

Phytoremediation Potential of *Chromoleana odorata* Leaf Powder, as a Feed Supplement against Glyphosate Damage on the Growth Traits, Reproduction and Biochemical Parameters of Japanese Quails

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Abstract

Background: Glyphosate-based herbicides (GBHs) are considered the world's most widely used herbicides in agriculture and households for controlling invasive weeds. Through bio-accumulation, residues of GBHs are detected in water, soil, crops, and food products, potentially exposing non-target organisms, such as humans, wildlife, and livestock, leading to food poisoning and even death. However, data on the potential of suitable medicinal plants to mitigate the toxic effects of GBHs on bird productivity and health are limited. Aims: This study aimed to evaluate the safety of Chromolaena odorata leaf powder (COLP) in mitigating the toxic effects of glyphosate on the production performance and biochemical parameters of Japanese quail. Materials and Methods: COLP was used as a supplement in the formulation of four experimental diets tested in this study. The diets were: T0 (only basal diet), T1 (basal diet supplemented with 1% COLP), T2 (basal diet + glyphosate 655 mg/L in drinking water), and T3 (basal diet + 1% COLP + glyphosate at 655 mg/L in drinking water). Each diet was fed to a total of 120 quails (male and female) for a 9-week period. Quail growth, reproduction, carcass characteristics, and blood biochemical parameters were collected and used as indicators for assessing COLP efficacy and safety. Results: The results showed significant differences ($P \le 0.05$) in feed intake, live weight, body weight gain, feed conver-

sion ratio, and carcass parameters (breast, thigh, wing, and neck weight). Biochemical parameters such as ALAT, ASAT, creatinine, urea, and protein levels also showed significant differences between the treatment groups. Glyphosate did not induce negative effects on the quails, while COLP supple-mentation improved growth parameters (feed intake, live weight, weight gain, and feed conversion ratio) and carcass traits (proportion of different body parts relative to live weight). Glyphosate increased serum concentrations of transaminases (ALAT, ASAT), urea, and creatinine. COLP-treated groups also showed improved egg laying, fertility, and hatchability compared to groups exposed to glyphosate alone, indicating that COLP supplementation enhances reproductive parameters. Conclusion: COLP can be used as a phytobiotic feed supplement to improve growth performance (feed intake, body weight gain, feed conversion ratio) and reproductive performance (egg laying rate, fertility, hatchability, and embryonic mortality) in Japanese quail. For animals intoxicated by GBHs, COLP as a feed additive can help mitigate the toxic effects of GBHs on growth and reproductive performance. However, further studies are needed to determine the optimal COLP dose for mitigating the harmful effects of GBHs on quail productivity. Additional implications of these fin-dings are discussed.

Keywords

Glyphosate, *Chromoleana odorata*, Japanese Quail, Toxicity, Growth, Reproduction, Herbal Medicine, Weeds, Herbicides, Food Poisoning

1. Introduction

Glyphosate-based herbicides (GBHs) are broad-spectrum herbicides, most frequently used worldwide, whose application has extended beyond agriculture to include the maintenance of green spaces, gardens, roads, and railroads [1]. It is a non-selective, systemic foliar herbicide absorbed by the leaves, exhibiting generalized action on the plant. GBHs are among the most controversial agrochemicals. Residues of this herbicide have been detected in soil, water, animal tissues across taxa, foodstuffs, and drinking water contaminated by rain and surface runoff, and may leach into groundwater. This suggests that the herbicide may increase exposure routes in animals and humans [2] [3].

Glyphosate has long been considered non-toxic to animals [4], as its primary mode of action is the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) enzyme in the shikimate pathway, present in plants and certain microbes. Since this pathway is absent in animals, it was initially believed that glyphosate does not affect them, making its mechanism of action exclusive to plants [5].

However, recent concerns about the adverse effects of GBHs have gained significant attention, both from the public and decision-makers. Evidence is rapidly accumulating regarding the negative impact of glyphosate on the development, phenotype, fitness, and behavior of various organisms, including reptiles, fish, amphibians, arthropods, mollusks, echinoderms, annelids, and mammals [4]. GBH residues are commonly found in the food chain due to their persistence in plants, water, and soil [6].

In rats, irreversible liver damage was reported after consuming 4.87 mg/kg body weight (bw) of glyphosate every other day for 75 days [7]. Hematological, biochemical, and oxidative alterations were observed at glyphosate doses of 50 and 500 mg/kg bw/day [8]. In the Japanese quail model, exposure to GBHs resulted in a decrease in embryonic development within the eggs [6] and delayed feather development in GBH-exposed parents compared to controls. Glyphosate residues were also detected in eggs, muscles, and the liver [4].

There is an urgent need to eliminate the use of the most toxic pesticides, including carcinogens, mutagens, reprotoxins (CMRs), and certain endocrine disruptors (EDs) [9]. As a result, traditional medicine has become an integral part of primary healthcare systems in many countries [10]. In livestock farming, various medicinal plants are used to control pathologies and serve as phytobiotic feed additives [11]. One such plant is *Chromolaena odorata* Linn., commonly known as Siam grass. It is an invasive herbaceous plant widely distributed in the humid tropics and subtropics of Africa and Asia, where it causes significant agroecological damage. Previous studies on *C. odorata* have identified bioactive compounds such as phenolics, flavonoids, and alkaloids, which exhibit anticancer, antioxidant, and anti-inflammatory properties [12]. *C. odorata* is also used in traditional medicine to treat diabetes, diarrhea, asthma, and skin infections. Moreover, its leaves are relatively rich in crude protein, dry matter, vitamins, and minerals [13].

The protective effects of plants against pesticide-induced damage to sexual characteristics and testicular histology in mammals have been demonstrated elsewhere [14]. While some studies have been conducted in birds [15] [16], data on this topic remain scarce, and few plants have been investigated. To provide a more comprehensive understanding of the potential of medicinal plants to mitigate the toxic effects of GBHs, Japanese quails were used as a model organism for experimental studies. Therefore, the primary objective of this study is to investigate the protective effects of *Chromolaena odorata* leaf powder (COLP) against glyphosate-induced toxicity in Japanese quails. More specifically, the effects of *C. odorata* feed supplementation on growth performance, biochemical parameters, and reproductive outcomes of glyphosate-intoxicated Japanese quails are assessed.

2. Materials and Methods

2.1. Ethical Approval

All animal care and protocol were carried out according to the guidelines (OIE ethical use of experimental animal) provided and supported by the University of Ngaoundere ethical community review committee. There was constant monitoring of the adherence to the procedures and resource provision.

2.2. Experimental Site

The experiments and laboratory analysis were conducted in the Ngaoundere which is the capital of the Vina Division in the Adamawa region, Cameroon. The geographical coordinate of the study area is located between 7°09' to 7°70' north latitude and 13°52' to 13°70' east longitude. The Adamawa Region has a Sudano-Guinean climate which is characterized by two seasons: a rainy season (April to October) and a dry season (November to March). Annual rainfall of the Adamawa region fluctuates between 900 and 1500 mm and the average annual temperature ranges from 23°C to 25°C [17]. The average annual humidity of the Adamawa is 70%. The Vegetation within the study area is essentially shrub or tree savannah [18]. The soil study area is characterized by ferritic texture, with brown or red, and hydromorphic structure. In the Adamawa region, soils are generally fertile and suitable for agro-pastoral activities. Agriculture in the Adamawa region is based essentially on the production of cereals (maize), tubers (cassava and sweet potatoes), fruit trees (mangoes and avocados) and legumes such as groundnuts. Livestock is mainly cattle, sheep and goats. Poultry farming remains underdeveloped [19]. A map of the study area is presented in Figure 1.



Figure 1. Map of the study area.

2.3. Preparation of Chromoleana odorata Feed Supplement

The plant material consisted of the *Chromoleana odorata* plant, which had been ground to a powder [13]. The fresh leaves of *Chromoleana odorata* were collected in the town of Ngaoundere and were then defoliated and dried for 2 to 3 days in the shade until they became crumbly [20]. They were then ground into flour using a mortar and pestle until a digestible powder was obtained. The powder obtained was stored in a plastic box in a dry place at room temperature until use [21] [22] [23].

2.4. Experimental Procedure

2.4.1. Animal Materials and Housing

A total of 120 quails, 35 days' old each with an average live weight of 120 ± 0.68 g, were sourced locally. They were divided into 12 batches of 10 birds each, to create comparable batches in terms of weight. The animals were housed in wire cages (70 cm × 90 cm) arranged in a 3-level system, made of board and low-mesh wire mesh (1.5 cm) at a density of 28 birds/m2, given the small size of the birds.

2.4.2. Herbicide Preparation

The herbicide used in our trials was glyphosate, known under the trade name GLYPHADER 36% (360 g/L), a light-yellow liquid herbicide distributed by SODEAC ("Société de Développement Agronomies du Cameroun") in Douala. This glyphosate base herbicide (GBH) was chosen since it is the most readily available herbicide in this region, so commonly used to control weeds but there is little awareness of its harmful effects. Preparation of the herbicide is in 4998.2 ml of water, 1.8 ml of herbicide solution was added, corresponding to 655 mg/L of glyphosate. This GBH concentration (655 mg/L) used, was referred to the previous work done by Mutwedu *et al.* [24] on guinea pigs where they showed that a concentration of 560 mg/kg body weight was considered as an LD_{50} on rats [25] [26] which is a range that can induce toxicity developmental in laboratory animals [27]. Further, the work of Kouamo *et al.* [28] also showed that using a concentration of 655 mg/L of Cypermethrin-based herbicide induces toxicity in female Japanese quails.

2.4.3. Preparation of the Experimental Diet

1) Basal diet

The basal diet contained 20.18% crude protein and 3013.78 kcal of metabolizable energy. The centesimal composition and calculated chemical values are summarized (Table 1).

2) Experimental design

From this basic diet, four experimental diets corresponding to treatments T0 (receiving the basic diet); T1 (basic diet with 1% *C. odorata* leaf powder); T2 (basal diet treated with glyphosate at 655mg/L water), and T3 (basal diet incorporated with 1% *C. odorata* leaf powder and treated with glyphosate at 655 mg/L of water).

120 4-week-old quails (60 \ddagger and 60 \updownarrow) of comparable weight (120 g ± 13.09 g) were divided into 12 batches. Each of the 4 experimental diets was randomly

Ingredients	Quantity in Kg
Corn	63
Wheat bran	4
Soya	14
Fish meal	5
Shell Fish	1
Bone meal	1
Concentrate 5%	5
Palm oil	2
Peanut cake	5
Total	100
Calculated chemical charact	eristics
Protein content (%)	20.18
Metabolizable Energy (kcal/kg)	3013.78
Energy/Protein	149.34
Fat (%)	5.45
Calcium (%)	1.41
Phosphorus (%)	0.62
Lysine (%)	1.16
Methionine (%)	0.44

Table 1. Growth-phase feed composition and calculated chemical characteristics (%DM).

DM: Dry mater; %: percentage.

assigned to 3 batches in a completely randomized design comprising 4 treatments (T0, T1, T2, and T3). Each treatment was repeated 3 times. Water and feed were distributed *ad libitum* throughout the trial, and animals in all batches benefited from similar rearing conditions.

2.5. Data Collection

Throughout the trial period, data were collected on feed intake, Body weight, Feed Conversion Ratio, laying rate, and egg measurements. At the end of the test period (at 12 weeks of age), 6 birds per treatment (01 male and 01 female per batch) were sacrificed on an empty stomach, then data were collected on carcass characteristics according to the method described by Genchev and Mihaylova [29], blood collected for biochemical assays and finally the weights and histology of the organs collected (heart, liver, kidney, testes).

2.5.1. Feed Intake

Feed was weighed at the beginning of the week and distributed daily. Leftovers from each experimental unit were also weighed at the end of the week, using an electronic scale with a 5000 g capacity and 1 g accuracy. Weekly feed intake (FI)

was calculated as the difference between the quantity of feed distributed and the quantity of leftover feed for each experimental unit.

2.5.2. Body Weight and Body Weight Gain

At the start of the trial and every 14 days thereafter, the animals were weighed fasting in the morning using an electronic scale with a 5000 g capacity and 0.1 g accuracy. Weight gain was calculated as the difference between two consecutive body weights.

2.5.3. Feed Conversion Ratio (FCR)

Data on feed intake (FI) and average weight gain every two weeks were used to calculate the feed conversion ratio throughout the growth phase (FCR) as follows:

$$FCR = \frac{Feed Intake(g)}{Weight gain(g)}$$

2.5.4. Carcass Characteristics

After the sacrifice and dissection of birds, data were collected on carcass weight, liver, heart, gizzard, head, thighs, breast, wings, and legs. The data collected were used to determine the following variables:

Carcass weight = Live weight - Untargeted (blood + feathers + viscera) Weight

Carcass yield (%) =
$$\frac{\text{Carcass weight}(g)}{\text{Life weight}(g)} \times 100$$

The relative proportion of organs (%) = $\frac{\text{Weight of part or organ}(g)}{\text{Life weight}(g)} \times 100$

2.5.5. Biochemical Characteristics

Blood sample was collected and centrifuged at 1500 rpm for 15 minutes. The serum was then collected and stored at -20 °C until for assay [13] [28]. Biochemical parameters assayed were creatinine, urea, Alanine Amino-Transferase (ALAT), Aspartate Amino-Transferase (ASAT), and proteins. Kinetic assays were performed according to standard procedures described by the manufacturer, using kits supplied by Sunshine Diagnostics [24]. ALT and ASAT were expressed in International Units (IU/I) and creatine urea in mg/dl.

2.5.6. Histology of the Organs

Right after sacrifice, the left testicle, liver, and kidneys of each animal were removed and immersed in a 10% neutral buffered formalin solution for fixation. Then, to prevent these organs from crumbling at the time of cutting, they were transferred to Bouin's solution until the histological assessment [30].

2.5.7. Reproductive Parameters

1) Weight and testicle measure

After the quails had been sacrificed, testes were removed and individually weighed using an electronic scale with a capacity of 100 g and a precision of 0.01 g. Diameter and height were then measured using a digital caliper with a 150 mm range and 0.01 mm accuracy. The data obtained enabled to calculate:

The shape index = $\frac{\text{Diameter}(\text{mm})}{\text{Height}(\text{mm})}$ Weight ratio = $\frac{\text{Weight left testicles}(g)}{\text{Weight right testicles}(g)}$ Gonado-Somatic Index (%) = $\frac{\text{Testis weight}(g)}{\text{Body weight}(g)} \times 100$

2) Laying performances and incubation characteristics

The egg-laying performance of the Japanese quails was assessed to verify the effects of different experimental diets on egg production. Data collected for laying performance evaluation included the age at the first egg laid or the age of sexual maturity, the number of eggs collected daily during the same period, and the weekly laying rate were determined as:

Weekly laying rate
$$(\%) = \frac{\text{weekly number of eggs laid}}{\text{Number of female } \times 7} \times 100$$

For 4 weeks, the eggs obtained in each batch were collected daily, counted, and weighed using an electronic balance with a capacity of 500 g and an accuracy of 0.01 g. Egg diameter and height will be measured (mm) using a digital calliper with a range of 0 - 150 mm and an accuracy of 0.01 mm. To assess fecundity and hatchability, incubation was carried out using an automatic turning incubator. Eggs were collected over seven days in the twelfth week. These eggs were subjected to a selection process, with small eggs (weighing less than 9 g), very large eggs (weighing more than 14 g), cracked eggs, and broken eggs. Eggs with mole shells were eliminated. The eggs selected for incubation were weighed, measured, marked, and stored before being introduced into the incubator.

Incubation was carried out at a temperature of 37.5 °C and a relative humidity of 65% [31]. Eggs were turned automatically, the temperature was reduced to 36.5 °C, and relative humidity was raised to 75%. At the end of incubation, the number of hatched eggs was determined by counting quail, confirmed by empty shells. No-hatched eggs were collected, counted, and opened to determine the level of embryo development. These various operations enabled to evaluate several parameters: the weekly laying rate (%) (WLR), Proportion of eggs to live weight (%) (PELW), Shape index (SI), Fecundity rate (FR), Apparent hatchability rate (AHR), Reel hatching rate (RHR), Embryo mortality rate (EMR) (%).

$$WLR = \frac{\text{Number of eggs laid}}{\text{Number of females} \times 7} \times 100$$

$$PELW = \frac{\text{Average egg weight}(g)}{\text{Average live weight of the female}(g)} \times 100$$

$$SI = \frac{\text{Large diameter}(\text{mm})}{\text{Height}(\text{mm})}$$

$$FR = \frac{\text{number of eggs hatched} + \text{number of eggs with embryos}}{\text{Rescale}}$$

number of eggs incubated

$$AHR = \frac{\text{number of eggs hatched}}{\text{number of eggs incubated}} \times 100$$
$$RHR = \frac{\text{number of eggs hatched}}{\text{number of eggs with one embryo} + \text{number of eggs hatches}} \times 100$$
$$EMR = \frac{\text{number of eggs with one embryo}}{\text{number of eggs incubated}} \times 100$$

At the end of the incubation period, unhatched eggs were opened to identify fertilized but unhatched eggs and the stage of development at which embryonic mortality occurred. Based on the embryo development stage, mortalities were classified into four categories: very early, early, late, and very late. The different incubation stages are described (**Figure 2**).



Figure 2. Different embryonic mortality stages: 1: very early; 2: early; 3: late; 4: very late.

2.6. Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 25 [6]. The normality of the data was assessed using the Shapiro-Wilk test. The effect of dietary treatment on growth performance, biochemical parameters [8] [13] [28], carcass traits, and reproduction parameters were analyzed using one-way ANOVA following the general linear model. Values of $P \le 0.05$ were considered statistically significant and they were separated by Duncan's test at the 5% significance level [9].

3. Results

3.1. Growth performances

The growth performances of the Japanese fed on different experimental diets are shown below (Table 2).

Table 2. Growth performance of Japanese quails fed with different experimental diets.

		Growth performances traits					
	Dietary treatment groups	FI (g)	BW (g)	WG (g/Week)	FCR		
	T0		298.76 ± 4.60^{ab}	19.53 ± 0.38^{ab}			
Females	T1		319.05 ± 14.61^{b}	$21.48\pm0.93^{\rm b}$			
	T2		288.24 ± 4.14^{a}	$18.26\pm0.82^{\rm a}$			

Continued					
	T3		309.34 ± 13.82^{b}	$20.65\pm0.05^{\text{b}}$	
remates	Mean		303.85 ± 15.01	19.98 ± 1.63	
	Т0		246.81 ± 2.55^{a}	$13.97\pm0.38^{\rm a}$	
	T1		$246.08\pm2.83^{\text{a}}$	$13.63\pm0.43^{\rm a}$	
Males	T2		244.70 ± 12.07^{a}	13.40 ± 0.85^{a}	
	T3		$247.20\pm14.73^{\text{a}}$	$13.87 \pm 1.14^{\rm a}$	
	Mean		246.20 ± 8.34	13.72 ± 0.69	
	Т0	$174.88 \pm 3.07^{\circ}$	272.79 ± 28.64^{a}	$16.75\pm3.06^{\rm a}$	10.44 ± 0.07^{b}
	T1	169.53 ± 1.05^{bc}	$282.57\pm41.06^{\text{a}}$	$17.55\pm4.34^{\rm a}$	$9.65\pm0.02^{\text{a}}$
Mix	T2	154.33 ± 5.13^{a}	266.47 ± 25.17^{a}	15.83 ± 2.76^{a}	$9.74\pm0.20^{\rm c}$
	T3	167.48 ± 2.53^{b}	278.27 ± 36.35^a	17.26 ± 3.99^{a}	$9.70\pm0.10^{\rm d}$
	Mean	166.55 ± 8.37	275.02 ± 31.75	16.85 ± 3.42	9.88 ± 1.48

FI: Feed Intake, BW: Body Weight, WG: Weight Gain, FCR: Feed Conversion Ratio for growth stage; a, b et c: in the same column and for the same gender, values with the same letter are not significantly different (P > 0,05). T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.

Table 3. Carcass yield and proportion of different body parts of the Japanese quails fed with different experimental diets.

Mariahla	Dietary treatment groups				
variable	Sex	T0	T 1	T2	Т3
	Male	71.40 ± 0.34^{a}	$72.13 \pm 1.38^{\text{a}}$	$70.56\pm0.98^{\text{a}}$	71.22 ± 0.67^{a}
Carcass yield	Female	66.39 ± 0.51^{ab}	$67.48 \pm 1.01^{\mathrm{b}}$	65.56 ± 1.27^{a}	67.75 ± 0.61^{b}
	Mean	68.90 ± 2.76^{ab}	69.80 2.77 ^b	68.06 2.96 ^a	69.48 1.98 ^b
	Male	26.48 ± 0.32^{a}	26.40 ± 1.15^{a}	$25.40\pm1.34^{\rm a}$	25.90 ± 0.67^{ab}
Breast	Female	$29.96\pm2.43^{\mathrm{b}}$	$30.98\pm0.79^{\rm b}$	$25.00\pm1.33^{\text{a}}$	$29.72\pm1.64^{\rm b}$
	Mean	$28.22\pm2.45^{\mathrm{b}}$	$28.69\pm2.25^{\mathrm{b}}$	$25.20\pm1.21^{\text{a}}$	28.91 ± 2.79^{b}
	Male	16.66 ± 0.23^{a}	$17.19\pm0.60^{\rm b}$	$16.24\pm0.88^{\text{a}}$	17.28 ± 0.70^{b}
Thigh	Female	$15.27\pm1.14^{\rm ab}$	$16.25\pm0.79^{\mathrm{b}}$	$14.49\pm0.47^{\text{a}}$	15.14 ± 0.70^{ab}
	Mean	$15.96\pm1.06^{\text{ab}}$	$16.72\pm0.80^{\rm b}$	$15.36\pm1.15^{\rm a}$	16.21 ± 1.33^{ab}
	Male	5.70 ± 0.22^{a}	$5.85\pm1.04^{\rm a}$	6.07 ± 0.40^{a}	$5.99\pm0.46^{\text{a}}$
Wing	Female	$5.54\pm0.40^{\rm b}$	5.35 ± 0.19^{ab}	$4.82\pm0.46^{\rm a}$	$5.69\pm0.13^{\rm b}$
	Mean	$5.62\pm0.30^{\rm a}$	$5.60\pm0.72^{\text{a}}$	$5.45\pm0.78^{\text{a}}$	$5.84\pm0.34^{\text{a}}$
	Male	3.68 ± 0.30^{a}	3.85 ± 0.24^{a}	3.80 ± 0.31^{a}	3.47 ± 0.15^{a}
Head	Female	$2.94\pm0.23^{\rm a}$	$2.95\pm0.23^{\text{a}}$	$2.80\pm0.39^{\rm a}$	$2.85\pm0.16^{\text{a}}$
	Mean	$3.31\pm0.47^{\rm a}$	$3.40\pm0.54^{\rm a}$	$3.30\pm0.63^{\text{a}}$	$3.16\pm0.37^{\text{a}}$
	Male	7.32 ± 0.22^{bc}	$7.70 \pm 0.40^{\circ}$	6.18 ± 0.67^{a}	6.64 ± 0.15^{ab}
Neck	Female	5.68 ± 0.92^{a}	5.69 ± 0.51^{a}	$8.26\pm1.16^{\text{b}}$	6.40 ± 1.28^{ab}
	Mean	6.50 ± 1.08^{a}	6.69 ± 1.17^{a}	7.22 ± 1.41^{a}	6.52 ± 0.82^{a}

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Continued					
	Male	1.74 ± 0.11^{a}	1.80 ± 0.31^{a}	$1.92\pm0.05^{\text{a}}$	$1.90\pm0.14^{\mathrm{a}}$
Legs	Female	1.65 ± 0.25^{a}	$1.67 \pm 0.10^{\mathrm{a}}$	1.50 ± 0.13^{a}	$1.59\pm0.20^{\mathrm{a}}$
	Mean	1.70 ± 0.18^{a}	1.74 ± 0.22^{a}	1.71 ± 0.24^{a}	$1.75\pm0.23^{\text{a}}$
Deals	Male	15.99 ± 0.71^{a}	17.02 ± 0.87^{a}	$16.94\pm0.85^{\rm a}$	15.82 ± 0.29^{a}
Dack	Female	$13.54\pm0.27^{\rm a}$	14.37 ± 1.70^{a}	$14.37\pm1.09^{\rm a}$	$14.40\pm0.91^{\text{a}}$
Voriable		Di	etary treatment	t groups	
variable	Sex	T0	T1	T2	T3
	Male	1.26 ± 0.04^{a}	1.18 ± 0.41^{a}	1.83 ± 0.35^{a}	1.45 ± 0.43a
Liver	Female	$2.32\pm0.33^{\text{a}}$	$2.34\pm0.50^{\rm a}$	3.22 ± 0.62^{b}	2.61 ± 0.07ab
	Mean	$1.79\pm0.62^{\rm a}$	$1.76\pm0.75^{\rm a}$	$2.52\pm0.89^{\rm b}$	2.03 ± 0.69a
	Male	0.42 ± 0.09^{a}	$0.33\pm0.13^{\mathrm{a}}$	0.52 ± 0.20^{a}	$0.43\pm0.05a$
Kidney	Female	0.36 ± 0.09^{a}	$0.49\pm0.14^{\rm a}$	$0.34\pm0.08^{\rm a}$	$0.40 \pm 0.16a$
	Mean	0.39 ± 0.09^{a}	0.41 ± 0.14^{a}	0.43 ± 0.17^{a}	$0.42 \pm 0.10a$
	Male	0.71 ± 0.22^{a}	$0.84 \pm 0.11^{\text{a}}$	$0.85\pm0.05^{\rm a}$	0.92 ± 0.01a
Heart	Female	$0.73\pm0.07^{\text{a}}$	$0.66\pm0.06^{\rm a}$	0.64 ± 0.04^{a}	$0.73\pm0.80a$
	Mean	0.72 ± 0.14^{a}	$0.75\pm0.12^{\rm a}$	0.75 ± 0.12^{a}	0.82 ± 0.11a
	Male	2.13 ± 0.46^{a}	1.99 ± 0.39^{a}	2.27 ± 0.05^{a}	$2.07 \pm 0.40a$
Gizzard	Female	$2.02\pm0.08^{\rm a}$	$2.10\pm0.77^{\text{a}}$	$1.97\pm0.16^{\rm a}$	2.32 ± 0.33a
	Mean	$2.08\pm0.30^{\rm a}$	$2.04\pm0.54^{\rm a}$	2.12 ± 0.19^{a}	$2.20\pm0.35a$

^{A, b, c} Means with similar letter indices on the same row are not significantly different (P > 0,05). T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.

There was a significant difference ($P \le 0.05$) in FI, BW, WG, and FCR mean between and among the treatment groups. Independent of sex, the highest and least FI were recorded in the T0 diet (174.88 ± 3.07 g) and T2 diet (154.33 ± 5.13 g) respectively. Hence feeding quail with the experimental diet showed a significantly higher live weight and female daily weight gain in T1 diets compared with that recorded in the T0 control batch (298.76 ± 4.60 g).

In males, there was no significant difference ($P \le 0.05$) in BW between experimental diets, although the glyphosate T2-only treatment recorded relatively lower values. The same variations were recorded for weight gain in both males and females. The feed conversion ratio (FCR) was similar in all batches, irrespective of treatment. However, the T0 negative control treatment showed the relatively highest FCR value.

3.2. Carcass Traits

Carcass yield, the proportions of breast, wings, thigh, neck, and liver were significantly $P \le 0.05$ affected by the different experimental diets (**Table 3**). Carcass yield was significantly ($P \le 0.05$) higher in the diet supplemented with COLP (T1

and T3), while the diet supplemented with glyphosate alone recorded the lowest carcass yield. The proportion of breast was significantly ($P \le 0.05$) higher in diets T0, T1, and T3 than in the glyphosate-only diet, which recorded the lowest value regardless of sex. Similarly, the proportion of thighs significantly ($P \le 0.05$) lower was recorded in the glyphosate-only batch, while the COLP-supplemented batch (T1) recorded the highest value, while remaining comparable to T0 and T3, irrespective of sex. A significant increase ($P \le 0.05$) in the proportion of neck was recorded in males in the T1 diet (7.70% ± 0.40%) compared to T2 while remaining similar to the control (T0) and T1 diets; in females, a significant difference ($P \le 0.05$) was recorded in T2 treatments (8.26% ± 1.16%) while remaining similar to T3, no significant difference (P > 0.05) was recorded for either sex.

Moreover, the different experimental diets had no significant effect (P > 0.05) on the proportions of the kidney, heart, and gizzard, regardless of sex. The proportion of liver in females in the glyphosate-only treatment (T2) ($2.52 \pm 0.89\%$) was significantly (P ≤ 0.05) higher than in the control treatment (T0) ($2.32 \pm 0.33\%$) and the COLP-treated treatment (T1) ($2.34\% \pm 0.50\%$), while that in the T3 treatment ($2.61\% \pm 0.07\%$) was comparable to that in the T2 treatment; in males, there was no significant difference in liver proportion, while the highest value was also recorded in the glyphosate-only batch (T2) ($1.83\% \pm 0.35\%$).

3.3. Serum Biochemical Markers

Table 4 reveals the variability in serum ALAT, ASAT, creatinine, urea, and protein concentrations of the Japanese quails fed with different experimental diets.

Waniahla	Dietary treatment groups				
variable	Sex	T0 (n = 6)	T1 (n = 6)	T2 (n = 6)	T3 (n = 6)
	Male	14.76 ± 0.21^{a}	$22.52 \pm 1.98^{\circ}$	$19.26\pm1.74^{\mathrm{b}}$	42.26 ± 2.18^{d}
ALAT (UI/L)	Female	$11.87 \pm 0.47^{\rm b}$	5.28 ± 0.81^{a}	$25.11\pm2.63^{\mathrm{b}}$	$13.57 \pm 1.26^{\circ}$
	Mean	13.31 ± 1.61^{a}	$13.90\pm9.54^{\rm a}$	22.19 ± 3.77^{ab}	27.92 ± 15.79^{b}
	Male	$123.04 \pm 2.65^{\mathrm{b}}$	123.76 ± 1.02^{b}	76.90 ± 12.28^{a}	132.33 ± 20.07^{b}
ASAT (UI/L)	Female	151.05 ± 0.72^{d}	$98.93 \pm 3.32^{\circ}$	$78.69\pm6.75^{\mathrm{b}}$	$38.38\pm6.58^{\text{a}}$
	Mean	$137.04 \pm 15.43^{\circ}$	111.34 ± 13.77 ^{bc}	$77.79\pm8.92^{\rm a}$	86.35 ± 85.16^{ab}
	Male	$19.86\pm0.85^{\text{a}}$	$20.73 \pm 1.55^{\text{a}}$	$24.60\pm2.62^{\mathrm{b}}$	$20.27 \pm 1.96^{\text{a}}$
Urea (mg/L)	Female	24.12 ± 2.31^{a}	$24.88\pm3.23^{\text{a}}$	$24.41 \pm 3.49^{\rm a}$	$21.78\pm1.45^{\text{a}}$
	Mean	$21.99\pm2.80^{\rm a}$	$22.80\pm3.21^{\text{a}}$	$23.84\pm2.76^{\text{a}}$	21.03 ± 2.22^{a}
	Male	2.11 ± 0.10^{a}	2.19 ± 0.10^{ab}	$2.25\pm0.16^{\rm b}$	2.15 ± 0.18^{a}
Creatinine (mg/L)	Female	$2.25\pm0.13^{\text{a}}$	2.10 ± 0.14^{a}	$2.16\pm0.05^{\text{a}}$	2.12 ± 0.12^{a}
	Mean	$2.18\pm0.12^{\text{a}}$	2.14 ± 0.12^{a}	$2.19\pm0.11^{\rm a}$	2.30 ± 0.21^{a}

Table 4. Serum biochemical markers of the Japanese quails fed with different experimental diets.

Continued					
	Male	$2.32\pm0.82^{\text{a}}$	2.48 ± 0.33^{a}	$2.31\pm0.46^{\rm a}$	1.87 ± 0.54^{a}
Protein	Female	$2.02\pm0.40^{\text{a}}$	3.25 ± 0.53^{ab}	2.20 ± 0.71^{ab}	$3.65 \pm 1.13^{\text{b}}$
	Mean	$2.17\pm0.60^{\mathrm{a}}$	$2.86\pm0.58^{\text{a}}$	$2.26\pm0.54^{\rm a}$	2.76 ± 1.25^{a}

^{a,b,c}Means with similar letter indices on the same row are not significantly different (P > 0.05). T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.



Figure 3. Histology of female Japanese quail liver (×100), kidney (×250), and testes (×100); Hematoxylin-eosin staining. T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP; Liver: Vp = hepatic portal vein; He = hepatocyte; Cs = sinusoidal capillary; Cb = Canaliculus biliary; VL = Vacuole lipid; Kidney: Gl = Glomerulus; Eu = Urinary space; Tcd = distal convoluted tubule; Tcp = proximal convoluted tubule; Tcp = collecting tubule.



Figure 4. Histology of the liver (×100), kidney (×250), and testes (×100); Hematoxylin-eosin staining. T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP; Liver: Vp = Hepatic portal vein; He = Hepatocyte; Cs = Sinusoidal capillary; Cb = Biliary canaliculus; VL = Lipid vacuole; Kidney: Gl = Glomerulus; Eu = Urinary space; Tcd = Distal convoluted tubule; Tcp = Proximal convoluted tubule; Tcp = Collecting tubule; Testes: Spz = Spermatozoa; Tcv = Connective vascular tissue; Ts = Seminiferous tubules; Dc = Germ cell disorganization; Ds = Sperm destruction.

Serum ALAT concentration increased significantly (P \leq 0.05) in glyphosateintoxicated treatments T2 (22.19 ± 3.77 mg/L) and T3 (27.25 ± 16.58 mg/L), compared with the control (T0), which remained comparable to the COLP-only treatment. There was a significant increase (P \leq 0.05) in serum ASAT concentration between treatments T0 (135.71 ± 17.12 mg/L) and T1 (112.34 ± 11.95 mg/L) compared with treatment T2 (76.13 ± 10.61 mg/L), which recorded the lowest concentration, while T3 remained comparable to T1 and T2, again irrespective of sex. Although no significant difference (P ≤ 0.05) in serum urea concentration was observed in females, in males it increased significantly (P ≤ 0.05) in the glyphosate-only treatment (T2) (24.50 ± 2.76 mg/L) compared with the other treatments (T0, T1, and T3), which recorded similar values. A significant increase (P ≤ 0.05) in serum creatinine concentration was noted in T3 (2.45 ± 0.18 mg/L), but this was comparable to T1 and T2, and also comparable in females. The highest protein concentration (P ≤ 0.05) was recorded between T3 (3.65 ± 1.13 mg/L) and T1 (3.25 ± 0.53 mg/L) in females only.

3.4. Histology of the Organs

The histological results of the Japanese quail females and males with different treatment diets are presented (Figure 3) and (Figure 4).

Analysis has shown that the histological sections of the livers of quails in treatment T0, as well as T1 treated with COLP and T3 treated with glyphosate and supplemented with COLP, showed normal liver parenchymal architecture, with a distinct centrilobular vein, hepatocytes, and sinusoidal capillaries, irrespective of sex. Only the glyphosate-only treatment (T2) showed histopathological changes marked by the onset of hepatocyte vacuolization. However, no renal alterations were observed in any of the treatments (**Figure 3**, **Figure 4**).

The histological structure of quail testes in control animals (T0) as well as T1 treated with COLP and T3 treated with glyphosate and supplemented with COLP, showed normal seminiferous tubule architecture. On the other hand, only the glyphosate-only treatment showed disorganization of germ cell arrangement and destruction of spermatozoa.

3.5. Reproduction Performances

3.5.1. Testis Characteristics

Variability in testicle weight and measurements for the Japanese quails fed with different experimental diets are shown (Table 5).

As demonstrated in (**Table 5**), a significant difference was recorded in the left and right testicle weight, diameter, height, and gonad-somatic index, which were higher in the T0 control dietary treatment and the T2 glyphosate-only dietary treatment. In terms of shape index, the different treatments were not significantly (P > 0.05) affected concerning the left testicles, but the shape index of the left testicles was significantly (P \leq 0.05) higher in dietary treatment T1 (0.74% ± 0.03 %). The weight ratio was not significantly (P > 0.05) affected by the different experimental treatments. Nevertheless, the highest values were found in batches T0 (1.18% ± 0.14%) and T2 (1.17% ± 0.09%).

3.5.2. Laying and Incubation Performances

The evolution of the egg-laying rate fed with different experimental diets is illustrated below (**Figure 5**) and (**Figure 6**).

Variable		Dietary treatment groups				
variable		T0 (n = 3)	T1 (n = 3)	T2 (n = 3)	T3 (n = 3)	
	Left	$3.48\pm0.40^{\rm b}$	2.83 ± 0.32^{a}	$4.22 \pm 0.18^{\circ}$	2.94 ± 0.26^{ab}	
Weight	Right	$2.95\pm0.80^{\rm a}$	2.71 ± 0.28^{a}	$3.59\pm0.27^{\text{a}}$	$2.98\pm0.64^{\rm a}$	
	Total	$6.43\pm0.96^{\rm a}$	$5.55\pm0.54^{\text{a}}$	$7.81\pm0.36^{\rm b}$	$5.93\pm0.64^{\text{a}}$	
	Left	$18.81\pm0.71^{\rm b}$	$15.14 \pm 1.00^{\mathrm{a}}$	$18.04\pm0.81^{\rm b}$	$15.23\pm0.92^{\rm a}$	
Diameter	Right	$16.82 \pm 1.26^{\circ}$	$14.12\pm0.09^{\text{a}}$	$16.38\pm0.52^{\text{bc}}$	15.15 ± 0.17^{ab}	
Height	Left	$25.02\pm2.21^{\mathrm{b}}$	22.83 ± 1.01^{ab}	$25.44\pm0.85^{\mathrm{b}}$	$21.83 \pm 1.92^{\rm a}$	
neight	Right	$24.53\pm0.27^{\text{a}}$	$22.55\pm0.42^{\text{a}}$	$24.94\pm0.71^{\text{a}}$	$23.22\pm2.24^{\rm a}$	
Shana indar	Left	0.72 ± 0.01^{a}	0.66 ± 0.01^{a}	0.70 ± 0.02^{a}	$0.69\pm0.02^{\mathrm{a}}$	
Shape mdex	Right	$0.63\pm0.02^{\text{a}}$	0.64 ± 0.01^{a}	$0.65\pm0.03^{\text{a}}$	$0.65\pm0.06^{\rm a}$	
Left/Right Weigl	ht Ratio	1.18 ± 0.14^{a}	1.15 ± 0.12^{a}	1.17 ± 0.09^{a}	1.01 ± 0.24^{a}	
Gonado-somatic index 2.68 ± 0.39^{a} 2.28 ± 0.19^{a} 3.54 ± 0.29^{b} 2.48 ± 0.29^{b}			2.48 ± 0.29^{a}			

Table 5. Testis characteristics of male Japanese quails fed with different experimental diets.

^{a,b,c}: on the same line, values with the same letter are not significantly different (P>0.05). T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.

Independent of the treatment diet, egg-laying rates evolved from week one to week four (**Figure 5**). However, the quails fed on the T2 treatment diet have decreased the egg-laying rate, fertility rate, and hatchability, and increased the embryonic mortality rate as compared to T0 and T1. This implies that a glyphosate-only treatment diet decreases the egg-laying rate, fertility rate, and hatchability rate while COLP used alone increases the egg-laying rate, fertility rate, and hatchability and decreases the embryonic mortality rate (**Figure 5**, **Figure 6**).

3.5.3. External Egg Characteristic

Variability in the external egg characteristics for the Japanese quail fed with different experimental diets is recorded (Table 6).



Figure 5. Evolution of egg-laying rates of the Japanese quails fed with different experimental diets. T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.



Figure 6. Average incubation performance of the Japanese quail eggs fed with different experimental diets. T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.

 Table 6. External egg characteristics of the Japanese quail fed with different experimental diets.

Variable –	Dietary treatment groups					
	T0	T 1	T2	T3		
Weight	11.84 ± 1.19^{a}	$12.63\pm0.87^{\rm a}$	11.75 ± 0.92^{a}	12.15 ± 0.77^{a}		
Diameter	$25.73\pm0.99^{\rm a}$	$26.25\pm0.72^{\text{a}}$	$25.82\pm0.68^{\text{a}}$	$25.98\pm0.80^{\text{a}}$		
Height	32.40 ± 1.11^{a}	$33.09\pm0.90^{\rm a}$	$32.19\pm1.47^{\rm a}$	33.41 ± 1.18^{a}		
Shape index	$0.79\pm0.01^{\rm a}$	$0.79\pm0.02^{\text{a}}$	0.77 ± 0.03^{a}	0.79 ± 0.02^{a}		

a, b, c: on the same line, values with the same letter are not significantly different (P > 0.05). T0: control, T1: 1% COLP, T2: glyphosate in drinking water and T3: glyphosate + 1% COLP.

It is shown in (Table 6), that egg weight, diameter, height, and shape index were not significantly (P > 0.05) affected by the different experimental treatments.

4. Discussion

In this study, feed intake in the glyphosate-only group was significantly ($P \le 0.05$) lower than in the control group. This decrease in feed intake in the glyphosate-treated group could be attributed to the effect of glyphosate on the gastrointestinal tract, resulting in loss of appetite and/or poor feed absorption, leading to reduced feed and water intake in treated quails [32]. This observation is consistent with that of Chan and Mahler [33] in rats after glyphosate administration for 13 weeks. An increase in feed intake (FI) was recorded in the glyphosate-treated group supplemented with COLP (T3), possibly due to the presence of phenolic compounds in COLP responsible for its anti-inflammatory properties [34]. These compounds may have acted on the walls of the gastrointestinal tract to promote intestinal transit and mitigate the adverse effects of glyphosate on feed intake. This result aligns with that of Ekenyem *et al.* [20], who found a higher FI value in broilers with

a 2.5% COLP incorporation rate compared to the control group. This contrasts with Aro [21] findings, who observed a decrease in FI when the COLP incorporation rate exceeded 5%. This also alleviates concerns raised in the literature about its high toxicity and unpalatability. The acceptability of COLP could be linked to the drastic reduction/elimination of anti-nutrients through effective processing methods (e.g., shredding, drying, etc.).

In this study, live weight and weight gain were not significantly affected in males between the different treatments. However, a relative reduction was observed in the group treated solely with glyphosate. On the other hand, a significant increase in live weight and weight gain was noted in females treated with 1% Chromoleana odorata leaf powder (COLP), and a significant reduction was observed in the group treated with glyphosate alone. The reduction in live weight and weight gain in both sexes corroborates the findings of Chan and Mahler [33], who observed similar results in rats exposed to glyphosate. This could be explained by the reduction in feed intake in these batches and the possible toxicity caused by glyphosate in the quails [35]. The increase in live weight could be attributed to the presence of nutritive compounds in the plant, such as crude protein, ash, dry matter, and flavonoids (e.g., quercetin), which protect the intestinal barrier and promote nutrient and mineral absorption. These results are similar to those of Bonos et al. [36], who supplemented quail feed with mannan oligosaccharides and observed an increase in live weight and weight gain compared to the control group, and contradictory to Ohanaka et al. [37], who found lower weights in layers given different rates of COLP inclusion than the control group.

Irrespective of sex, carcass yield and the proportions of breast and thigh showed significant variation between the control and glyphosate-treated groups. The reduction in carcass yield could be attributed to a decrease in feed intake, live weight, and alterations in the health status of the animals. However, there was an increase in carcass yield and the proportions of breast and thighs in the COLP-supplemented groups. This could be explained by the fact that, with the increase in live weight in these groups, carcass yield and the proportions of certain parts also increased. Similar results were obtained by [37], who reported higher percentages of breast and thigh cuts at 5% and 10% COLP inclusion, and Bonos *et al.* [36], who supplemented quail feed with 2% mannan oligosaccharides.

In this study, an increase in the relative liver weight was observed in glyphosatetreated animals compared to the control group. Similar results were reported by Kouamo *et al.* [28] in female quails and by Mayang [35] in male quails treated with the same dose, as well as by Chan and Mahler [33] and Cox [38], who observed an increase in liver weight following glyphosate administration to rats. This could be due to intense detoxification activity and the accumulation of glyphosate in this detoxification target organ. However, a reduction in relative liver weight was observed in the COLP-supplemented groups. This could be explained by the high bioavailability of the plant's phenolic metabolites in the liver, which prevent dysbiosis and xenobiotic synthesis and enhance antioxidant functions. No significant difference in kidney proportion was observed between the different experimental treatments. These results align with the histology of the kidneys, which showed no alterations at the kidney level. These findings contradict those of Mallem *et al.* [39], who reported a decrease in kidney weight in rabbits exposed to glyphosate, and Wunnapuk *et al.* [40], who observed necrotic and apoptotic cells in the tubular epithelium and renal cortex of rats exposed to glyphosate at a dose of 2500 mg/kg. This could be explained by the fact that glyphosate is rapidly eliminated, with only 1% of the administered dose remaining stored in the kidneys [41], and thus the kidneys do not undergo intense activation that would likely cause toxicity.

Determining biochemical parameters is crucial for assessing the toxic effects of pesticides on the liver and kidneys. Our results indicate that glyphosate exposure led to a significant increase in ALAT levels, while ASAT levels decreased. Since ALAT is localized in the liver, it is more specific for liver damage than ASAT, which is present in a wide range of tissues. This could indicate hepatotoxicity, which leads to altered permeability of the liver plasma membrane and leakage of enzymes into the plasma, or the onset of liver necrosis [42]. The elevated transaminase levels were corroborated by histopathological changes in the liver, including the presence of lipid vacuoles, reflecting intensified liver detoxification activity to eliminate glyphosate accumulating in the liver. Administration of COLP did not significantly reduce ALAT and ASAT levels in the glyphosate-intoxicated T3 treatment, but these levels remained constant in the COLP-only treatment. This may be due to the relatively low incorporation rate of COLP.

Creatinine and urea levels are used as indicators of kidney damage and kidney function. Our results show a relative increase in serum urea and creatinine concentrations in the glyphosate-only treatment compared to the control. This could be explained by the fact that the low dose of glyphosate remaining stored in the kidneys is likely to create potential toxicity. In quails treated with glyphosate and supplemented with COLP, there was a reduction in serum urea and creatinine concentrations. This result may be due to the bioactive compounds in COLP that promote kidney function. In general, total protein levels were not significantly affected by the experimental treatments. Similar results were obtained by Peter-Damian *et al.* [13], who found higher protein levels in laying birds fed COLP compared to the control, possibly reflecting better protein quality and quantity in the diets.

In our trial, there was an increase in testicular characteristics in the T2 treatment with glyphosate alone. This result correlates with histological findings showing disorganization of germ cell arrangement and destruction of spermatozoa. This corroborates the work of Folarin *et al.* [43], who observed significant reductions in sperm count, motility, and severe degenerative lesions in testicular architecture in rats exposed to glyphosate at concentrations of 3.6, 50.4, and 248.4 mg/kg for 12 weeks. The destruction of spermatozoa can be attributed to the action of POEA, which alters hormones that regulate reproductive function (GnRH, LH, FSH, estradiol, progesterone) or the expression of their receptors at various levels of the reproductive axis, including the hypothalamus, pituitary, and testes [44], inhibiting spermatogenesis and resulting in testicular hypertrophy. The reduction in testicular weight and histological reorganization observed in COLPsupplemented treatments can be explained by the presence of specific compounds that regulate testicular function, thus promoting sperm progression.

laying rates increased up to week 4 before decreasing at week 5 in the glyphosate treatment (T2). Our results corroborate those of other studies showing that female fish experience changes in their sex hormones following glyphosate exposure [45], as well as reductions in egg production and embryo viability [46]. This could be due to glyphosate interfering with the activity of aromatase, the enzyme responsible for converting androgens into estrogens, thus inhibiting egg formation in the oviduct [47]. The delayed egg-laying observed in the T3 treatment could be due to the negative synergistic effect of glyphosate and *C. odorata*, which could have affected the development and histological structure of the genitalia, thereby delaying egg-laying onset [16] [42] [48]-[51].

The results of our study showed that the external characteristics of the eggs (weight, diameter, and height) were not significantly influenced by the experimental diets. However, the glyphosate-only treatment showed lower values compared to the control. The reduction in live weight of females, coupled with lower feed intake, suggests that lower egg weights in the glyphosate-treated groups are a consequence. Conversely, treatments supplemented with COLP recorded higher values. This work made by Aro [21], who found the heaviest eggs in diets incorporated with 2% COLP, and Williams *et al.* [52], who observed similar improvements in hens consuming a diet supplemented with 1% and 2% *Moringa oleifera* leaf powder. These improvements are thought to result from better feed intake and improved health in birds fed a diet supplemented with *C. odorata.* The live weight of fully grown quails is a determining factor for egg growth [53].

The results of our study show that COLP-supplemented diets induced the best incubation parameters compared to those of the cypermethrin-only treatment. This may be explained by the positive effect of *C. odorata* leaf powder on embryo development, hatchability, and an increase in normal sperm count. These results are consistent with findings from other studies, such as those with *Moringa oleif-era* leaf powder in quail [54].

Despite the broad potential of COLP leaf to reduce the harmful effects of GBHs, the current study is limited in regard to the optimal dosage of COLP that can be used by farmers to mitigate glyphosate-induced toxicity in Japanese quails. However, previous studies on the ethanolic extract of *Chromoleana odorata* leaves suggest that a dosage of 250 mg/kg significantly mitigates oxidative stress and biochemical alterations caused by methotrexate-induced nephropathy in rats [53]. An LD50 of 2154 mg/kg was also recorded when evaluating the toxic effects of aqueous extracts of *Chromoleana odorata*.

leaves in Wistar rats [54]. Although specific studies on the use of COLP in Japanese quails are scarce, the antioxidant and protective effects observed in rats suggest that a similar dosage range (250 - 500 mg/kg body weight) could be effective in mitigating glyphosate-induced toxicity in quails [4]. Nevertheless, while the COLP dosage used in this study improved growth traits, reproduction, and biochemical parameters, the exact dosage and efficacy in Japanese quails require further investigation. Therefore, the potential for direct application of COLP in Japanese quail farms should be approached with caution, considering species-specific responses to glyphosate-based herbicides.

5. Conclusions

This study aims to investigate the protective effects of *Chromoleana odorata* leaf powder (COLP) against glyphosate-induced toxicity in Japanese quails. The findings of this study demonstrate the feasibility of utilizing *Chromoleana odorata* leaf powder as a suitable feed supplement to mitigate glyphosate-induced toxicity in quails, with no adverse effects on feed intake, live weight, weight gain, feed conversion ratio, or carcass characteristics, including the proportions of certain parts, such as the thigh and breast.

However, there was a reduction in liver weight in quails treated with COLP, accompanied by a significant decrease in biochemical markers of liver function (ALAT and ASAT) and kidney function (creatinine and urea) when compared to the glyphosate-only treatment group.

Additionally, improvements in male reproductive characteristics were observed, marked by a significant reduction in testicular weight and normal sperm production. In females, the *Chromoleana odorata* treatment led to increased fertility and hatchability while reducing embryonic mortality.

Considering all the findings from this study, further confirmatory research is needed to determine the optimal COLP dosage that will mitigate the harmful effects of glyphosate and enhance productivity.

Conflicts of Interest

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

Declaration of Authors Contributions

FDK and LBA performed data curation and formal analysis; FDK and ANT designed the methodology, and wrote the original draft; FDK, ANT, HWM, LBA, HM, and ME reviewed and edited the manuscript. FDK and ME supervised, validated, and visualized the entire study. All the authors have read and approved the final version of the manuscript.

Data Availability Statement

The authors confirm that the data contained in this article is from our study and it is accessible upon inquiry with the approval of the corresponding authors who possess the data.

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Conflicts of Interest

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

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