported the same result in the female Japanese quail who received orally 100 µl/kg b.w of essential oil of ginger. In fact, the *Curcuma longa* rhizome essential oil contains a number of antioxidants compounds such as tetrahydro-curcuminoids [10]; curcumin, dimethoxy-curcumin, and bisdemethoxy-curcumin [61]. These properties increased oxidative metabolism, especially in the final few days before hatching, as a normal result of embryonic growth. It is reported that over increment lipid peroxidation may lead to tissue damage [23], whereas diets with antioxidants properties as ginger may protect the embryo and therefore increase hatchability and chick's survival rate. A negative and significant correlation was recorded between the MDA and: the embryonic mortality ($\rho = -0.92$; $p < 0.05$); the hatchability of fertile eggs ($\rho = -0.75$; $p < 0.05$) and the fertility ($\rho = -0.95$; $p < 0.05$).

## 6. Conclusion

The results of this study suggest that the inclusion of *Curcuma longa* rhizome essential oil in Japanese quail diet can improve the reproductive performances and oxidative yolk parameters in female quails. The effects on the quail production performance seemed to be dose dependent and *Curcuma longa* rhizome essential oil at the highest tested level (225 µl/kg.b.w) was the most effective treatment. Based on these findings, *Curcuma longa* rhizome essential oil can be considered a promising feed additive for managing reproductive processes in female poultry at level of 225 µl/kg.b.w. However, further studies are needed to conclude on the effect of *Curcuma longa* rhizome essential oil on egg quality and the quality of the poultry products as well as its mechanism of action.

## Author Contributions

Conceptualization, H.V.N.; methodology, H.V.N., H.T., and F.N.; software, H.V.N., H.T. and F.N.; validation, H.V.N., H.T., and F.N.; formal analysis, H.V.N., H.T., and F.N.; investigation and resources, H.V.N., H.T., S.N.D., D.A.K., S.T.N., and F.N.; data curation, H.V.N., H.T., S.N.D., D.A.K., S.T.N., and F.N.; writing original draft preparation, Z.M. and Z.N.; writing—review and editing, H.V.N., H.T., S.N.D., D.A.K., S.T.N., and F.N. All authors have read and agreed to the published version of the manuscript.

## Institutional Review Board Statement

Experimental protocols used in this study were approved by the ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [39].

## Data Availability Statement

Qualified researchers can obtain the data from the corresponding author.

## Funding

The authors declare that no financial help was received.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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