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Effects of the Ethanolic Extract of *Dacryodes edulis* (Safoutier) Leaves on Reproductive Traits, Oxidative Stress and Toxicity Indicators in *Gallus gallus domesticus* (Brahma) Roosters

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Abstract

Plant extracts possess antimicrobial and anti-inflammatory properties that help reduce the incidence of diseases, improve gut health and enhance immune response in favor of growth and reproduction while limiting the use of synthetic antibiotics in poultry production. A total of 60 10-week-old Brahma roosters with an average weight of 1.2 ± 0.15 kg were partitioned into 5 experimental units following a complete randomized design. From the control group without additive ($T0^-$), 4 other rations were formulated by adding 200 mg of vitamin C ($T0^+$) per kg of feed to $T0^-$ or the ethanolic extract of *Dacyodes edulis* leaves at 0.55% (T1), 0.75% (T2) or 0.95% (T3) per kg of feed. The data collected were subjected to one-way analysis of variance. The results revealed a significant increase in the serum level triglyceride and total protein levels in the 0.75% and 0.95% extract groups compared with those in the control groups. Similarly, a nonsignificant difference ($P > 0.05$) in the serum albumin, globulin, total cholesterol, alanine and aspartate amino transferase enzymes and creatinine levels was observed regardless of the treatment. Significant increases ($P < 0.05$) in white blood cell, red blood cell, platelet, haemoglobin and haematocrit concentrations were observed with 0.95% extract. The incorporation of the extract at a rate of 0.75% in the feed significantly ($P < 0.05$) decreased the activity of malondialdehyde, increased the level of catalase in the treated groups compared with those in the control groups and decreased the level of glutathione in the

treated groups. The testosterone concentration was significantly ($P < 0.05$) greater in animals receiving the 0.75% of ethanolic extract of *D. edulis* than in the other groups. The animals receiving 0.75% of ethanolic extract of *D. edulis* leaves in their feed presented a significantly ($P < 0.05$) greater level of viable spermatozoa compared to all other treated groups. In view of these results, the ethanolic extract of *Dacryodes edulis* can be used to ameliorate the reproductive performance of Brahma rooster birds at a rate of 0.75%.

Keywords

Ethanolic Extract, Brahma Birds, *Dacryodes edulis* Leaves, Reproductive Performance

1. Introduction

In rural areas, village poultry farming represents an essential activity for obtaining income through the sale of eggs and birds. In Cameroon, local chicken populations with low productivity represent 70% of the poultry population, whereas other poultry species represent only 6% [1]. Local consumption of chickens provides a valuable source of protein. These birds are particularly vulnerable to mycotoxins because of the massive use of cereals in their diet and the absence of a ruminal reservoir containing microorganisms capable of degrading toxins before their intestinal absorption [2].

However, current production methods, while allowing greater yield, have probably introduced risk factors that are intrinsic to them, and the performance of the poultry sector is hampered by several health obstacles [3].

To provide solutions to these problems, animal production researchers have embarked on a quest for alternatives to antibiotics that would be available, accessible to all breeders and without harm to the environment. Since then, plant-based products, owing to their great diversity and varied biological activities, have significantly developed [4]. Currently, the use of plant extracts is considered an alternative in poultry production. Previous work by Forat *et al.* [5] has shown the interest in the use of the leaf extracts in the context of disease prevention and as a feed additive that acts on the regulation of the digestive flora. One of the plants that can be used to combat poultry diseases is safoutier.

The safoutier (*Dacryodes edulis*) is an oil-bearing fruit tree belonging to the Burseraceae family. It is cultivated in Africa in equatorial, humid tropical and tropical zones of altitude, from Nigeria to Uganda in the East, and up to Angola in the South. It is native to the Gulf of Guinea [6].

The roots, leaves and bark of safoutier include numerous therapeutic recipes in traditional pharmacopoeia for the treatment of wounds, anaemia and dysentery, digestive tract disorders, toothache and earache, and leprosy [7].

Phytochemical studies carried out on *Dacryodes edulis* revealed that this plant contains substances such as triterpenoids, tannins, xanthones, anthocyanins, fla-

vonoids, phenolic acid, alkaloids and steroids [8] [9]. These compounds are responsible for pharmacological properties such as antioxidant, antifungal, antiviral, antibacterial, anti-inflammatory, anticarcinogenic [10], antiprotozoal, hepatoprotective, immunomodulatory properties, growth and fertility [11]. The presence of phenolic compounds helps prevent degenerative pathologies such as diabetes, cancer, inflammation and cardiovascular diseases [12] [13]. Compared to other plants, *Dacryodes edulis* is rich in fatty acids such as oleic and linoleic acid that are known for their health benefits. The ethanolic extract of *D. edulis* contains phenols and flavonoids that have strong antioxidant properties in combating oxidative stress and lowering the risk of chronic diseases. This extract has shown to possess antimicrobial activity against clinical bacteria isolates such as *Escherichia coli* and Staphylococcus. *D. edulis* contains compounds such as phenols, flavonoids and vitamin C that exhibit antioxidant properties, helping to combat oxidative stress [14].

In view of these properties, this plant would have a zootechnical interest in strengthening the immune system and limiting infections in chickens to benefit of their growth and reproduction. The main objective of this work was to increase the reproduction of roosters and, more specifically, to determine the effects of the ethanolic extract of *Dacryodes edulis* leaves on biochemical and hematological characteristics, markers of oxidative stress and spermatozoa characteristics.

2. Materials and Methods

2.1. Animal Material

The animal material consisted of 60 Brahma Roosters from COSEPVI M (Cooperative Companies of Village Chicken Breeders of Mifi). These birds were divided into 5 groups of 12 animals each. These groups corresponded to the 5 treatments in a completely randomized design. Each group was subdivided into 3 subgroups of 4 birds corresponding to the group's repetitions. When the animals arrived at the farm, they were allowed to adapt for one week. At the start of the test, the animals were 10 weeks old, with an average weight of 1.2 ± 0.15 kg.

2.2. Plant Material

The plant material consisted of the leaves of *Dacryodes edulis* "Safou" (**Figure 1(a)**) collected at campus G of the University of Dschang Cameroon. These samples were dried and ground to obtain a powder (**Figure 1(b)**) which was used to prepare the ethanolic extract (**Figure 1(c)**).

Figure 1. (a) Fresh Safou leaves and fruits; (b) Dried blended safou leaves; (c) Ethanolic extract of safou leaves.

The ethanolic extract obtained were sent to the Laboratory of Chemistry of Natural Substances of the University of Dschang for phytochemical analysis according to the method described by Hildebert *et al.* [15]. The results of the phytochemical tests revealed the ethanolic extract of the presence of flavonoids, steroids, tannins etc, in the ethanolic extract of the leaves of *Dacryodes edulis* (Table 1). However, the results of the saponin and mucilage tests were negative in the leaf extract.

Table 1. Qualitative tests/phytochemical screening: ethanolic extract of powders of *D. edulis* leaves.

Constituent	Concentration
Flavonoid	+
Saponins	–
Steroids	+
Glycosides	+
Free Quinones	+
Anthracene derivatives	+
Mucilage	–
Tanins	+
Catechic tannins	+
Gallic tannins	–

–: absent, +: present.

2.3. Housing and Equipment

The animals were raised in a permanent breeding building. The boxes were built from plywood measuring 2.25 m × 1 m × 0.66 m (length × width × height), and a mesh which allowed ventilation of the boxes was placed above. The animals were raised on litter for a period of 3 months. Each box had a wooden feeder of 0.9 m × 0.10 m × 0.07 m (length × width × height) and a 10 L drinker.

2.4. Feeding

The birds were provided with locally available feed and fresh water ad libitum. The feed was made up of ingredients such as: maize, wheat bran, wheat middling, bone meal, cotton seed cake, soyabean meal, fish meal, powder shell, palm oil and 5% broiler concentrate which were purchased from a local market. The feed composition and the chemical characteristics are presented in Table 2.

Table 2. Bromatological composition and characterization of rations based on age [16].

Ingredients	Starter phase 1 - 12 weeks (kg)	Grower phase 13 - 21 weeks (kg)
Maize	55	55
Wheat bran	4	14

Continued

Palm kernel cake	4	5
Coton cake	9	11
Groundnut meal	7.5	6.5
Soya beans meal	15	3
Calcium (Coquillage)	1.5	1.5
Bone meal	1.5	1.5
Poulette concentrate 2.5%	2.5	-
Layer Concentrate 2.5%	-	2.5
Total	100	100
Organic matter	90.89	94.21
Ash	9.11	5.79
Crude protein	17.20	14.75
Fat	4.27	4.75
Crude fibre	7.63	6.38
Gross energy	4275.36	4400.82
Metabolisable energy	3135.03	3407.46

2.5. Preparation of the Building and Prophylaxis

Two weeks before the arrival of the birds, the breeding building and the various equipment were cleaned and disinfected with Cresyl (20 ml for 1 liter of water) and Virunet solution (50 g for 5 liters of water), which were spread in the room and on all the cages. The building was also disinfected by applying calcium carbonate to the walls and floor. After disinfection, a crawl space of two weeks was established before the introduction of the birds. As soon as they arrived at the farm, the birds received antistress Aliseryl (1 g per 2 liters of water) for three consecutive days during the adaptation period. The birds were vaccinated against Newcastle disease, Avian Cholera and Thyhose (Multivax) and Avian Smallpox (Diftosec) at two months of age before the start of the trial.

2.6. Trial Conduction and Experimental Design

For this test, sixty Brahma roosters aged approximately 60 days with an average weight of 1.2 ± 0.15 kg were divided into 5 groups of 12 birds each. These groups corresponded to the 5 treatments in a completely randomized design. Each group was subdivided into 3 subgroups of 4 birds corresponding to the repetitions of the group. The birds in each group of the following rations each morning for 90 days:

- Group 0: control ration without a feed supplement;
- Group 1: control ration + 200 mg Vitamin C;
- Group 2: control ration + 0.55% ethanolic extract of *Dacryodes edulis* leave powder;
- Group 3: control ration + 0.75% ethanolic extract of *Dacryodes edulis* leave powder;

- Group 4: control ration + 0.95% ethanolic extract of *Dacryodes edulis* leave powder.

From the start of the trial, feed consumption and weight gain were assessed weekly.

2.7. Ethanolic Extract Protocol and Choice of the Various Extract Doses Incorporation

2.7.1. Ethanolic Extract Protocol

Fresh matured leaves of *D. edulis* were harvested at campus G of the University of Dschang. The leaves were washed and air dried at an ambient temperature under a shade until a constant weight was obtained and they were milled into fine powder using a grinding machine. The powder was sieved to obtain fine powder which was used to make the ethanolic extract following the procedure described by Yakubu *et al.* [17]. For this, 250 g of the *Dacryodes edulis* powder was introduced in a recipient containing 1.5 litter of ethanol and the content allowed to rest for 72 hours. Furthermore, the maceration was filtered using a Whatman paper number 3. The filtrate obtained was dried in an oven at 45˚C until a solid extract was obtained which was conserved in an opaque air-tight container and stored in a cool dry place till used.

2.7.2. Choice of the Various Doses Incorporated

The choice of the various doses administered to the birds was based on the works of Tchoffo *et al.* [18] who used the doses of 0.25%, 0.50% and 0.75% of *D. edulis* powder leaves per killogram of feed on Brahma birds for the evaluation of growth performance and toxicity indices. In his study, the best performances were observed at doses of 0.75%. With this regard, the doses used for this study were 0.55%, 0.75% and 0.95% of ethanolic extract of *D. edulis* leaves which will help us to determine the most effective dose on various parameters studied and which has no toxic effect on the animals.

2.8. Studied Parameters and Data Collection

After 90 days of the trial, 06 roosters per treatment were chosen at random and fasted for 24 hours. They were then weighed individually using a scale with a capacity of 7000 g and an accuracy of 10^{-3}. The weight of each rooster was recorded. Each bird was sacrificed via decapitation using a knife and immediately blood was collected in 02 test tubes, one containing anticoagulant (EDTA) and the other not. These tubes were labeled with tape bearing the animals number. The blood collected in the tube containing the anticoagulant was placed in a rack and the other tube was placed in a cooler containing ice. Subsequently, the bird was scalded and plucked, then the birds were opened using a razor blade to isolate the various organs such as the liver, kidney, testis, gizzard, and heart. The isolated organs were freed from fatty tissue and weighed and volumes taken.

2.8.1. Relative Weight and Volume of Organs

At the end of the experiment (90^{th} day), six birds per treatment were randomly

selected and fasted for 24 h, weighed, and slaughtered by decapitation. After slaughtering of the animals, the abdominal cavity was opened and organs, such as the liver, kidney, epididymis, testicles and abdominal fats of the various birds, were carefully removed free of all adipose tissues and weighed separately using a balance of capacity of 160 g and precision 10^{-3} g. The relative weight of each organ was calculated using the following formula.

Relative organ weight (%) = Organ weight (g)/Live body weight of rooster (g) × 100

The volumes of the liver, kidney and testicles were determined via the water displacement method which consisted of pouring 0.9% NaCl in a graduated cylinder at an initial known volume and reading the volume (V_1). The organ was subsequently introduced, and the volume (V_2) was noted. The volume of the organ was then calculated via the following formula:

$$V_{organ} = V_2 - V_1$$

2.8.2. Biochemical Analysis

At the end of the trial, 6 roosters per treatment were randomly selected and fasted for 24 hours. They were then weighed individually and sacrificed via decapitation. Blood from each rooster was collected during sacrifice in a labelled test tube without anticoagulant. This blood was centrifuged at 3000 revolutions per minute for 15 minutes and the serum was isolated and then stored at −20˚C till use

- **Serum biochemical analysis**

- The level of serum triglycerides, total cholesterol, total protein, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALT) were analysed following the CHRONOLAB commercial kit (Ref: 101-0281).
- Testosterone, LH and FSH by the solid-phase immunoenzymatic method (ELISA) following the instructions for the Omega DIAGNOSTICS commercial kit (Ref: OD337);
- Creatinine by the kinetic colorimetric method following the instructions for the CHRONOLAB commercial kit (Ref: 101-0281).

- **Oxidative stress markers**

A portion of the serum was used for the determination of the serum levels of the oxidative stress markers: malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) via commercial methods. poultry 2023.2 192 special CHRONOLAB kits, Ref. 101-0255, CHRONOLAB, Ref. 101-0256, CHRONOLAB, Ref. 101-0281, CHRONOLAB, Ref. 101-0575, and CHRONOLAB, Ref. 101-0240, respectively, by spectrophotometric methods, as described in the commercial CHRONOLAB kits.

2.8.3. Hematological Analysis

For the evaluation of the hematological parameters, blood taken from the tubes containing the anticoagulant was sent to the Biochemical Laboratory of the Dschang District Hospital, where the hematological analyses were carried out. The blood count was performed via a hematology analyser and the target parameters were as follows: red blood cell indices, white blood, platelet indices, hemo-

globin, hematocrit, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell velocity and platelet count.

2.8.4. Analysis of Spermatozoa Characteristics

The epididymis was isolated, weighed and dilacerated in a petri dish containing 5 ml of 0.9% NaCl and this preparation made it possible to determine the characteristics of the spermatozoa (mobility, vitality, normality and concentration).

- **Sperm motility and concentration**

Sperm motility and other parameters were evaluated via a computer-assisted sperm analyser (CASA). After the animals were decapitation, the left tail of the epididymis was isolated, weighed, and then dilacerated in a solution of 0.9% NaCl preheated in a bath. Individual sperm motility was assessed via direct examination of the preparation. After homogenization of the mixture, the solution was deposited via a micropipette in order to fill the chamber of the Thoma cell by capillary action, without air bubbles. The slide was subsequently placed under a CASA microscope for the analysis of the following sperm parameters: percentage of mortile sperm, rapid-motility sperm (WHO A); slow motility sperm (WHO B); immobile sperm (WHO C); average velocity of progression (APV); curvilinear velocity (CLV); straight line velocity (VSL); and percentages of sperm moving in a straight line (PLIN).

- **Viability**

The viability test made it possible to evaluate the proportion of live spermatozoa in the initial preparation [19]. It is based on the coloring of the nucleus of living spermatozoa with a so-called vital dye. During this study, nigrosin-eosin staining was used. Thus, 40 µl of the previously diluted preparation was mixed with 80 µl of nigrosin-eosin dye and maintained at 37˚C. Subsequently, 20 µl of this mixture was used to prepare a seed smear on a slide preheated to 37˚C. Observation of this smear at 100 × magnification made it possible to determine the percentage of dead spermatozoa out of the 200 spermatozoa counted. The nuclei of dead spermatozoa were completely or partially colored pink or red, whereas those of the living sperm remained colorless. Indeed, eosin is a "vital" dye that crosses the membrane of dead cells to color the nucleus. The nigrosin used here serves as a background dye. To determine the number of viable sperm, the following formula was used:

$$\text{Percentage of viable spermatozoas } (\%) = \frac{\text{number of viable spermatozoas}}{\text{total number of spermatozoas}} \times 100$$

- **Morphology**

Among the 200 spermatozoa previously counted, the percentages of normal spermatozoa and those with tail and head abnormalities were determined. To determine the proportion of normal sperm, the following formula was used:

$$\%\ \text{Normal spermatozoas} = \frac{\text{number of normal spermatozoas}}{\text{total number of spermatozoas counted}} \times 100$$

2.9. Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) with a factor

of 1, and the Duncan test was used to separate the means where there was a significant difference. The Bravais Pearson correlation coefficient was used to determine the relationships among the different parameters. The data are expressed as the means ± standard deviations. SPSS 22.0 (Statistical Package for Social Sciences) software was used for the analysis, and the limit of significance was fixed at 5%.

3. Results and Discussion

3.1. Results

3.1.1. Effect of the Ethanolic Extract of *Dacryodes edulis* Leaves on the Relative Weight of Organs of the Reproductive Organs and the Volume of Testicles in Brahma Roosters

Table 3 presents the relative weights of the reproductive organs and volumes of the testicles in the Brahma rooster exposed to the ethanolic extract of *D. edulis* leaves in the feed rations. The incorporation of the ethanolic extract of the leaves of *D. edulis* regardless of its concentration in the feed did not significantly effect ($P > 0.05$) the weight and volume of the testicle or the weight of the right epididymis. Nevertheless, a significant decrease ($P < 0.05$) in the weight of the left epididymis was detected in birds subjected to the plant extract compared to those subjected to the negative control.

Table 3. Effects of the ethanolic extract of *Dacryodes edulis* leaves on the reproductive organs of Brahma roosters.

Relative weight of organs (g)	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P value
	T0⁻	T0⁺	0.55	0.75	0.95	
L Test	0.56 ± 0.08	0.52 ± 0.09	0.57 ± 0.19	0.69 ± 0.24	0.58 ± 0.11	0.46
R Test	0.53 ± 0.09	0.58 ± 0.16	0.58 ± 0.19	0.62 ± 0.18	0.49 ± 0.14	0.69
L Epid	0.08 ± 0.02^{a}	0.05 ± 0.01^{b}	0.04 ± 0.02^{b}	0.07 ± 0.01^{ab}	0.05 ± 0.01^{b}	0.01
R Epid	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.02	0.06 ± 0.04	0.04 ± 0.02	0.33
V L Test	16.93 ± 2.44	15.40 ± 3.13	19.00 ± 0.82	18.89 ± 7.05	14.60 ± 2.88	0.34
V R Test	15.38 ± 3.38	16.50 ± 5.01	16.33 ± 5.85	14.20 ± 4.55	15.40 ± 4.56	0.93

a, b: means carrying the same letters on the same line are not significantly different ($P > 0.05$); L Test: left testicles; R Test: right testicles: L; left; R: right; V: volume; L Epid: left epididymis; R Epid: Right Epididymis; V L Tes: volume of left testicles; V R Tes: volume of right testicles; T0⁺: postitive control with 200 mg of vitamin C; T0⁻: negative control without feed additive.

3.1.2. Effect of the Ethanolic Extract of *Dacyodes edulis* Leaves on the Relative Weight of Detoxification and the Volume of the Liver and Kidneys of Brahma Roosters

Table 4 summarizes the relative weights of detoxification organs, abdominal fat and the volumes of the liver and kidneys in Brahma roosters depending on the level of incorporation of the ethanolic extract of *D. edulis* leaves in their feed. The administration of the ethanolic extract of *D. edulis* leaves at all concentrations in

the feed induced organ weights and volumes comparable to those recorded in the control groups. A significant decrease ($P < 0.05$) in abdominal fat was observed in rooster birds subjected to the ethanolic extract of *Dacryodes edulis* leaves compared with those in the positive control group.

Table 4. Effects of the ethanolic extract of *Dacryodes edulis* leaves on the relative weights of detoxification organs, abdominal fat and the volumes of the liver and kidney of Brahma roosters.

R.W organs (g)	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P value
	T0⁻	T0⁺	0.55	0.75	0.95	
Liver	0.96 ± 0.07	0.97 ± 0.18	1.01 ± 0.4	0.98 ± 0.20	1.13 ± 0.24	0.37
L kidney	0.19 ± 0.06	0.20 ± 0.05	0.19 ± 0.02	0.26 ± 0.18	0.19 ± 0.03	0.58
R kidney	0.17 ± 0.02	0.20 ± 0.06	0.18 ± 0.03	0.31 ± 0.13	0.18 ± 0.03	0.39
Abd fat	1.66 ± 0.52^{b}	4.41 ± 0.54^{a}	1.41 ± 0,49^{b}	1.33 ± 0.26^{b}	1.56 ± 0.18^{b}	0.00
v. L kidney	5.21 ± 1.04	6.00 ± 0.63	5.50 ± 0.77	5.74 ± 0.95	4.83 ± 0.98	0.22
v. R kidney	5.00 ± 1.09^{b}	6.17 ± 0.75^{a}	4.71 ± 0.81^{ab}	5.16 ± 0.98^{a,b}	4.58 ± 0.79^{b}	0.04
v. liver	25.40 ± 1.81	25.33 ± 3.44	26.60 ± 2.40	26.40 ± 3.05	29.80 ± 3.19	0.12

a, b: means carrying the same letters on the same line are not significantly different ($P > 0.05$); L: left; R: right; v: volume; Abd fat: abdominal fat; v L Kidney: volume of left kidney; v R Kidney: volume of right kidney; T0⁺: positive control with 200 mg de vitamin C; T0⁻: negative control without feed additive.

3.1.3. Effects of the Ethanolic Extract of *Dacryodes edulis* Leaves on the Biochemical Parameters of Brahma Roosters

Table 5 shows the results of the biochemical parameters of Brahma roosters according to the rate of incorporation of the ethanolic extract of D. edulis leaves into the feed rations. The addition of the ethanolic extract of *D. edulis* leaves, regardless the concentration, did not affect the serum Albumin, globulin, total cholesterol, ALAT, ASAT or creatinine concentrations. A significant decrease ($P < 0.05$) in the serum urea concentration was observed in the roosters receiving 0.75% and 0.95% ethanolic extracts of *Dacryodes edulis* leaves compared with those in the control groups. Moreover, a significant increase in total protein was observed in the 0.95% incorporation group and the positive group compared to the negative control group. A significant increase in triglyceride concentration was observed with the incorporation of 0.95% ethanolic extract of *Dacryodes edulis* leaves compared to the control groups.

Table 5. Effects of the ethanolic extract of *Dacryodes edulis* leaves on biochemical parameters of Brahma roosters.

Control	Concentrations of ethanolic extracts of *D. edulis* leaves (%)					
Biochemical Parameters	T0⁻	T0⁺	0.55	0.75	0.95	P value
Total protein (g/dl)	2.59 ± 0.38^{b}	3.53 ± 0.24^{a}	3.09 ± 0.59^{ab}	2.95 ± 0.44^{ab}	3.57 ± 0.38^{a}	0.01

Continued

Albumin (g/dl)	1.79 ± 0.29	1.43 ± 0.36	1.76 ± 0.49	1.50 ± 0.36	1.48 ± 0.15	0.28
Globulin (g/dl)	1.79 ± 0.38	1.80 ± 0.43	1.56 ± 0.08	1.77 ± 0.17	2.09 ± 0.67	0.52
Total cholesterol (mg/dl)	87.78 ± 3.34	91.56 ± 2.98	94.22 ± 8.79	89.53 ± 1.87	93.79 ± 3.86	0.24
Triglycerides (mg/dl)	84.37 ± 9.61^{c}	75.41 ± 11.29^{c}	89.12 ± 10.67^{bc}	102.55 ± 15.23^{ab}	110.16 ± 5.62^{a}	0.00
ALAT (U/I)	20.25 ± 3.57	20.08 ± 4.03	17.46 ± 1.32	18.04 ± 2.07	18.85 ± 2.46	0,40
ASAT (U/I)	109.99 ± 7.68	103.71 ± 2.59	108.42 ± 7.16	114.05 ± 9.35	113.39 ± 8.27	0.22
Urea (mg/dl)	13.56 ± 0.25a	12.42 ± 0.81b	12.79 ± 0.39ab	9.22 ± 0.89d	11.48 ± 0.32c	0.00
Creatinine (mg/dl)	0.21 ± 0.04	0.23 ± 0.05	0.25 ± 0.06	0.17 ± 0.02	0.21 ± 0.08	0.36

a, b, c, d: means carrying the same letters on the same line are not significantly different ($P > 0.05$); ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase; $T0^{+}$: positive control with 200 mg of vitamin C; $T0^{-}$: negative control without feed additive.

3.1.4. Effects of the Ethanolic Extract of *Dacryodes edulis* Leaves on the Hematological Parameters of Brahma Roosters

Table 6 presents the effects of the incorporation of the ethanolic extract of *D. edulis* leaves into the feed on the hematological parameters of the Brahma roosters. There was a significant increase ($P < 0.05$) in the WBC, RBC, PLT, HGB and HTC with 0.95% ethanolic extract of *Dacryodes edulis* leaves in the feed compared with those of the positive control group. A significant ($P < 0.05$) increase in PCT and MCHC was observed in roosters receiving 0.75% of ethanolic extract of *Dacryodes edulis* leaves in their feed. The MCH was significantly ($P < 0.05$) greater only in birds receiving 0.55% ethanolic extract of *Dacryodes edulis* leaves in their feed. A significant decrease in MCV and LYM was observed in birds receiving the ethanolic extract of *Dacryode edulis* leaves in their feed compared with those in the control groups.

Table 6. Effects of the ethanolic extract of *Dacryodes edulis* leaves on the hematological parameters of Brahma roosters.

Control	Concentrations of ethanolic extracts of *D. edulis* leaves (%)					
Hematological Parameters	**$T0^{-}$**	**$T0^{+}$**	**0.55**	**0.75**	**0.95**	**P value**
WBC	142.02 ± 1.38^{e}	143.42 ± 0.81d	156.18 ± 0.35c	158.82 ± 0.25b	183.45 ± 0.30^{a}	0.00
RBC	2.80 ± 0.00^{a}	2.34 ± 0.21^{c}	2.48 ± 0.04^{b}	2.67 ± 0.01^{a}	2.77 ± 0.08^{a}	0.00
PLT	111.83 ± 0.41^{d}	109.20 ± 0.44^{e}	117.20 ± 0.45^{c}	137.00 ± 0.81^{b}	153.80 ± 1.78^{a}	0.00
HGB (g/dl)	19.15 ± 0.37^{a}	14.15 ± 0.51^{d}	15.47 ± 0.25^{c}	16.66 ± 0.45^{b}	19.43 ± 0.24^{a}	0.00

Continued

MCV	166.48 ± 0.04[a]	161.96 ± 0.05[b]	156.65 ± 0.92[c]	157.05 ± 0.05[c]	157.67 ± 4.53[c]	0.00
MCH	61.02 ± 0.47[d]	62.24 ± 0.60[c]	64.43 ± 0.16[a]	62.83 ± 0.08[b]	59.75 ± 0.39[e]	0.00
MCHC (g/dl)	36.41 ± 0.09c	37.67 ± 0.49[b]	36.16 ± 0.11c	40.93 ± 0.08[a]	38.12 ± 0.64[b]	0.00
LYM (%)	78.83 ± 0.47[c]	82.57 ± 0.40[a]	82.37 ± 0.16[ab]	82.06 ± 0.05[b]	79.17 ± 0.22[c]	0.00
HCT (%)	48.00 ± 0.57[b]	37.70 ± 0.64[d]	39.25 ± 0.34[c]	37.17 ± 0.54d	49.07 ± 0.05[a]	0.00
PCT	0.091 ± 0.004[d]	0.14 ± 0.004[b]	0.120 ± 0.008[c]	0.172 ± 0.004[a]	0.14 ± 0.005[b]	0.00

a, b, c, d, e: means carrying same letters on the same line are not significantly different (P > 0.05); WBC: white blood cells, RBC: red blood cells; PLT: platelets; HGB: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; LYM: lymphocytes; HCT: haematocrit; PCT: platelet count: p: probability: $T0^-$: feed without feed additive: $T0^+$: feed with 200mg of Vitamin C; T1: feed containing 0.55% of ethanolic extract of *D. edulis*; T2: feed containing 0.75% of ethanolic extract of *D. edulis*; T3: feed containing of 0.95% of ethanolic extract of *D. edulis*.

3.1.5. Effect of the Ethanolic Extract of *Dacyodes edulis* Leaves on Markers of Oxidative Stress in Brahma Roosters

Table 7 presents the effects of the incorporation of the ethanolic extract of D. edulis leaves into the feed rations of Brahma roosters on stress markers. The level of malondialdehyde (MDA) recorded in the animals receiving the feed containing the ethanolic extract of *Dacryodes edulis* leaves was significantly (P < 0.05) lower than that in the control groups. In contrast, the incorporation of the ethanolic extract of *D. edulis* leaves into the feed at a rate of 0.75%, induced significantly increase in catalase activity compared to that of the control groups.

Table 7. Effects of the ethanolic extract of *Dacryodes edulis* leaves on markers of oxidative stress in Brahma roosters.

Markers of Oxidative stress	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P value
	$T0^-$	$T0^+$	0.55	0.75	0.95	
MDA	3.60 ± 0.21[a]	2.41 ± 0.65[C]	3.05 ± 0.23[b]	1.92 ± 0.16[e]	2.16 ± 0.12[d]	0.00
Catalase	0.46 ± 0.33[b]	0.33 ± 0.06[b]	1.10 ± 0.83[ab]	1.57 ± 0.99[a]	0.81 ± 0.08[ab]	0.00
Peroxydase	19.03 ± 4.02c	42.31 ± 3.76a	26.28 ± 22.15b	31.06 ± 17.98b	19.58 ± 12.57c	0.00
Glutathion	41.62 ± 5.94[b]	59.45 ± 3.21[a]	40.97 ± 2.04[b]	40.63 ± 4.65[b]	17.12 ± 4.57[C]	0,00
SOD	0.56 ± 0.08	0.54 ± 0.35	0.61 ± 0.12	0.61 ± 0.12	0.56 ± 0.09	0.95

a, b, c, d, e: means caring the same letters on the same line are not significantly different (P > 0.05); MAD: malondialdehyde, SOD: superoxide dismutase, $T0^+$: positive control with 200 mg of vitamin C; $T0^-$: negative control without feed additive.

Peroxidase and glutathione activity were reduced in the roosters fed with diets containing the ethanolic extract of *D. edulis*, leaves regardless of the concentration compared to the positive control group. Superoxide dismutase (SOD) activity was not affected by the incorporation of the ethanolic extract of *D. edulis* leaves regardless of their concentration in the bird feed.

3.1.6. Effect of the Ethanolic Extract of *Dacryodes edulis* Leaves on the Spermatozoa Characteristics of Brahma Roosters

- **Effects of the ethanolic extract of *Dacryodes edulis* leaves on the spermatozoa quality of Brahma roosters**

Table 8 summarizes the qualities of spermatozoa in Brahma roosters depending on the level of incorporation of the ethanolic extract of *D. edulis* leaves into the feed rations. The rates of fast progressive motile spermatozoa (WHO A), slow progressive motile spermatozoa (WHO B) and immobile or nonmotile spermatozoa (WHO C) were affected in a significantly high manner ($P < 0.05$) in the control treatment, followed by birds whose incorporation rate of the ethanolic extract was 0.75% in the ration for WHO A and WHO B and 0.55% for WHO C. The rate was lower in animals receiving 95% of the extract in the feed ration for WHO A, positive controls for WHO B, and negative controls for WHO C. Linearity and rectitude were significantly greater in roosters whose incorporation of the ethanolic extract was 0.95% than in the control.

With respect to the velocity of spermatozoa, Table 8 shows that the ethanolic extract of the leaves of *D. edulis* had a significantly greater effect ($P < 0.05$) on the average path velocity (APL), curvilinear velocity (VCL) and straight line velocity (VSL) for the negative control treatment animals, followed by the roosters whose rate of incorporation of the ethanolic extract was 0.75% in the feed and was lowest for the animals that received 0.55% of ethanolic extract of Safou leaves for VCL and VSL, whereas the animals in the treatment which received 0.95% of ethanolic extract had the lowest APV. The straightness, which is the VSL/VCL ratio, was affected significantly greater ($P < 0.05$) in the 0.95% treatment than in the negative control treatment.

Table 8. Effects of the ethanolic extract of *Dacryodes edulis* leaves on the sperm quality of Brahma roosters.

Quality of spermatozoa	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P Value
	T0⁻	T0⁺	0.55	0.75	0.95	
% mo sperm	83.75 ± 3.77^{a,}	77.34 ± 6.19^{a,b}	72.99 ± 12.21^{b}	80.63 ± 6.66^{a,b}	74.81 ± 8.46^{a,b}	0.01
WHO A	10.60 ± 0.55^{a}	5.20 ± 0.45^{c}	7.00 ± 0.89^{b}	7.50 ± 1.05^{b}	3.00 ± 0.63^{d}	0.00
WHO B	31.91 ± 1.75^{a}	22.53 ± 2.16^{d}	23.80 ± 1.64^{c,d}	27.50 ± 3.67^{b}	26.80 ± 2.28^{bc}	0.00
WHO C	43.50 ± 1.38^{c}	51.17 ± 1.47^{a}	47.50 ± 1.22^{b}	45.50 ± 2.74^{b}	47.00 ± 0.89^{b}	0.00

Continued

APV	21.67 ± 1.37[a]	15.50 ± 0.55[c]	16.40 ± 0.89[c]	18.67 ± 0.52[b]	15.17 ± 1.47[c]	0.00
VCL	35.17 ± 0.52[a]	25.17 ± 0.98[d]	24.83 ± 0.75[d]	32.33 ± 1.21[b]	26.33 ± 1.21[c]	0.00
VSL	8.00 ± 0.00[a]	6.00 ± 0.00[c]	5.50 ± 0.55[d]	7.00 ± 0.00[b]	5.67 ± 0.52[c,d]	0.00
P LIN	35.00 ± 0.63[a]	25.33 ± 0.52[d]	23.33 ± 0.52[e]	30.17 ± 0.75[b]	29.00 ± 1.00[c]	0.00
Linearity	22.67 ± 0.52[a,]	23.00 ± 0.00[a,b]	22.50 ± 0.84[b]	21.67 ± 0.82[c]	23.67 ± 0.52[a]	0.00
Rectitude	35.83 ± 0.75[b]	36.60 ± 0.55[b]	37.17 ± 0.75[a,b]	36.17 ± 0.41[b]	38.00 ± 2.00[a]	0.02

a, b, c, d, e: means that the same letters on the same line are not significantly different (P > 0.05); % mo sperm: percentage of motile sperm; WHO A: spermatozoa with fast progressive motility; WHO B: spermatozoa with slow progressive motility; WHO C: local motile spermatozoa; APV: average path velocity; VSL: straight-line velocity ; VCL: curvilinear velocity; PLIN: percentage of line moving spermatozoa.

The incorporation of the ethanolic extract of *D. edulis* leaves into the diet of Brahma roosters had a significant effect on the percentage of spermatozoa in rectilinear movement (PLIN). It was greater in the negative control treatment, followed by 0.75% incorporation of the extract into the food. In addition, it was lower in birds where the ethanolic extract was incorporated at 0.55% in the feed.

- **Effects of the ethanolic extract of *Dacryodes edulis* leaves on the sperm viability and morphology of Brahma roosters**

Table 9 presents the effects of incorporating the ethanolic extract of *D. edulis* leaves into rations on the viability and morphology of spermatozoa. The incorporation of the ethanolic extract of *D. edulis* leaves regardless of the concentration, and the positive control apparently induced viable sperm levels comparable to those of the negative control. When we consider only the roosters subjected to the ethanolic extract of the leaves of *D. edulis*, those exposed to a concentration of 0.75% in the feed presented a significantly greater percentage of viable spermatozoa significantly than did the rooster fed a ratio containing 0.55% of the ethanolic extract of the leaves of *D. edulis*. Furthermore, the average numbers of dead and normal spermatozoa were comparable among the treatment groups. The number of dead spermatozoa was lowest with roosters receiving 0.75% ethanolic extract of *Dacryodes edulis* leaves in their feed.

Table 9. Effects of the ethanolic extract of *Dacryodes edulis* leaves on sperm viability of Brahma roosters.

Sperm Concentration	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P value
	T0⁻	T0⁺	0.55	0.75	0.95	
Alive	79.83 ± 3.07[ab]	79.58 ± 3.04[ab]	78.67 ± 3.03[b]	83.42 ± 3.10[a]	80.33 ± 2.13[ab]	0.01

Continued

Dead	20.17 ± 3.90	20.42 ± 3.04	20.92 ± 3.23	16.75 ± 3.43	19.67 ± 3.11	0.25
Normal	98.42 ± 0.38	98.33 ± 0.61	98.66 ± 0.52	98.83 ± 0.26	98.75 ± 0.27	0.23

a, b: means that the same letters on the same line are not significantly different ($P > 0.05$); $T0^+$: positive control with 200 mg of vitamin C; $T0^-$: negative control without feed additive.

3.1.7. Effects of the Ethanolic Extract of *Dacryodes edulis* Leaves on the Sex Hormones of Brahma Roosters

Table 10 summarizes the effects of the ethanolic extract of *D. edulis* leaves on the hormonal levels of Brahma roosters. The testosterone level increased significantly ($P < 0.05$) in the Brahma roosters that received the ethanolic extract of *D. edulis* leaves at 0.75% in the feed compared with those in the control roosters. The ethanolic extract of D. edulis leaves in the feed did not have a significant effect on FSH or LH levels ($P > 0.05$) regardless of the incorporation rate which was comparable to that of the controls. Nevertheless, an increase in LH concentration was observed with roosters receiving the ethanolic extract of *D. edulis* leaves in their feed compared with the control groups.

Table 10. Effects of the ethanolic extract of *Dacryodes edulis* leaves on the sex hormones of Brahma roosters.

Sex Hormones	Control		Concentration of ethanolic extract of *Dacryodes edulis* leaves (%)			P value
	$T0^-$	$T0^+$	0.55	0.75	0.95	
Testosterone	0.66 ± 0.11^c	0.50 ± 0.12^c	1.26 ± 0.33^b	1.64 ± 0.34^a	0.78 ± 0.07^c	0.00
FSH	0.90 ± 0.39	1.00 ± 0.47	0.82 ± 0.38	0.85 ± 0.29	0.98 ± 0.59	0.95
LH	1.90 ± 0.79	1.43 ± 0.67	2.88 ± 1.26	2.33 ± 1.14	2.67 ± 0.61	0.32

a, b, c: means carrying the same letters on the same line are not significantly different ($P > 0.05$); $T0^+$: positive control with 200 mg of vitamin C; $T0^-$: negative control without feed additive; LH: luteinizing hormone; FSH: follicle stimulating hormone.

3.2. Discussion

The incorporation of the ethanolic extract of *D. edulis* leaves at concentrations of 0.55, 0.75 and 0.95% into the feed of Brahma roosters for 12 weeks did not result in a significant difference ($P > 0.05$) in the weight of the liver, kidney, testes and right epididymis. This result is consistent with that of Martinez *et al.* [20], who reported a nonsignificant effect of the incorporation of *Anacardium occidental* powder, and it is also consistent with that reported by Herve *et al.* [18], who reported a nonsignificant effect ($P > 0.05$) on liver and heart weight with the incorporation of *Dacryodes edulis* powder in the feed of Brahma birds. Conversely, a significant reduction in the weight of left epididymis and abdominal fat was ob-

served in birds that received the extract. This reduction in the percentage of the fat in the abdomen could be attributed to the presence of tannins and flavonoids present in the plant, which are inhibitors of fat absorption in the intestine, and have been reported to have lipid-lowering effects [21]. The reduction in the left epididymis could be explained by the fact that, the ethanolic extract of *Dacryodes edulis* leaves contained properties such as tannins and flavonoids that could have lowered the excess fat deposition on the epididymis and hence lowering the overall weight of the epididymis. Indeed, an increase in epididymal weight due to increase adipocytes can impair fertility [22].

In the present work, the ethanolic extract of *D. edulis* leaves in rooster feed led to a significant decrease ($P < 0.05$) in the serum levels of malondialdehyde, peroxidase and glutathione. Compared to the control, it also induced a significant increase ($P < 0.05$) in the serum catalase level. Furthermore, it induced a nonsignificant decrease ($P > 0.05$) in superoxide dismutase levels. Indeed, the low level of MDA recorded could reflect the antioxidant activity mediated by the compounds present in the ethanolic extract of *D. edulis* leaves, which would have induced a reduction in lipid peroxidation via neutralization of free radicals through the participation of these molecules in the fight against free radicals. These results corroborate those of Arius [23], who reported that avocado pit powder caused a significant decrease in the serum level of malondialdehyde and a nonsignificant increase in the levels of reduced glutathione and superoxide dismutase in young rabbits.

Serum ALT and AST levels are used to assess liver function. The present study revealed that supplementation with the ethanolic extract of *D. edulis* leaves in a rooster diet had no effect on the serum AST or ALT levels. This could therefore reflect the fact that the ethanolic extract of D. edulis leaves was not hepatotoxic. These results are in agreement with those of Arius [23], who reported that the supplementation of avocado pit powder in the rabbit diet had no effect on the serum levels of AST or ALAT.

The serum urea level significantly decreased with the incorporation of the ethanolic extract of *D. edulis* leaves into the rooster diet. Our results contradict those of Josiane *et al.* [24], who reported that the serum urea concentration significantly increased with the addition of 1.5 g of methenamine/L of water. This contrasting result could be due to the difference in the plant material used for the study (specie-specificity). The reduced serum urea concentration could be attributed to the nephroprotective activities of the rich phenols and flavonoids content in the leaves of *Dacryodes edulis* leading to improve kidney functions [25]. The creatinine level was significantly greater in the roosters that consumed the feed containing 0.75% of the ethanolic extract of the leaves of *D. edulis* than the control group. These results contrast with those of Herve *et al.* [26] who reported that compared with those of birds in the control group, the creatinine level decreased significantly in quails treated with essential oil regardless of sex. This finding suggests that the kidney function of the roosters was not disrupted by the ethanolic extract of *D. edulis* leaves.

In this study, total protein in the serum was significantly affected in roosters receiving the ethanolic extract of *Dacreodes edulis* which was incorporated into their feed. These results contrast with those of Herve *et al.* [26], who reported that oral administration of the essential oil of ginger rhizomes to Japanese quails did not significant effect total protein. Furthermore, these findings contrast with those of Nahed *et al.* [27] who reported a significant decrease in total protein and globulin levels in the serum of broiler hens at doses of 200 and 300 mg/kg of ginger powder. The increase in total protein levels in the serum could be associated with the bioactive compounds such as flavonoids, tannins and saponins present in *D. edulis* leaves [28].

Sperm density, motility and morphology are indicators of sperm quality and therefore fertility. In the present work, the ethanolic extract of *D. edulis* leaves did not significantly affect sperm motility ($P > 0.05$) in roosters. There was a strong trend in the negative control group, followed by birds that received the 0.75% ethanolic extract in the feed. The results obtained in this study revealed that the linear velocity (VCL) is superior to the curvilinear velocity (VSL) and can be classified as follows: negative control treatment, 0.75%; positive control, 0.95% and 0.55%. The sperm from the two roosters that were analyzed had a rounded path rather than a straight line. These results contradict those reported by Masindi *et al.* [29], who reported that sperm were more mobile. The difference in the results that we obtained compared with those recorded by the latter could be explained by the 0.9% NaCl diluent used in the maceration of the epididymis, which is not specific for the dilution of rooster semen, whereas other authors have used a specific dilution, particularly BPSE (buffered phosphate saline solution with egg yolk).

In this work, the roosters subjected to the ethanolic extract of the leaves of *D. edulis* exposed to a concentration of 0.75% in the feed presented a significantly greater percentage of viable spermatozoa ($P < 0.05$), whereas this incorporation had no significant effect on the percentage of normal spermatozoa. Our results are in agreement with those of Herve *et al.* [26], who reported that the percentage of live spermatozoa increased significantly ($P < 0.05$) in quails treated with essential oil regardless of the dose, compared with that of quails from the control group, but was not significantly different from the level of normal spermatozoa. These results are in line with those of Severin *et al.* [30], who showed that the treatment of rats with the aqueous extract of the roots of *H. acida* promoted a significant increase in the number of spermatozoa in the caudal epididymis. This increase can be attributed to the antioxidant properties of the ethanolic extract of *D. edulis* leaves and to the stimulatory effects of some of its constituents on male reproductive functions.

Testosterone is the predominant gonadal hormone in males. Its synthesis takes place from cholesterol, in Leydig cells located in the interstitial spaces of the seminiferous tubules [31]. It appears from this work that roosters subjected to the ethanolic extract of the leaves of *D. edulis* at a concentration of 0.75% in the feed had a significant increase ($P < 0.05$) in the serum level of testosterone compared with the control groups and a decrease testosterone concentration with 0.95% in-

corporation of the ethanolic extract of *Dacryodes edulis* leaves. Our results are in agreement with those of Brice *et al.* [32], who reported that the extracts of *Polygonum limbatum meism* from guinea pigs poisoned with cadmium increased the testosterone level in these guinea pigs compared with that of animals exposed exclusively to cadmium chloride. This observed increase in testosterone levels could be explained by the presence of antioxidant elements such as phenol in the leaves of *Dacryodes edulis*, which are capable of neutralizing the overproduction of free radicals. A decrease in testosterone concentration with 0.95% incorporation could be justified by the fact that, an increase in white blood cell concentration was observed in this group, which shows an activation of the immune system in response to diseases. Disease is said to be one of the major factors that affects testosterone concentration in animals and its presence suppress testosterone production by the Leydig cells. This decline in testosterone during disease is adaptive, because energy is allocated from reproduction into immune function and reproductive behaviors are reduced to improve survival [33]. The ethanolic extract of *D. edulis* leaves in feed led to an increase in the serum levels of luteinizing hormone (LH) in roosters. These results were comparable to those of Herve *et al.* [26], who reported elevated LH and FSH values in the quail diet with the use of ginger essential oil.

4. Conclusion

The results of this study revealed that the ethanolic extract of *Dacryodes edulis* leaves improved the reproductive characteristics of Brahma rooster birds by increasing the levels of reproductive hormones and live spermatozoa. It induced an increase in serum hematological and biochemical (total protein and triglyceride) parameters and equally led to an increase in antioxidant enzyme (catalase and SOD) activities and a decrease in MDA, peroxidase and glutathione levels. It also led to a decrease in the serum urea concentration. On the basis of these findings, the ethanolic extract of *Dacryodes edulis* leaves can be used in male animals at a rate of 0.75% in feed to improve reproductive performance.

Ethical Approval and Consent to Participate

Experimental protocols used in this study were approved by the Ethical committee of the Department of Animal Science of the University of Dschang and was in conformity with the internationally accepted standard ethical guidelines for laboratory animals use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

Consent for Publication

All the authors reviewed, revised and approved the final draft for publication.

Availability of Data and Materials

Not applicable.

Authors' Contributions

FG, HT and MMMC conceived, designed and supervised the research, NN, DBK conducted the experiment, collected, analyzed the data and wrote the manuscript. ABND, MA, NV carried out the data analysis. All the authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

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