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Research Article

Effect of Incorporating Soursop (*Annona muricata*) Leaves Powder on Reproductive Performance of Japanese Quail (*Coturnix coturnix japonica*)

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ABSTRACT

Introduction: Soursop leaves are rich in various molecules, including total phenols, terpenes, and steroids, which possess a range of pharmacological properties that can be utilized in animal production to enhance both the growth and reproduction of animals. The present study aimed to evaluate the effect of incorporating soursop (*Annona muricata; A. muricata)* leaves powder into feed on the reproductive performance of Japanese quail.

Materials and methods: A total of 80 Japanese quails (64 females and 16 males) aged two weeks were randomly divided into four experimental groups, labeled T0, T1, T2, and T3, and received feed additives with 0 mg/kg body weight (bw), 250 mg/kg bw, 500 mg/kg bw, and 750 mg/kg bw of powdered soursop leaves, respectively. Additionally, water was provided *ad libitum*, and the quails' weights were measured every 7 days for 75 days. At the end of the period, 12 female quails from each group were sacrificed after a 24-hour fasting period. The blood sample was collected for hematological (Leukocyte, Erythrocyte, and Platelet indices) and serum biochemical (total serum cholesterol, total proteins, albumin, globulin, Aspartate aminotransferase, Alanine aminotransferase, Urea, and Creatinine) analysis. The males were also sacrificed to evaluate the spermatozoa characteristics (mobility, concentration, and viability).

Results: No statistically significant changes in growth characteristics or hematological parameters were observed. However, biochemical parameters increased significantly with the inclusion of Soursop (*A. muricate)* in quail feed, including increased total cholesterol, total protein, and globulin levels, and decreased malondialdehyde levels. This effect was most significant at a dosage of 500 mg/kg bw. Serum levels of urea, Alanine aminotransferase, and Aspartate aminotransferase were not significantly affected by *A. muricata* whatever the concentration considered. A significant increase in fast progressive spermatozoa, along with a decrease in immotile spermatozoa, was observed with *A. muricate* at a dosage of 500 mg/kg bw compared to the control. Sperm viability also increased significantly, particularly in live at a dosage of 500 mg/kg bw. A significant increase was observed in fertility parameters, including increased fertility rate, hatchability rate of fertile eggs, total hatchability rate, and chick weight, along with decreased embryonic mortality at 500 mg/kg bw treatment compared to the control.

Conclusion: In conclusion, the findings indicated that incorporating *A. muricata* leaf powder at 500 mg/kg bw into quail feed positively influences reproductive cells and boosts fertility growth promoters.

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1. Introduction

Soursop (Annona muricata) is a tropical fruit belonging to the Annonaceae family, native to tropical countries, also known according to the producing countries as graviola, guanabana, sauersak, guayabano, and several other regional names[1]. It has economic importance and growth in the Caribbean as well as the equatorial belt of the Americas, mostly in the Bahamas, Bermuda, Cuba, Dominican Republic, Grenada, Mexico, Costa Rica, St. Vincent, Puerto Rico, Colombia, Venezuela, Equator, and Brazil. It is distributed throughout the tropics of the world, including the Caribbean, Africa, and Southeast Asian countries such as Thailand, Malaysia, Indonesia, and the Philippines, with Mexico as the principal producer country[2].

However, this method has been associated with several setbacks, including high cost, environmental contamination, allergic reactions in humans, residue in animal products, harm to both target and non-target species, and development of antibiotic resistance which could potentially be transferred to humans[3,4]. These concerns have prompted the search for alternative solutions.

One potential avenue is the utilization of phytogenic feed additives[5,6,7]. Soursop leaves (*A. muricata*) have been traditionally used in medicine and are known for their bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, which possess antioxidant, anti-inflammatory, and antimicrobial properties[8]. While their specific effects on birds have not been extensively studied, some research[9,10] has examined their nutritional composition, and recent studies by Indah et al.[11] and Rachael[12] have investigated their phytochemical properties and impact on digestion and nutrient absorption. The Soursop leaves are large, dark-green with a distinct slightly waxy appearance, which for centuries have been used as traditional medicine and has gained attention for its nutritional properties. Although the specificity of the soursop leaves in birds has not been extensively studied, among the various poultry species, the Japanese quail (*Coturnix coturnix japonica*) has gained popularity due to its adaptation, resilience, rapid growth, and reproductive potential. However, optimizing the reproductive performance of quail remains a challenge for farmers in Cameroon[13]. Exploring innovative approaches to enhance quail reproductive performance is crucial for sustainable and profitable farming. Hence, the main objective of the present study is to improve birds' reproductive performance.

2. Materials and Methods

2.1. Ethical approval

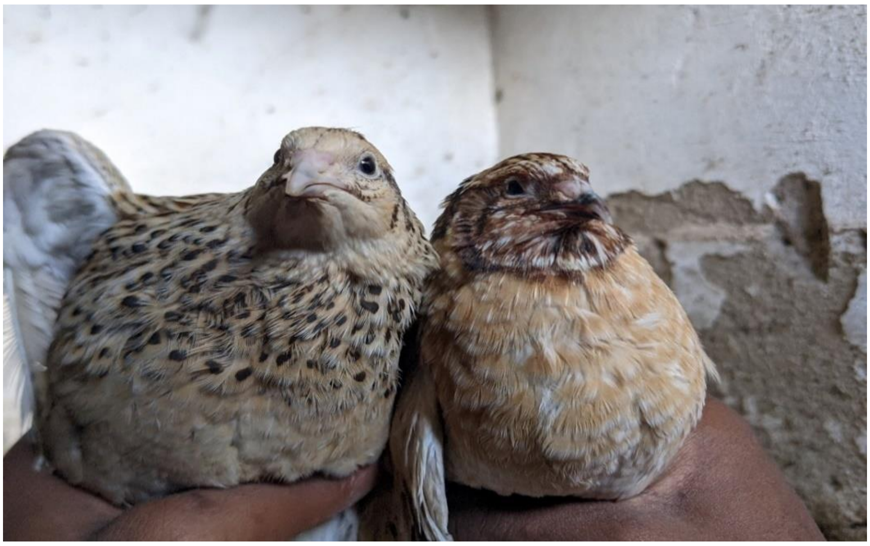
This study was carried out in strict accordance with recommendations of institutional guidelines for the care and use of laboratory animals. Quails were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.1. Study area

The experimental work was carried out at the poultry unit of the Application and Research Farm (ARF) and the Animal Physiology and Health laboratory of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, between March to May 2024.

2.2 Animal material

A total of 80 healthy Japanese quail birds (16 males and 64 females) aged two weeks were purchased from poultry in Douala and were raised over a period of 75 days (Figure 1). The birds were then identified with a tape bearing their number on one of their paws. The birds had an adaptation period of one week.



A B

Figure 1. Adult female Japanese quail (A) and Adult male Japanese quail (B)

Table 1. Composition of diet (grower and laying phases) in quails

Ingredients (%)	Grower phase (1-4 weeks, fattener)	Finisher phase (5-10 weeks)
Maize	60.0	63.0
Wheat bran	4.5	4.5
Soya bean meal	22.0	18.0
Fish meal	4.5	4.5
Bone meal	2.0	2.5
Shell	2.0	2.5
Concentrate 5% (*)	5.0	5.0
Total	**100.0**	**100.0**
Calculated chemical composition		
Raw protein (%)	23.20	20.70
Metabolizable energy (kcal/kg)	2913.00	3013.00
Calcium (%)	1.48	1.51
Phosphorus (%)	0.69	0.73
Lysine (%)	1.29	1.10
Methionine (%)	0.43	0.40
ME/CP	125.00	145.00

* Broiler concentrate 5%; Crude protein: 40%; Metabolizable Energy: 2078 kcal/kg; Calcium: 8%; Available Phosphorus: 2.05%; Lysine: 3.30%; Methionine: 2.40%. ME: Metabolizable Energy; CP: Crude protein

2.2.1 Housing

The birds were raised in a semi-open permanent breeding building to allow ventilation. The cages were made of wire mesh (45 cm long, 40 cm wide, and 44 cm high). Each cage was equipped with a 50 ml plastic drinker and a 50 ml plastic feeder. The birds were raised on bedding for 75 days.

2.2.2 Feeding

The birds received commercial feed and freshwater ad libitum. The feed was formulated with ingredients, such as maize, wheat bran, bone meal, soya beans meal, fish meal, and powdered shell, along with a 5% concentrate purchased from the Dschang market. The feed was served in 50cl plastic feeders, while water was provided in 50cl containers. Each bird received a feed mixture supplemented with *A. muricata* leaf powder in varying percentages. The composition and chemical composition of the feed during the grower and finisher phases are presented in Table 1.

2.3 Sanitary protection and prophylaxis

Two weeks before the arrival of chicks, the brooding house and various equipment were washed and disinfected using bleach, Cresyl (20 ml per 1 litter of water), and virunet solution (50 g per 5 litter of water), spread in the room and on all the cages. The room and equipment used were cleaned on a daily basis during breeding. Upon arrival of the birds on the farm, they were provided with anti-stress (1g per 2 liters of water) for two consecutive days during the period of adaptation.

2.4 Plant material and powder preparation

Fresh matured Soursop leaves were harvested on the same tree in Bafang (LN 5 ° 09 ′ 23″, LE 10 ° 10 ′ 44″), a district of the Upper-Nkam division Western region of Cameroon. The leaves were washed and dried at room temperature (25°C) in the shade to reach a constant weight, then they were milled into fine powder using a grinding machine. The powder was sifted to achieve a consistent fine texture, which was subsequently combined with ground feed.

2.4.1 Chemical constituent of Soursop leaf

A portion of the *A. muricata* powder was used for phytochemical screening at the chemistry laboratory of natural substances at the University of Dschang, following the methodology outlined by Banso and Ngbede[14] and Ngbede et al.[15]. Table 2 shows the results of the phytochemical test in the presence of tannins, alkaloid, flavonoids, phenols, saponins, steroids, and triterpenes.

Table 2. Constituent of *Annona muricata* leaf powder

Constituent	(+) Present; (-) absent
Tannins	+
Alkaloid	+
Flavonoids	+
Phenols	+
Saponins	+
Steroids and triterpenes	+

(+) Present; (-) absent

2.4.2 Choice of the various powdered doses administered

The selection of various concentrations for administration to the birds was obtained from the study of Elly et al.[16], who investigated the effects of 0.5 mg/kg, 10 mg/kg, and 15 mg/kg of soursop leaf powder (*A. muricata*) on the reproductive performance of Tegal duck fed with graded levels of the powder.

2.5 Experimental design

The animals were kept in rows of cages made from galvanized metal mesh (45 cm long, 40 cm wide, and 44 cm high for a set of five quails). Each cage housed five quails, including four females and one male. The cages were equipped with a suitable 50cl drinker and a linear feeder of 50cl. A total of 80 quails aged two weeks with an average weight between 116g-118g were distributed into four groups of 16 birds corresponding to four treatments in a completely randomized design (T0, T1, T2, and T3). Each group was subdivided into four subgroups of five birds (4 females and 1 male) corresponding to the group's repetitions. The birds in each group received one of the following treatments:

In the first group (control group) birds received the basic ratio without *A. muricata* leaf powder, whereas the second group received the basic ratio with 0.25 mg/kg bw powder of *A. muricata* leaf. The third group received 0.50 mg/kg bw powder of *A. muricata* leaf while the fourth group received 0.75 mg/kg bw powder of *A. muricata* leaf.

In birds between 3 and 12 weeks of age, food intake and weight gain were assessed weekly. Furthermore, after 9 weeks of treatment, eight females were chosen randomly to fast for 24 hours and sacrificed according to the method described by Jourdain[17]. Blood was collected to evaluate biochemical parameters. In addition, four males of each treatment were sacrificed using the same method, and the vas deferens removed and dilacerated for the evaluation of sperm characteristics.

2. 6 Data collection and studied parameters

2.6.1 Growth performance

2.6.1.1. Feed intake

At the beginning of each week, the weight of the feed was recorded, then it was distributed daily to the birds. To monitor feed intake, the remaining feed for each experimental treatment was weighed weekly using a precision balance (0.01g accuracy, 500g capacity). Feed intake was calculated by subtracting the weight of feed refused during a given week from the total weight of feed served during the same week.

2.6.1.2. Life body weight (LBW) and weekly body weight gain (WBWG)

The live body weight of each bird was recorded weekly. The weekly body weight gain was then calculated by subtracting the live body weight from the previous week from the live body weight of the current week, thereby obtaining the difference in weight between the two consecutive weeks.

2.6.2 Biochemical and hematology analysis

At the end of the trial, 32 females were taken and fasted for 24 hours. Their weights were taken individually and were sacrificed by decapitation. The 4 ml of blood of each subject was collected in two labelled test tubes, one with anticoagulant and the other anticoagulant-free. The blood with anticoagulant was used for hematological analyses and the anticoagulant-free samples had produced serum which was used for evaluation of serological parameters.

2.6.2.1 Serum biochemical analysis

During the animal sacrifice, approximately 4 ml of blood was collected from each selected animal into anticoagulant-free tubes for biochemical analysis. The blood was then centrifuged at 3000 rpm for 15 minutes to separate the serum from the clotted blood. The serum was carefully collected using a micro pipette and transferred using test tubes. The collected serum was stored at -20°C for further use. The serum was used to evaluate various biochemical parameters, including total serum cholesterol, total proteins, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (Ur), and creatinine (Cr). These biochemical characteristics were assessed using spectrophotometry methods, as described in the CHONOLAB commercial kits, Spain.

2.6.2.2 Analysis of hematological parameters

Blood samples (2-3 ml) were collected from sacrificed animals in each group. The blood was collected in anticoagulant tubes and analyzed for various hematological parameters using an automated hematimeter (Genius Mark, model KT 6180, Germany). The evaluated parameters contained Hemoglobin, hematocrit, packed cell volume (PCV), blood platelets, red blood cell (RBC), and white blood cell (WBC). The PCV was also measured using a micro-hematocrit capillary tube and a hematocrit reader. To provide a comprehensive assessment of the animal's hematological profile, within 24 hours of blood collection, the following additional parameters were measured using an automated cell counter, USA. The RBC count, WBC count, platelet count, hemoglobin (Hgb), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH).

2.6.3 Malondialdehyde (MDA)

The serum was used to determine malondialdehyde (MAD) concentration by the thiobarbituric acid method[16].

2.6.4 Reproductive parameters

2.6.4.1 Semen collection

After 10 weeks of experimentation, the two epididymal tails of 4 quails from each treatment group were removed by opening the abdomen, then minced in 10 ml of 9% NaCL pre-incubated in a water bath at 36°C. Thus, spermatozoa diffuse into the solution, including 20 µl in HCB029 Veterinary Mammal Sperm Analyzer at 40 X magnification to evaluate spermatozoa characteristics (Motility, concentration, Viability).

2.6.5 Laying performance and characteristics of eggs

2.6.5.1. Laying rate and weight of eggs

At the start of laying, the eggs were collected every morning per treatment for four weeks, then weighed using an electronic balance of 500g capacity and of 0.01g precision to evaluate the laying rate and weight of eggs using the following formulas[18].

$$\text{Laying rate (\%)} = \frac{\text{Number of eggs of a week}}{\text{Number of hen} \times 7} \times 100$$

$$\text{Average weight of eggs (g)} = \frac{\text{Total weight of eggs of one week (g)}}{\text{Number of eggs for the week}}$$

2.6.5.2. Characteristics of eggs

The internal and external quality of eggs were evaluated[19]. To achieve this purpose, when the birds were 9 weeks, 12 eggs per treatment were randomly collected for three times. The weight of the egg yolk, weight of egg white, and shell weight were evaluated using an electronic balance of 500g capacity and of 0.01 g precision.

2.6.6 Fertility indices

A total of 34 healthy eggs per treatment were randomly collected on three separate occasions, each spanning six consecutive days, at 10, 11, and 12 weeks old. The eggs were weighed individually and then incubated. After 18 days of incubation, the eggs hatched. Any unhatched eggs were then examined and cracked and were classified as either infertile or having experienced embryonic mortality.

At hatching after incubation, the following indices were calculated [18] for each replicate according to the following formulas.

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

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

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

$$\text{Embryonic mortality rate (\%)} = \frac{\text{Number of embyos dead}}{\text{Number of fertile eggs}} \times 100$$

Chicks per treatment were collected after hatching and individually weighed using an electronic scale of 500g capacity with a precision of 0.01g. The average weight of chicks was determined by dividing the total weight of chicks of the treatment group by the number of chicks obtained.

2.7 Statistical analysis

Data was analyzed through one-way analysis of variance (ANOVA) to test the effect of *A. muricata* powder concentrations leaves (25 mg/kg, 500 mg/kg, and 750 mg/kg bw) on the various parameters. Means showing significant differences were compared using Duncan's Multiple Range Test. Data is expressed in means ± standard deviation form. The IBM Statistical Package for Social Science (SPSS) software version 26.0 was used for the statistical analysis. The limit of significance was fixed at $p < 0.05$.

3. Results

3.1.1. Effects of Annona muricata powder on the growth parameters of female Japanese quail

Table 3 presents the growth parameters of Japanese quail exposed to *A. muricata* powder. It showed that the concentrations of *A. muricata* in the feed of quails did not significant affect their growth characteristics compared to control.

3.1.2 Effect of Annona muricata powder on female hematology parameters of Japanese quail

3.1.2.1. Leukocyte indices

Table 4 illustrates the leukocyte indices of female Japanese quail exposed to *A. muricata* powder. It resulted that the incorporations of *A. muricate* powder

Table 3. Effects of *A. muricata* leaf powder on growth characteristics in Japanese quail

Growth characteristics	Rate of *Annona muricata* powder in the ration (mg/kg bw)				P-value
	Control	250	50	750	
Feed consumption M-F (kg)	7.65 ± 0.29	7.40 ± 0.54	7.52 ± 0.49	7.77 ± 0.34	0.64
Live weight Male kg)	0.25 ± 0.04	0.26 ± 0.03	0.26 ± 0.04	0.26 ± 0.04	0.93
Female live weight (kg)	0.29 ± 0.01	0.27 ± 0.03	0.26 ± 0.02	0.29 ± 0.02	0.34
Live weight M-F (kg)	1.39 ± 0.09	1.36 ± 0.11	1.32 ± 0.09	1.41 ± 0.08	0.49
Average male weight gain (kg)	0.12 ± 0.04	0.14 ± 0.03	0.14 ± 0.04	0.14 ± 0.04	0.91
Average female weight gain (kg)	0.17 ± 0,01	0.16 ±0,03	0.15 ±0,02	0.18 ±0,02	0.36
Average M-F weight gain (kg)	0.82 ± 0,08	0.78 ±0,11	0.74 ±0,08	0.84 ±0,08	0.46
Male average daily gain (g)	2.06 ± 0,67	2.27 ± 0,53	2.33 ± 0,65	2.33 ± 0,59	0.91
Female average daily gain (g)	2.88 ± 0,23	2.68 ± 0,44	2.51 ± 0,35	2.92 ± 0,34	0.36
Average daily gain M-F (g)	13.59 ± 1,39	13.00 ±1,84	12.39 ± 1.28	14.00 ± 1.26	0.46
Feed conversion ratio MF (g)	9.44 ± 0.83	9.56 ± 0.85	10.16 ± 0.49	9.29 ± 0.60	0.37

M-F: Male to female, P-value: Threshold of significance typically set at 0.05

Table 4. Effects of *Annona muricata* powder on leukocytes indices in the female Japanese quail

Parameters	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P value
		250	500	750	
WBC (10^3/ul)	123.15 ± 75	159.38 ± 30	170.51 ± 38	146.26 ± 46	0.345
LYM %	64.53 ± 33	84.20 ± 6.8	81.53 ± 7.6	87.51 ± 4.29	0.150
MID %	14.06 ± 9.36	14.38 ± 5.65	16.13 ± 6.5	11.63 ± 3.45	0.705
GRAN %	4.73 ± 6.96	1.41 ± 1.23	2.33 ± 1.20	0.85 ± 0.89	0.287

WBC: White blood cell, LYM: Lymphocytes, MID: Mid-size-cell, often Monocytes, GRAN: Granulocytes. P-value: Threshold of significance typically set at 0.05

Table 5. Effects of *Annona muricata* powder on erythrocytes indices in the female Japanese quail

Parameters	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P value
		250	500	750	
RBC (10^6/ul)	3.15 ± 1.45	3.52 ± 0.84	3.98 ± 1.17	3.11 ± 0.49	0.467
HGB (g/dl)	16.51± 6.3	21.03 ± 5.35	22.38 ± 5.89	18.38 ± 1.4	0.227
HCT (%)	41.13 ± 18.35	52.26 ± 12.23	58.91 ± 16.74	47.90 ± 4.34	0.198
MCV (10^6-15 L)	138.13 ± 3	148.20 ± 6.21	149 ± 7.91	155.26 ± 11	0.447
MCH (10^6-12 g)	60.81 ± 28	59.88 ± 7.42	57.06 ± 4.91	59.66 ± 4.99	0.977
MCHC (g/dl)	42.76 ± 12.52	40.18 ± 3.44	38.18 ± 1.43	38.38 ± 1.86	0.609

RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, P-value: Threshold of significance typically set at 0.05

concentration in the quails' feed, did not significantly affect the leukocytes indices ($p < 0.05$, WBC, LYM, MID, GRAN).

3.1.2.2. Erythrocyte indices

As can be seen in Table 5 the inclusion of *A. muricata* leaf powder concentration in the feed of the quail did not scientifically affect the erythrocyte indices compared to the control ($p > 0.05$).

3.1.2.3. Platelet indices

Table 6 provides the platelet indices of female Japanese quail exposed to *A. muricata* powder. Accordingly, the incorporations of *A. muricata* powder concentration in the feed, did not significantly affect the platelet indices ($p < 0.05$).

3.1.3. Effect of Annona muricata powder on female biochemical parameters of Japanese quail

Table 7 presents the effect of incorporating *A. muricata* leaf powder in the feed of Japanese quail in the serological parameters. It was found that incorporating *A. muricata* leaf powder at a concentration of 500 mg/kg bw significantly increased total cholesterol levels in the serum compared to other concentrations and the control group. Similarly, administering *A. muricata* leaf powder at concentrations of 500 mg/kg bw and 750 mg/kg bw resulted in significantly higher levels of total protein and albumin compared to the lowest concentration (250 mg/kg bw) and the control group ($p < 0.05$). The globulin levels in quails treated with *A. muricata* leaf powder were higher across all concentrations compared to the control group.

Table 6. Effects of *Annona muricata* powder on the platelet indices in female Japanese quail

Parameters	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P value
		250	500	750	
PLT (10^3/ul)	127.36 ± 113	72.66 ± 47	121.83 ± 127	85.33 ± 20	0.655
MPV (f L)	5.45 ± 1.25	5.46 ± 0.96	5.60 ± 1.03	5.16 ± 0.61	0.894
PDW (%)	6.18 ± 1.83	5.58 ± 0.71	6.08 ± 0.81	5.51 ± 0.60	0.643
PCT (%)	0.817 ± 0.08	0.383 ± 0.35	0.717 ± 0.09	0.383 ± 0.01	0.597
P_LCR (%)	7.36 ± 5.08	7.55 ± 4.63	8.28 ± 4.78	4.98 ± 2.66	0.600
P_LCC (10^3/ul)	13.83 ± 20.82	6.16 ± 9.76	13.33 ± 23.44	5.16 ± 2.85	0.709

PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, P_LCR: Platelet large cell ratio, P_LCC: Platelet large cell count. P-value: Threshold of significance typically set at 0.05.

Table 7. Effects of *Annona muricata* powder on female biochemical parameters of Japanese quail

Parameters	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P-value
		250	500	750	
Total cholesterol (mg/dL)	147.29 ± 94^b	154.94±58^b	285.94±78^a	218.18±118^b	0.03
Total protein (g/dL)	3.47 ± 1.28^b	2.68±0.50^b	4.31 ±0.83^a	3.79±1.09^a	0.02
Albumin (g/dL)	1.11 ± 0.44^b	1.19±0.29^b	1.67±0.48^a	1.78±0.30^a	0.03
Globulin (g/dL)	2.35 ± 1.43^{ab}	1.48±0.62^b	2.00±0.97^{ab}	2.64±0.53^a	0.01
Urea (mg/dL)	58.24 ± 33	30.10± 0.40	23.83±45	25.23±16	0.20
Creatinine (mg/dL)	1.00 ± 1.00	0.84 ± 1.87	0.76±1.04	1.31±1.17	0.84
ALT (U/I)	8.18 ± 18	2.18 ± 0.65	2.83±2.45	1.96±0.40	0.49
AST (U/I)	217.02 ± 75	340.89±319	164.21±81	158.55±59	0.14
MDA (µM)	5.51 ± 5.74^a	2.51 ±2.37^{ab}	1.59±0.77^b	3.33±2.99^{ab}	0.02

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MDA: Malondialdehyde, P-value: Threshold of significance typically set at 0.05. ^{a,b} the mean with the same letter on the same line are not significantly different ($p > 0.05$).

Table 8. Effects of *Annona muricata* powder on sperm parameters of the Japanese quail

Spermatozoa	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P-value
		250	500	750	
Total detected (M/ml)	216.75 ± 102^{b}	210.69±108^{b}	250.75±63^{a}	250.75±63^{a}	0.003
Concentration (10^6/ml)	119.06 ± 56^{b}	119.17 ± 53^{b}	137.73 ± 34^{b}	174.44 ± 30^{a}	0.003
Total of mobile sperm (millions)	143.44 ± 88^{b}	149.13 ± 83^{b}	192.94 ± 58^{ab}	212.00 ± 37^{a}	0.017
Percent of mobile spermatozoa (%)	61.50 ±15^{b}	68.87 ± 15^{ab}	75.13 ± 8.5^{a}	67.36 ± 8.9^{b}	0.005
Fast progressive (%)	3.57 ± 6.97^{b}	7.60 ± 12.1^{b}	16.65 ± 11.77^{a}	10.58 ± 8.21^{ab}	0.004
slow progressive (%)	13.08 ± 7.40^{b}	17.19 ± 5.12^{ab}	19.66 ± 5.26^{a}	17.15 ± 5.04^{ab}	0.020
Local motile (%)	44.88 ± 9.16^{a}	44.07 ± 11.11^{a}	39.42 ± 7.63^{a}	42.01 ± 9.16^{a}	0.196
Immotile (%)	38.52 ± 15^{a}	31.12 ± 8.51^{ab}	23.86 ± 8.74^{b}	32.61 ± 11^{a}	0.004

P-value: Threshold of significance typically set at 0.05. a,b: the mean with the same letter on the same line is not significantly different ($p > 0.05$)

Table 9. Effects of *Annona muricata* powder on the spermatozoa viability

Percentage of Spermatozoa	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P-value
		250	500	750	
Alive	78.00 ± 0.816^{b}	77.50 ± 1.291^{b}	80.00 ± 0.168^{a}	76.75 ± 0.957^{b}	0.04
Dead	22.00 ± 0.816^{a}	22.50 ± 1.291^{a}	20.00± 0.816^{b}	23.25 ± 0.957^{a}	0.04
Abnormal	15.50 ± 1.291^{b}	18.50 ± 1.291^{a}	16.50 ± 1.291^{ab}	18.50 ± 1.291^{a}	0.01

P-value: Threshold of significance typically set at 0.05. a,b: The mean with the same letter on the same line is not significantly different ($p > 0.05$)

Notably, within the *A. muricata* treated groups, the 500 mg/kg bw group showed a significant increase in serum globulin levels compared to the 250 mg/kg bw group. The serum content in malondialdehyde decreased with the addition of *A. muricata* leaf powder in the bird feed, however, this reduction was significant only at the concentration of 250 mg/kg bw in the feed ($p < 0.05$). The other characteristics (Urea; Creatinine, ALT, and AST), were not significantly affected by the concentrations of *A. muricata* added in the feed of the Japanese quail compared to the control.

3.1.4. Effect of Annona muricata powder on the reproductive parameters of the male Japanese quail

3.1.4.1. Effect of Annona muricata powder sperm parameters of the Japanese quail

Table 8 describes the effects of *A. muricata* leaf powder on sperm parameters of Japanese quail. The total detected spermatozoa show a significant increase at the *A. muricata* concentrations of 500 mg/kg bw and 750 mg/kg bw compared to the control geoup. However, the concentration of spermatozoa in 750 mg/kg bw treated birds increased significantly compared to the other concentration of *A. muricata* and the control ($p < 0.05$). The total number of mobile spermatozoa increased with the concentrations of *A. muricata* in the feed, with a significant increase observed only at the concentrations 750 mg/kg bw compared to the control group ($p < 0.05$). The percentage of mobile spermatozoa increased with the concentrations of *A. muricata* in the feed, with a significant increase of 500 mg/kg bw (concertation of *A. muricata* leaf powder) compared to the highest concentration (705mg/kg bw) and the control group. Fast progressive and slow progressive spermatozoa showed an increase at all the concentrations with a significant increase at 500 mg/kg bw as compared to the control ($p < 0.05$). Inversely, the *A. muricata* leaf powder in the feed of Japanese quail at all the concentrations decreased immobile spermatozoa count, but this was significant only at 500 mg/kg bw compared to the control ($p < 0.05$). The local mobile spermatozoa count was not significantly affected by the graded levels of *A. muricata* leaf powder referring it to that of the control.

3.1.4.2. Effect of Annona muricata powder on the viability rate of sperm in the Japanese quail

Table 9 presents the effect of graded concentrations of *A. muricata* powder on the viability parameters of Japanese quail. Accordingly, the *A. muricata* leaf powder at a concentration of 500 mg/kg bw introduced in the feed of Japanese quails significantly increased the number of spermatozoa alive, compared to the control group and other *A. muricata* powder concentrations ($p < 0.05$). Inversely, the same concentration significantly decreased the number of dead spermatozoa. The percentage of abnormal spermatozoa in birds treated with *A. muricata* powder increased across all concentrations compared to the control group. However, the increase was only statistically significant at concentrations of 500 mg/kg bw and 750 mg/kg bw, indicating a notable effect on sperm quality at these specific doses.

3.1.5. Effects of Annona muricata powder on fertility and egg parameters of female Japanese quail

3.1.5.1. Effect of Annona muricata powder on fertility parameters of Female Japanese quail

As can be seen, Table 10 presents the effects of *A. muricata* powder on the fertility parameters of female Japanese quail. The *A. muricata* leaf powder at a concentration of 500mg/kg bw induced a significant increase of fertility rate compared to the other concentrations of *A. muricata* ($p < 0.05$). The concentration

Table 10. Effects of *Annona muricata* powder on fertility parameters of female Japanese quail

Parameters	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P-value
		250	500	750	
Fertility rate (%)	73.13±10.99[ab]	57.66±12.99[bc]	88.73±10.43[a]	42.30±21.98[c]	0.023
Hatchability rate of fertile eggs (%)	95.46±4.02[ab]	91.73±5.26[ab]	98.73±2.19[a]	80.73±15.10[b]	0.120
Total hatchability Rate (%)	70.36±26.87[ab]	53.90±12.06[bc]	87.73±11.82[a]	35.73±10.75[c]	0.026
Embryonic Mortality rate (%)	3.93±3.40[b]	3.47±3.08[b]	3.20±2.98[b]	10.93±0.80[a]	0.025
Weight of chick (g)	8.54±0.22[b]	8.04±0.14[c]	7.94±0.06[c]	8.96±0.46[a]	0.01

P-value: Threshold of significance typically set at 0.05. [a,b,c] the mean with the same letter on the same line are not significantly different ($p > 0.05$)

Table 11. Effects of *Annona muricata* powder on egg parameters of female Japanese quail

Parameters (g)	Control	Concentration of *A. muricata* leaf powder (mg/kg bw)			P value
		250	500	750	
Weight of egg	12.86 ± 0.11	12.65 ± 0.96	13.21 ± 0.18	12.34 ± 0.36	0.31
Weight of shell	1.81 ± 0.99[a]	1.71 ± 0.02[ab]	1.80 ± 0.10[a]	1.62 ± 0.38[b]	0.04
Weight of yolk	3.25 ± 0.43	3.03 ± 1.14	3.62 ± 0.18	3.46 ± 0.25	0.70
Weight of albumen	7.69 ± 0.54	7.69 ± 0.93	7.73 ± 0.33	7.27 ± 0.29	0.40

P-value: Threshold of significance typically set at 0.05. [a,b] the mean with the same letter on the same line are not significantly different ($p > 0.05$)

of 500 mg/kg bw of *A. muricata* leaf powder significantly increased the hatchability rate compared to the concentration of 750 mg/kg bw ($p < 0.05$). The total hatchability rate significantly increased in 500 mg/kg bw *A. muricata* treated quails compared to the other *A. muricata* concentrations ($p < 0.05$). The embryonic mortality rate recorded in the quails fed with 250 and 0.50 mg/kg bw of *A. muricata* was comparable to that of the control but decreased significantly to that of the animals fed with 750 mg/kg bw *A. muricata* leaf powder. The concentration of 750 mg/kg bw of *A. muricata* leaf powder significantly increased the chick's weight in comparison with other concentrations and control groups ($p < 0.05$).

3.1.5.2. Effect of Annona muricata powder on egg parameters of female Japanese quail

Table 11 describes the effects of *A. muricata* leaf powder on egg parameters of Japanese quail. The weight of eggs, weight of shell, weight of yolk, and weight of albumen increased with 500 mg/kg bw of *A. muricata* concentrations in the quail feed compared to the value of the considered characteristics recorded in the other treated quails and the control groups. However, the increase was significant only with the weight of the shell.

4. Discussion

According to the obtained data, the different concentrations of *A. muricata* powder in feed do not result in statistically significant changes in various growth characteristics measured, compared to the control groups. This means that *A. muricata* powder does not significantly impact the feed consumption, live weight, weight gain, and feed conversion in the quails under these conditions and over 75 days. The findings of the present study are in line with those of Chongsi et al.[20], who investigated the impact of *Mangifera indica* (mango) leaf powder on growth characteristics in Brahma chicken. Nevertheless, the obtained results contradict to those of Tchoffo et al.[21], who observed significant differences in chickens exposed to a higher dose of 750 mg/kg bw of *Mangifera indica* (mango) leaf powder. The effect may be attributed to variations in the bioactivity and metabolic processing of the active compounds present in the mango leaves (phenols, steroids, and terpenes). While soursop leaves powder contains beneficial phenolic and flavonoid compounds, these substances may not be readily bioavailable or efficiently metabolized in the quail digestive system. The bioavailability of polyphenols varies due to the influence of factors, such as gut microflora composition, digestive enzyme activity, and the presence of other dietary components that can affect absorption.

The current study's hematological analysis revealed that incorporating *A. muricata* leaf powder into quail feed, regardless of concentrations, did not significantly affect the counts of leukocytes, erythrocytes, and platelets ($p < 0.05$). These findings align with those of Rahab et al.[22], who observed no significant change in feed intake, mortality rates, and hematological parameters. Consequently, *A. muricata* leaf powder has no adverse effects on the blood profile of quails, even at varying concentrations. The lack of significant change in leucocytes, erythrocyte, and platelet counts in quails fed with *A. muricata* leaf powder is likely due to the specific bioactive compounds in *A. muricata* leaves primarily exerting antioxidant and anti-inflammatory effects compared to the directly influencing hematopoiesis (blood cell production). The regulation of blood cell count is a complex process controlled by various growth factors, cytokines, and regulatory molecules. The phenolic compounds and flavonoids in *A. muricata* leaves do not strongly impact this regulatory pathway in a way that would significantly affect leucocyte, erythrocyte, and platelet count.

The present study revealed that *A. muricata* leaf powder significantly affects the biochemical parameters, including increasing total cholesterol, total protein, and globulin levels, and decreasing MDA levels indicating an antioxidant effect. However, the increase in cholesterol at higher concentrations may be a concern. The findings are consistent with that of Oluwayinka et al.[23] with concentrations of 50 mg/kg bw, 100 mg/kg bw, and 200 mg/kg bw of *A. muricata* leaf in broiler chickens. It can be justified by the presence of bioactive antioxidants in soursop leaves, which reduce oxidative damage, leading to lower malondialdehydes levels, while simultaneously enhancing immune function and protein synthesis, resulting in increasing serum globulin and total protein levels. The modulation of lipid metabolism by these compounds can also explain the rise of total cholesterol. The serum content in urea, creatinine, AST, and ALT did not significantly affect the quail fed on *A. muricata* whatever the concentration of the control birds. The transaminase levels suggest that the doses of powder administered were not toxic and regulated the liver activity of the quails. According to Dodji et al.[24], an increase in serum transaminase level indicates hepatic cytolysis. The malondialdehyde (MDA) as an indicator of lipid peroxidation and oxidative stress significantly decreased in all the treatment groups compared to the control ($p<0.05$).

The results indicated that *A. muricata* leaf powder has a beneficial effect on several sperm parameters, including total sperm count, sperm density, and sperm motility. The significant increase in both fast progressive and slow progressive sperm, coupled with a reduction in immotile sperm, indicates a general enhancement in sperm quality and fertility potential resulting from the application of the leaf powder concentration. It highlights the potential of *A. muricata* leaf powder as a supplement in improving male reproductive health. Following the result, Zancan et al.[25] revealed that Soursop has protective effects against cisplatin-induced testicular damage and oxidative stress in animals. The Soursop bioactive molecules, such as phenols with antioxidants improved testicular structure by increasing the thickness of the seminal tubules and the germ cell membrane and consequently increasing sperm quality and motility of the spermatozoa[26]. According to Lenzi et al.[27], The membrane of sperm cells is notably abundant in polyunsaturated fatty acids, making them susceptible to lipid peroxidation caused by reactive oxygen species, a factor linked to male fertility. Lenzi et al.[27] confirmed that the supplementation of the male quail diet with *A. muricata* rich in antioxidant properties reduce the impairment of sperm membrane. The observed effects may be attributed to the synergistic action of the antioxidant properties, including phenolic compounds and flavonoids, anti-inflammatory components, such as acetogenins, and the nutrient-rich profile, including amino acids, vitamins, and minerals found in *A. muricata* leaves. The antioxidant properties, collectively contribute to improved sperm viability, motility, count, and density, while simultaneously decreasing the quantity of immobile spermatozoa. These effects highlight the potential of *A. muricata* leaf powder as a natural supplement for improving male reproductive health.

The obtained results show that the 500 mg/kg bw concentration of *A. muricata powder* seems to have a relatively better effect on the viability of spermatozoa compared to other concentrations and the control groups. It increases the number of live spermatozoa while reducing the number of dead spermatozoa.

Notably, none of the concentrations of *A. muricata* powder demonstrated any significant enhancements in both normal and abnormal spermatozoa when compared to the control group. This finding aligns with Olatungi et al.[28], who also reported no significant differences in normal and abnormal spermatozoa. Nevertheless, *A. muricata* powder demonstrated advantages in both live and dead sperm characteristics, likely attributable to its antioxidant properties. The phenolic compounds present in *A. muricata* leaves may reduce oxidative stress, a major contributor to sperm cell damage and death. These antioxidants enhance sperm viability by neutralizing free radicals and safeguarding sperm cells from oxidative damage, leading to a higher count of live sperm and a reduction in dead sperm. According to Tchoffo et al.[5], the phenols compounds rich in antioxidant property could reduce the impairment in sperm membrane and DNA, and subsequently improve the spermatozoa characteristics. Sperm morphology, encompassing both normal and abnormal forms, is predominantly determined by genetic factors as well as the spermatogenesis process. Since the primary function of antioxidants is to protect against oxidative damage rather than to influence genetic or developmental process, the ratios of normal and abnormal sperm remain unchanged. Therefore, although the antioxidants improve the overall viability of sperm, they do not have a substantial impact on the fundamental factors that influence sperm shape and structure.

The present study revealed that *A. muricata* leaf powder has several significant effects on fertility parameters, including increasing fertility rate, hatchability rate of fertile eggs, total hatchability rate, and chick weight and decreasing embryonic mortality indicating relatively better effect on the fertility parameters of female Japanese quails compared to the control group. The findings of the current study are in agreement with Ndubuisi et al.[29], who recorded a significant increase in fertility, total hatchability by using soursop powder for Turkeys. The various bioactive compounds in *A. muricata* leaves provide a comprehensive strategy for enhancing reproductive health, combining antioxidant, anti-inflammatory, nutritional, antimicrobial, and adaptogenic effects. This comprehensive support highlights the potential of *A. muricata* leaf powder as an effective supplement for enhancing fertility parameters in quails.

The findings indicate that incorporating *A. muricata* into the diet of laying quails led to improve egg production metrics. Notably, the weight of eggshells was significantly higher in the group fed with various levels of *A. muricata* compared to the control group. These findings align with those of Nguyen et al.[30], who found significant improvement in egg weight, egg mass, and number of eggs due to supplementing Moringa leaf powder on ISA Brown lying hens' diet. Qana et al.[31] also reported significant increases in egg weight and mass in quails fed with *A. muricata.* The improvement in egg production can be attributed to the nutrient-rich properties of soursop leaf powder, which contains essential substances, such as amino acids, fatty acids, minerals (iron, calcium, magnesium, selenium, and zins), and vitamins (E and C).[32].

These nutrients likely played a significant role in the enhancement of the egg production parameters.

5. Conclusion

The current study's hematological analysis showed that incorporating *A. muricata* leaf powder into quail feed, at any concentration did not have a significant impact on the counts of leukocytes, erythrocytes, and platelets. However, the 500 mg/kg bw dose of *A. muricata* leaf powder had notable effects on biochemical parameters, including elevated levels of total cholesterol, total protein, and globulin. Reduced malondialdehyde levels indicate a potent antioxidant effect. Accordingly, *A. muricata* leaf powder at a specific dose, can influence certain biochemical markers with no hematological parameter changes. The *A. muricata* leaf powder at 500 mg/kg bw has a beneficial effect on several sperm parameters, including total sperm count, sperm density, and sperm motility. The notable rise in fast progressive and slow progressive sperm, along with a reduction in immotile sperm, indicates a general enhancement in sperm quality and fertility potential resulting from the application of the leaf powder concentration. The *A. muricata* leaf powder at 500 mg/kg bw has positive impacts on fertility parameters, demonstrating significant increases in fertility rate, hatchability rate of fertile eggs, total hatchability rate, and chick weight. Conversely, a reduction in embryonic mortality indicates a comparatively more favorable effect on fertility parameters. Additionally, *A. muricata* leaf powder appears to influence egg characteristics in quails, including egg weight, shell weight, yolk weight, and albumen weight. The findings suggest that *A. muricata* leaf powder may be a valuable adjunct to support reproductive health and fertility in quails. According to the obtained results, it is therefore recommended that further studies focus on the extraction of the *A. muricata* active molecules that are effective on quail reproductive performance.

Declarations

Competing interest

The authors declare that they have no competing interests.

Authors' contribution

Herve Tchoffo, Chongsi Margaret Mary Momo, Pride Forsoh Ayemle, Roussel Manfouo, and Ferdinand Ngoula conceived, designed the research, and reviewed the manuscript. Camile Kondo Nyembo, Mohamadou Adamou, Byamungu Kasomo Dedieu, and Arius Baulland Nguedia Dongmo collected the data, carried out data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

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