Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 9 September 2022

Research Article

Effects of *Chromolaena odorata* Leaf Meal Supplementation on Egg Quality, Semen Characteristics, and Some Stress Markers on Brahma Chicken (*Gallus gallus* Domesticus)

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DOI: 10.31080/ASVS.2022.04.0501

Received: August 01, 2022

Published: August 29, 2022

Abstract

Chromolaena odorata leaf meal (COLM) has been used to feed poultry without adverse effects on growth, however, studies on its effect on the fertility of local chicken are scarce. This study aimed at evaluating the effect of COLM-based diets on egg fertility, semen characteristic, and tissue biochemistry parameters (Protein, cholesterol, catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) concentration) of Brahma chickens. Sixty hens and fifteen cocks, aged 4-5 months were fed three dietary treatments T0, T1, and T2, as containing 0, 1 and 2% COLM, respectively in five replications for two months. Semen samples were collected once a week by double hand massage technique and semen quality parameters (volume, motility, concentration, and spermatozoa viability) were determined. Data on testicular and epididymis histology and biochemical parameters in testes were also collected during the eighth week of the experiment. During the incubation phase, fertility, and hatchability of eggs, as well as embryonic mortality of fertile eggs were recorded. The semen analysis showed that spermatozoa concentration and spermatozoa motility ($p < 0.05$) increased significantly with increased COLM supplementation levels. The biochemical analysis showed an increase in protein, cholesterol, and GSH levels while MDA levels decreased in males supplemented with COLM. The fertility percentage for T1 was significantly higher than in the other treatments, while hatchability and mortality of the embryo showed non-significant differences among the groups. The histopathological analysis also revealed a positive effect of the plant on spermatogenesis. Altogether, the addition of COLM up to 1% in local chicken diets improves semen quality, oxidative status level of testes, and egg fertility.

Keywords: *Chromolaena odorata*; Semen; Tissue Biochemistry; Fertility; Local Chicken

Abbreviations

CAT: Catalase; CE: Crude Energy; CF:Crude Fiber; CHOP-PAP method: Enzymatic Colorimetric Determination of Serum Cholesterol; COLM: *Chromolaena odorata* Leaf Meal; CP: Crude Protein; DM: Dry Matter; E :Epididymis; EE: Ether Extract; F: ANOVA; GSH: Reduced Glutathione; H: Kruskal Wallis Test; IRAD: Institute of Agricultural Research for Development; LTS: Lumen of Seminiferous Tubule; MDA: Malondialdehyde; ME: Metabolizable Energy; OM: Organic Matte; SPZ: Spermatozoa; T0: Control; T1: COLM Supplemented at 1%; T2: COLM Supplemented at 2%; TI: Interstitial Tissue

Introduction

In many parts of rural and urban Africa, chicken production has made a substantial contribution to livestock production as a source of protein, food security, employment, and income in resource-constrained communities [26,29]. However, for successful poultry breeding, high fertility rates are crucial. Successful hatchability of chick production also depends on the fertility and reproductive performance of male birds (cocks), which is determined to a large extent by the quality of the semen produced [19,23,26]. Many factors, including poor nutrition and management, have been blamed for the decline in the semen quality of chickens or poultry [21]. The use of natural or synthetic antioxidants in diets of chicken to reduce oxidative stress in sperm, improve sperm quality, and boost sperm fertility has been widely documented [4,15,35]. Today, several plants have been extensively researched for potential benefits in various aspects of animal production, including nutrition, health, and reproduction [14]. Medicinal plants contain pharmacologically active chemicals that may be useful in animal production [24]. The possibility of using plant materials to improve reproduction in farm animals is currently receiving a lot of attention including *Chromolaena odorata* King and Robinson (Asteraceae) [4,7]. *C. odorata* is used in traditional medicine in Cameroon to treat abdominal pains, headaches, and malaria [28]. It has been revealed to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antibacterial, and antioxidant activities [33]. The use of the plant meal as a supplement in the diets of chickens is documented [1,27,37], however, there is a paucity of information on its effect on reproduction parameters. It is known that the inclusion of *Chromolaena odorata* leaf meal as feed supplements was non-toxic and did not suppress the growth of broilers but studies on the fertility of poultry are scarce.

The aim of this work was to evaluate the effect of *Chromolaena odorata* leaf meal (COLM) in diets of local fowls or chicken *(Gallus gallus domesticus)* on egg fertility, semen characteristics, and testes tissue biochemistry parameters (Protein, cholesterol, catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) concentration).

Material and Methods

Study site

The feeding trial was conducted at the experimental farm of the Institute of Agricultural Research for Development (IRAD) Nkolbisson. The institution is situated in an area of medium-altitude (730 m above sea level) between 3° 51' to 3° 53' North latitude and 11° 25' to 11° 27' East longitude. The area is in agro-ecological zone V of bimodal humid forest, characterized by an average annual temperature of 25°C, a bimodal rainfall varying between 1500 to 2500 mm/year, and relative humidity of 70 to 90%. Its climate is the Equatorial Guinean type with an average rainfall of 1600 mm/year divided into four seasons, 2 of which are rainy (March to June and September to November) alternating with 2 other relatively dry (December to February and July to August) [18].

Plant material

The plant material for this study was leaves of *Chromolaena odorata* (L.) R. M. King and Robinson (*Asteraceae*). The fresh leaves were collected during the period of April-May 2019 corresponding to the rainy season when biomass is abundant, on the campus of the IRAD. The choice of this plant was based on its different uses, its availability, and its invasiveness as gathered from ethnomedicinal information obtained from traditional healers in certain localities. The identification of this plant was carried out at the National Herbarium (IRAD). The chemical composition of *C. odorata* leaf was analyzed according to standard analytical methods [5] and is shown in table 1.

The harvested leaves were weighed, washed with plenty of water, drained, and dried in the laboratory at room temperature (22 ± 2°C) to avoid any degradation of the active ingredients as described by [30]. During drying, the leaves were regularly aerated to avoid contamination by fungi. Once dried, the leaves were ground using an electric grinder and kept in plastic bags for further use.

Preparation of experimental diet

Maize, wheat bran, soybean cake, cotton seed cake, fish meal, oyster shell, concentrate, premix, and bicalcic phosphate, were also purchased from a local market. Three experimental diets were formulated and in each diet, COLM was supplemented at 0%, 1%, and 2% and coded T0, T1, and T2 respectively. The weights of ingre-

Nutrients	Composition (% DM)
DM	91.545
EE	8.015
OM	91.245
CP	20.585
Fat	5.400
CF	14.365
CE	4495.530
ME	2643.575
DM: Dry Matter; CP: Crude Protein; ME: Metabolizable Energy; CF: Crude Fiber; EE: Ether Extract, Crude Energy	

Table 1: Proximate composition (%) of COLM.

dients that were mixed to obtain the experimental diets and their respective chemical compositions were calculated as presented in table 2.

Ingredients (Kg)	T0 (0% COLM)	T1 (1% COLM)	T2 (2% COLM)
Maize	51.8	51.8	51.8
Wheat Bran	7.0	7.0	7.0
Soya meal	15.0	15.0	15.0
Cotton seed cake	7.0	7.0	7.0
Fish meal	5.0	5.0	5.0
oyster shell	8.0	8.0	8.0
Concentrate 5%	5.0	5.0	5.0
Bone meal	2.0	2.0	2.0
Elitox	0.1	0.1	0.1
Bicalphos	0.1	0.1	0.1
Chromolaena odorata Powder	0	1	2
Total	100	101	102
Calculated chemical Composition			
EM (Kcal/Kg)	2673.00	2700.00	2726.50
Crude Protein %	21.49	21.48	21.15
Fat Mater	4.11	4.12	4.13
Calcium %	4.37	4.33	4.39
Phosphorus %	0.78	0.78	0.77
Total Lysine%	1.13	1.13	1.12
Total Methionine	0.47	0.47	0.47

Table 2: Weight of ingredients used for production of experimental diets at supplementation levels of COLM.

Animals and experimental design

Seventy-five breeding chickens (Brahma) aged 4-5 months were randomly allocated to three groups of fifteen chickens. Each group was then subdivided into five replicates of five chickens each (1 male and 4 females). A control ration (T0) and 2 other rations containing 1% (T1) and 2% (T2) of COLM were allocated to the experimental units in a completely randomized design. Water and feed were offered *ad-libitum* throughout the eight weeks experimental period. Egg's collection was carried out every day.

Data collection

Semen sample collection and evaluation

After the first two weeks of acclimatization and training, semen collection and evaluation were carried out for eight weeks. Semen samples were collected once a week by the double hand massages technique [12]. Immediately after semen collection, the following spermatological parameters were estimated: semen volume, sperm motility, and concentration of spermatozoa live and dead spermatozoa.

Semen from each cock was collected using a collection tube and semen volume was determined using 2 mL Eppendorf tubes. Colored sperm that wasn't completely white was eliminated since it could have been tainted with feces or blood. To eliminate investigator bias, semen was collected and examined by a single investigator throughout the study period.

Sperm motility was estimated subjectively under the microscope, by placing a drop of raw undiluted semen on a pre-warmed slide and covered with a slip to spread the semen into uniform thickness and viewed under a light microscope at ×100 magnification. Motility was estimated by subjectively assessing the wave pattern and ranked according to the standard method [3].

Spermatozoa concentration was determined using a Neubauer hemocytometer and a light microscope and the technique involved mixing semen with sterile saline solution at a dilution ratio of 1: 200. Sperm count was carried out according to [16]. The formula used to determine spermatozoa concentration was:

$C = n \times d \times 50\,000$

Where C= Spermatozoa concentration, n=number of sperm cells counted in 5 diagonal squares, d= dilution factor, and 50 000 a constant.

Percentage live/dead spermatozoa were evaluated using the eosin/nigrosin staining procedure [10] by mixed 10 µL semen with 50 µL of eosin/nigrosin smear, then making a thin smear, immediately air-dried and viewed under a light microscope (×400). The proportion of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in a sample were assessed based on 200 cells counted [40].

At the end of the experiment, three cocks were taken randomly from each group, fasted for 24 hours, weighed and slaughtered, and testicles were carefully removed. One and its epididymis were fixed in formaldehyde 10% buffered for histopathological analysis. The other was homogenized to 10% homogenate with ice-cold physiological saline and centrifuged at 3700 rpm at 4°C for 15 min, then stored at - 20°C for biochemical analysis.

Biochemical analysis

The testicular protein was determined by the biuret method using the BIOLABO kit and total cholesterol contents by the CHOP-PAP method. Catalase activity (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined using the commercial kits according to the manufacturer's instructions (BIOLABO SAS).

Histological study

After dehydration and embedding in paraffin, a 5 µm section of each organ was cut with a microtome, deparaffinized, and stained with hematoxylin-eosin. Photomicrography of tissues was obtained using a light microscope (Leitz wetzlar Germany 513) connected with a Celestron 44421 camera linked to a computer.

Fertility parameters

Eggs were collected and properly marked, stored at room temperature, and set at a 7 days interval for 4 weeks. During the incubation phase, fertility and hatchability of eggs, as well as embryogenic mortality of fertile eggs were recorded. Eggs from every treatment (T0, T1, and T2) were collected and stored, and incubated after a week in an automatical incubator with a capacity of 1000 eggs, automatic regulation with temperature and relative humidity. Eggs were automatically turned every 12 hours. Hatching eggs were fumigated with formalin gas (generated by mixing 40 mL formaldehyde 40% and 20 g potassium permanganate on a 1 m^3 room capacity). Fertility was determined as 100[number of fertile eggs]/number of total eggs set; hatchability as 100[number of chicks hatched]/number of fertile eggs and hatchability from totally eggs set were both calculated as the number of chicks hatched/number of total eggs set. Embryonic mortalities were estimated by dividing the number of dead embryos by the number of fertile eggs multiplied by 100.

Statistical analysis

Data collected were expressed as mean ± standard error of the mean (SEM). The statistical differences in egg quality and semen characteristics between treatments were assessed using the Kruskal-Wallis' test (H) followed by a Mann-Whitney pairwise test among treatments. The statistical differences in other variables between treatments were assessed ANOVA (F) followed by a Duncan pairwise test among treatment by using SPSS Statistic 20 software. Furthermore, Pearson correlation coefficients for some parameters were also performed with the same software. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Results

Effect of supplementation of COLM on semen volume, sperm concentration, sperm motility, live/dead sperm

As shown in table 3, COLM significantly affected sperm concentration, sperm motility, live/dead sperm ($p < 0.05$), but no significant ($p > 0.05$) differences were observed with semen volume.

Parameters	Control (T0)	T1	T2	H/F	p-value
Semen volume (mL)	0.42 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	2.42	0.11
Sperm concentration (10^9 mL^{-1}	2.2 ± 0.06^a	3.11 ± 0.07^b	2.78 ± 0.11^b	30.17	0.001
Sperm motility (%)	61.24 ± 0.34^a	77.32 ± 0.35^b	76.33 ± 0.27^b	17.17	0.001
Live sperm (%)	65.03 ± 0.39^a	82.5 ± 0.76^b	78.08 ± 0.44^b	30.17	0.001
Dead sperm (%)	34.98 ± 0.39^a	17.5 ± 0.76^b	21.93 ± 0.44^b	266.9	0.001

Table 3: Supplementation of COLM on semen volume, sperm concentration, sperm motility, and spermatozoa viability.

The same letter in a row shows no significant difference between treatments ($p > 0.05$).

Effect of supplementation of COLM on testes biochemical parameters

The effects of supplementation of COLM on testes biochemical parameters of cock are shown in table 4. The amount of testicular cholesterol in cock was highest ($p < 0.05$) in the T2 diet and least in the control diet group. Moreover, the testes concentration reduced glutathione (GSH) and protein as well as catalase activity in cocks fed T1 and T2 diets were similar but significantly higher ($p < 0.05$) than cocks fed the control diet. The concentration of malondialdehyde (MDA) decreased with dose-dependently to COLM supplementation levels.

Effect of supplementation of COLM on egg production, fertility, hatchability, and embryo mortality

Data in table 5 shows the effect of supplementation of COLM on egg production, fertility, hatchability, and embryo mortality. Hens fed 1% COLM exhibited the highest fertility rates ($p < 0.05$) while no significant differences ($p > 0.05$) were observed with respect to hatchability and embryo mortality among the different dietary treatments.

	Control	T1	T2	H	p value
Cholesterol (mmol/L)	0.18 ± 0.01^a	0.27 ± 0.02^a	0.63 ± 0.15^b	7.2	0.027
Catalase (mmol H_2O_2/g organ)	12.62 ± 0.75^a	15.60 ± 0.51^b	14.99 ± 0.71^b	5.66	0.04
GSH Concentration (µmol/g organ)	2.14 ± 0.12^a	4.2 ± 0.14^b	3.81 ± 0.08^b	87.96	0.001
Protein (mg/dL)	0.08 ± 0.01^a	0.17 ± 0.01^b	0.17 ± 0.01^b	1022	0.001
MDA (mol/g organ)	6.85 ± 0.05^a	4.00 ± 0.04^b	3.77 ± 0.06^b	1101	0.001

Table 4: Effects of supplementation of COLM on testicular biochemical parameters.

The same letter in a row shows no significant difference between treatments ($p > 0.05$). GSH: reduced glutathione; MDA: Malondialdehyde; T1: COLM supplemented at 1%: T2: COLM supplemented at 2%.

	Control (T0)	T1	T2	H/F	p-value
Fertility (%)	67.01 ± 2.30^a	79.85 ± 2.32^b	69.31 ± 3.64^a	5.88	0.009
Hatchability (%)	42.77 ± 3.32	39.75 ± 4.42	44.26 ± 2.52	0.43	0.66
Embryo mortality (%)	57.53 ± 3.21	60.41 ± 4.29	55.43 ± 2.38	0.54	0.59

Table 5: Effects of supplemented of COLM on egg production, fertility, hatchability and embryonic mortality.

The same letter in a row shows no significant difference between treatments ($p > 0.05$).
T1: COLM supplemented at 1%: T2: COLM supplemented at 2%.

Correlation between sperm motility, dead sperm, live sperm, fertility and hatchability

Positive relationships were observed between live sperm and fertility ($r = 0.66$, $p < 0.05$) and between live sperm and sperm motility ($r = 0.83$, $p < 0.05$).

Effect of supplementation of COLM on micrography of testes and epididymis

The effect of supplemented of COLM on photomicrography of testes and epididymis is illustrated in figure 1.

	Fertility	Hatchability	Sperm motility	Live sperm	Dead sperm
Fertility	1				
Hatchability	0.08	1			
Sperm motility	0.44*	-0.20	1		
Live sperm	0.66***	-0.05	0.83***	1	
Dead sperm	-0.66***	0.05	-0.83***	-1***	1

Table 6: Correlation between sperm motility, dead sperm, live sperm, fertility, and hatchability.

*: significance at 0.05 threshold

***: significance at 0.001 threshold

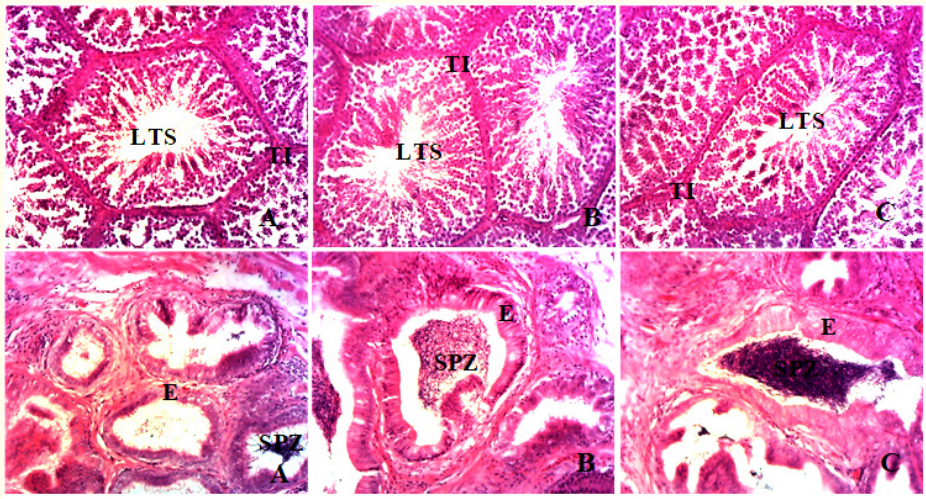

Figure 1: Microphotography of testes and epididymis (X100) in poultry cock of T1 and T2 treatment.
A: Normal Treatment (T0); B and C: Cock Fed Dietary Supplementation with COLM at the level of 1% (T1) and 2% (T2).
LTS: Lumen of Seminiferous Tubule; TI: Interstitial Tissue; E: Epididymis: SPZ: Spermatozoa

The figure below shows the effects of T1 and T2 treatment on the microarchitecture of the testis and epididymis in the cock. The figure shows that T1 and T2 treatments compared to normal animals resulted in an increase in sperm density in the epididymis and in the lumen of the seminiferous tubules. The testicular sections also show germ cells at all stages from spermatogonia to spermatozoa.

Discussion

The fact that sperm concentration, percentage of sperm motility, and live/dead sperm cell values obtained higher values in T1 and T2 fed cocks could indicate that COLM therapy enhanced sperm production in cocks. It can also be due to the action of natural compounds like flavonoids present in the plants, which play the role of natural antioxidants reducing oxidative stress and modulating harmful biological pathways. This collaborates with [6,34,38] who revealed that some plants can improve semen parameters of animals and men. Additionally, histological observation revealed that incorporation of COLM improved spermatogenesis. This is similar to what was observed in local Kabir rooster and local cock, such as *Thymus vulgaris* and *Xylopia aethiopica*, respectively reported by [17,38], who evaluated the effect of these plants on the semen quality.

In this study, the increased amount of the testicular cholesterol concentration with dietary supplementation of the cock's diet with COLM up to 2% induce the novo synthesis of cholesterol in germ cells [36]**.** This means also that 2%COLM during eight weeks leads to an increase in the production of cholesterol which positively affected testes of cock, while improving spermatogenesis and the number of spermatozoa. Our findings corroborate those of [2] who found that the testes of high cholesterol diet-cinnamon co-treated mice looked to be as normal as those of negative control animals. It is known that cholesterol is a precursor to steroid synthesis, which is required for proper sperm production and plays a determinant role in spermatogenesis [13].

That testes concentration of catalase (CAT) and reduced glutathione (GSH) were significantly higher in cocks fed COLM supplemented diets could be explained by the fact this plant can increase enzymatic antioxidants such as the aforementioned ones. The obtained results are similar to [22] who found that *Cirsium arvense* had beneficial impacts on enzymatic antioxidants. Decrease testes concentration of malondialdehyde in COLM fed cocks could be attributed to the high antioxidant activity of *Chromolaena odorata* [25]. The enhancement in egg fertility in hens fed COLM supplementation could be explained by the fact that *Chromolaena odorata* has beneficial bioactive compounds such as flavonoids, essential oils, saponins, and alkaloids [31]. These bioactive are known to have positive impacts as antioxidant, antimicrobial, and immunestimulatory action [11,32,39] which excite the excretion of digestive enzymes in the gastrointestinal system, increasing bird appetite and maintaining physiological stability. Also, improved fertility in this study could be linked to improved semen quality, and this is related to the findings of [8]. Who observed that ginger improved semen quality. Hatchability and embryonic mortality were not affected by COLM in this study. This result is thorough consistency with [9] who reported that Chamomile, Wild mint, and Oregano herbal to laying Japanese quails had no effect on hatchability.

Conclusion

In conclusion, *Chromolaena odorata* leaf meal (COLM) supplemented in the Brahma chicken diet improved semen quality, egg fertility, and antioxidative enzymes (catalase, reduced glutathione, and malondialdehyde). It is recommended that *Chromolaena odorata* leaf can be offered to local chicken at a 1% level without adversely affecting reproductive performance, especially spermatogenesis.

Acknowledgment

The authors are grateful to the Institute of Agricultural Research for Development. Cameroon through its Public Investment Budget for funding and providing the necessary facilities for this work, as well as the Animal Physiology Laboratory of the University of Yaoundé 1 for carrying out the histological and biochemical analysis.

Conflict of Interest

No.

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