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Research Article
Effects of *Dacryodes edulis* and *Mangifera indica* Leaves Powders on Immune System and Growth Performances in Brahma Hen

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Abstract

Background and Objective: Medicinal plants are rich in bioactive molecules with diverse pharmacological activities that can be used in the animal sector to improve productive performances. Due to the cancelling of antibiotics used in the poultry field and the regression of bird production performances, the study assessed safou and mango leaves powder's effect on Brahma hens' immune systems and growth performances. **Materials and Methods:** A total of 84 Brahma hens aged 45 days old with an average weight of 400±12 g were divided into seven groups of 12 hens each. The hens of the control group received a diet without additives and those of groups 1, 2 and 3 received, feed with 0.25, 0.5 and 0.75% of *Dacryodes edulis* leaves powder, respectively while the hens of groups 4, 5 and 6 in addition to the control ration received, 0.25, 0.5 and 0.75% powder from *Mangifera indica* leaves, respectively. After 60 days of treatment, all studied characteristics were evaluated. **Results:** The live body weight except that of birds fed with 0.5% of *D. edulis* and the carcass yield 2 increased in the hens exposed to *D. edulis* and *M. indica* powder leaves with reference to the control. However, this increase was significant only in hens exposed to 0.75% of *M. indica* leaf powder. The relative weight of the Fabricius bursa recorded in 0.25% of *M. indica* leaves treated hens was significantly higher compared to that of the control hens. Conversely, the relative weight of the spleen, the level of total protein and serum globulin recorded in powder leaves treated hens decreased. **Conclusion:** The powder of safou and mango leaves due to their bioactive compounds with antimicrobial and antioxidant properties can be used as an alternative for improving the immune system in birds.

Key words: Brahma hen, *Dacryodes edulis*, growth performance, immune system, *Mangifera indica*, phytobiotics

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In rural areas, local chicken production represents a primordial activity to obtain revenue through the sale of eggs and birds. In Cameroon, the local chicken population, of low-productivity represents 70% of the poultry population, while other poultry species represent only 6%[1]. The consumption of local chickens is a valuable source of protein. These birds are particularly vulnerable to mycotoxins due to the massive use of cereals in their diet and the absence of the ruminal reservoir containing micro-organisms capable of degrading toxins before their intestinal absorption[2]. In addition, they are exposed to difficult environmental conditions such as high densities in livestock, poor water and feed quality and temperature fluctuations. All these factors induce oxidative stress responsible for depression of the immune system and increased susceptibility to various diseases and consequently a general decline in zootechnical performance[3].

The adverse effects of environmental stress in poultry are numerous (hepatotoxic, nephrotoxic, dermotoxic or reprotoxic effects)[4]. They lead to a decrease in the synthesis of coagulation factors and hemostasis disorders due to impaired liver function. Similarly, stress alters the number of different lineages of white blood cells and immune cells present in the tissues. Moreover, one of the notable effects of stress would be the reduction in size and cell depletion of lymphoid organs (thymus, spleen and Peyer's patch). According to Herve *et al.*[5] a stress provokes and a reduction of an organ cell thickness and the effect is linked to the organ weight. In addition to these obvious effects on immunity, there are more discreet adverse effects such as digestive toxicity and changes in animal performance.

To find solutions to this situation, breeders have resorted to the use of high quantities of chemicals, amounting to more than 80% of antibiotics and hormones to fight against various infections and as a growth activator to boost production. Unfortunately, the use of these antibiotics and hormones in animal husbandry has been banned in many countries due to the resistance of highly pathogenic bacteria induced by antibiotics[6] and the storage of hormones in meat which can affect the health of consumers. Therefore, the use of plant-based products because of their diversity, accessibility, availability and also their varied biological activities is now obvious.

The mango (*Mangifera indica*) and the Safou (*Dacryodes edulis*) oldest cultivated fruit trees found in many countries including Cameroon. The phytochemical studies of *Mangifera indica* and *Dacryodes edulis* carried out by several researchers like Márquez *et al.*[7], Lukubye *et al.*[8], Guiekep *et al.*[9] and Tangomo *et al.*[10] revealed that these plants contain many natural substances from the group of triterpenoids, tannins, xantones, anthoxyan, flavonoids, phenol acid, alkaloid and steroids. These secondary metabolites are responsible for pharmacological properties such as antioxidant, antifungal, antiviral, antibacterial, anti-inflammatory, anti-carcinogenic[10,11], antiprotozoal, hepato-protective, immunomodulatory properties, growth and fertility stimulators[12,13]. In view of these properties, these plants would have a zootechnical interest in strengthening the immune system, limiting infections in the hen for the benefit of its growth and reproduction.

The general objective of this work was to increase immunity and growth performance in poultry, in order to improve their productivity.

MATERIALS AND METHODS

Study area and period: This study started from October to December, 2022 at the Application and Research Farm (FAR-UDs) of the University of Dschang, Cameroon. The farm is located at Latitude 5°44-5°36 and 5°44-5°37 North and Longitude 10°06-9°94 and 10°06-9°85 East.

Animal material: A total of 84 healthy Brahma hens aged 45 days and weighing 400±12 g were purchased in west region of Cameroon from Cooperative Society of Mifi Village Chicken Breeders for this study. Each hen was labeled at the level of the paw with a ring bearing his number.

Plant material: The plant material used consisted of Safou and mango leaf powders (1.5 kg of each powder). These leaves were harvested between 8 am and 10 am each from a mature tree located on campus G of the University of Dschang. They were washed, dried in the shade separately for a period of 10 days and then crushed using a grinding machine to obtain homogeneous powders which were incorporated into the hens' feed at different concentrations). Figure 1(a-b), respectively show the dried and powder leaves of *M. indica* while Fig. 1(c-d), respectively represent the dried and powder leaves of *D. edulis*.

An amount of 50 g of each powder obtained was sent to the Laboratory of Chemistry of Natural Substances from the University of Dschang for phytochemical qualitative analysis according to the method described by Wagner *et al.*[14] and Hussain *et al.*[15].

Housing and equipment: The animals were reared in galvanized wire mesh cages. The density was 0.12 m^2/chick.

Table 1: Composition and bromatological characterization of the ratio according to age[16]

Composition (%)	Starter (1-12 weeks)	Grower (13-20 weeks)
Maize	48.00	45.00
Wheat bran	2.50	0.00
Wheat middlings	14.00	22.00
Cotton cake	8.00	6.00
Soybean meal	15.00	13.00
Fish meal	6.00	3.00
Bone meal	0.00	1.50
Powder shell	1.50	1.00
Concentrate 5% (*)	5.00	5.00
Palm oil	0.00	3.50
Total	100.00	100.00
Bromatological characterization		
Crude protein (%)	23.2	20.7 0
Metabolizable energy (kcal kg^{-1})	2913	3013.00
Calcium (%)	1.48	1.51
Phosphorus (%)	0.69	0.73
Lysin (%)	1.29	1.10
Methionine (%)	0.43	0.40
ME/CP	125	145.00
P/Ca	1.87	1.51

*Broiler concentrate 5%: Crude protein = 40%, Metabolizable energy = 2078 kcal kg^{-1}, Calcium = 8%, available Phosphorus = 2.05%, Lysine = 3.30% and Methionine = 2.40%

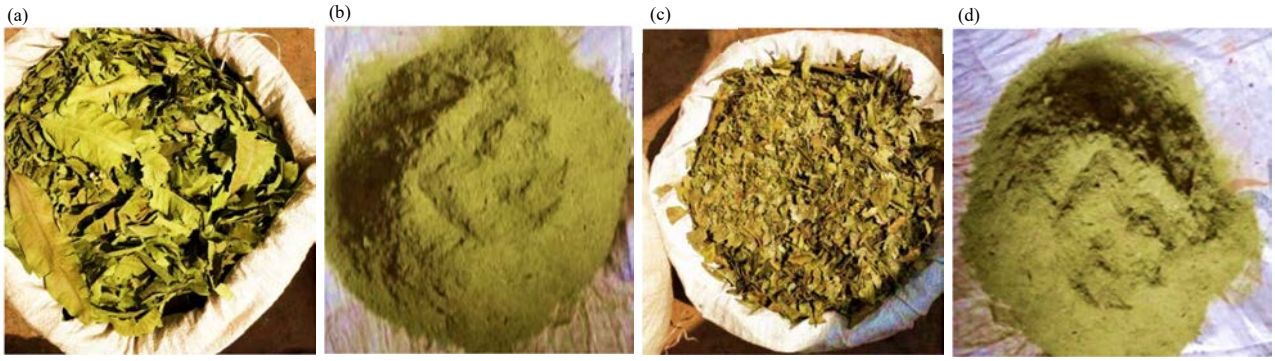

Fig. 1: Dried and powder leaves of (a-b) *Mangifera indica* and (c-d) *Dacryodes edulis* leaves
(a) Dried leaves and (b) powder of *Mangifera indica*, dried leaves (c) and powder (d) of *Dacryodes edulis* leaves

Each cage housed three chicks and was equipped with a one-liter capacity drinker and a 1.5-liter feeder. Throughout the treatment period, the lighting was 12 hrs/12 throughout the treatment period.

Feeding: During the trial period, the birds received feed rations whose ingredients and chemical composition were summarized in Table 1. To the control ratio were added leaves powders of *D. edulis* and *M. indica* at the rates of 0.25, 0.50 and 0.75%. The animals had free access to this food and drinking water.

Preparation of cages and prophylaxis: Two weeks before the arrival of the chicks, a sanitary space was carried out in the breeding building. To do this, the building and the cages were swept and cleaned from the ceiling to the floor, then washed with water containing detergent, feeders and drinkers were also washed. Then, the rooms and equipment were sprayed with cresyl diluted in 2% tap water, 2cl for 21 liters of water. Two days before the chicks arrived, feeders and drinkers were placed in the cages. When the animals arrived, an anti-stress was administered to the chicks for 3 days (1g/2 litres/day). Then, the birds were placed in the cages for a 14 day adaptation period before the start of the test.

Evaluation of biochemical parameters linked to immunity: The evaluation of biochemical parameters was carried out at the Laboratory of Animal Physiology and Health of the University of Dschang. To this effect, the blood of each hen collected during the sacrifice in labelled tubes without

anticoagulant was centrifuged at 3000 trs/min for 15 min to isolate serum. It was subsequently stored in labelled Eppendorf tubes and conserved in a freezer at -20°C until the dosage of total protein and albumin based on instructions on Chronolab kits (Barcelone, Espagne). Globulin levels were calculated based on the method preconized by Abdel Fattah *et al.*[17].

Trial conduction: For this test, eighty-four Brahma hens aged about 45 days with an average weight of 400±12 g were divided into seven groups of 12 birds each. These groups corresponded to the seven treatments in a completely randomized design. The 12 birds of each group were kept in 3 in 4 birdcages corresponding to the repetitions of the group. The birds of each group received each morning and for 60 days, one of the experimental rations. The control group received a diet without additives and test groups 1, 2 and 3 received in addition to the control ratio, 0.25, 0.5 and 0.75% of *D. edulis* leaves powder, respectively, while the hens of groups 4, 5 and 6 in addition to the control ration received, 0.25, 0.5 and 0.75% powder from *M. indica* leaves, respectively.

At the beginning of the trial, an animal received 70 g of food, this quantity of feed was adjusted with the evolution of the weight of the birds.

From the beginning of the experiment, feed intake and weight gain were assessed weekly.

Sacrifice and data collection: After 60 days of the experiment, hens of each treatment were fasted for 24 hrs. They were then weighed individually using a scale with a capacity of 7000 g and 10^{-3} precision. Each bird was sacrificed by decapitation using a knife and immediately blood was collected in 02 test tubes, one containing anticoagulant (EDTA) and the other not. These tubes were labelled with tape bearing the animal number. The bird was scalded and plucked, then the autopsy was performed using a razor blade to isolate the bursa of Fabricius, spleen, gizzard, liver and heart. The isolated organs were freed from fatty tissue and weighed.

Measurements and data collection: The data collected and the various measurements made were to evaluate the live body weight, the weight of the carcasses, the development and histology of the bursa of Fabricius and the blood parameters of the treated hens.

Evaluation of live body weight and carcass weight: Before scarifying the birds, they were weighed with 7000 g capacity and precision 10^{-3} scale to determine the live body weight. The ready-to-cook carcass plus the legs, gizzard, liver, head and heart of each hen were weighed using the same scale to obtain the carcass yield 2. For the carcass yield 1, the ready-to-cook carcass was weighed.

The relative carcass weights were calculated by the formula described by Herve *et al.*[18]:

$$\text{Carcass yield 1} = \frac{\text{Ready-to-cook carcass weight (g)}}{\text{Live body weight (g)}}$$

$$\text{Carcass yield 2} = \frac{\text{Ready-to-cook carcass weight (g)} + \text{(legs, gizzard, liver, head and heart) weight}}{\text{Live body weight (g)}}$$

Development of the spleen and bursa of Fabricius: The spleen and bursa of Fabricius of each hen collected after sacrificing were weighed using a scale of 160 g capacity and 10^{-3} g precision. The relative weight (mg/100 g b.wt.) of each organ was calculated using the following formula[18]:

$$\text{Relative weigth (\%)} = \frac{\text{Organ weight (mg)}}{\text{Body weight (g)}} \times 100$$

The volumes of the spleen and the bursa of Fabricius were determined by the water displacement method which consists of pouring a quantity of water into a graduated cylinder and reading its volume (V_1). Subsequently, introduce the organ and note the volume V_2. The volume of the organ was calculated according to Tchoffo *et al.*[16].

Histology of the bursa of Fabricius: Realization and observation of histological sections of the bursa of Fabricius were performed using the method described by Tchoffo *et al.*[16].

Evaluation of hematological and biochemical parameters: The blood taken in the anticoagulant tube during the sacrifice of the birds was used for the hematological analyses. While serum collected from blood without anticoagulant was used for biochemical examinations.

Evaluation of hematological parameters: For the evaluation of the hematological parameters, the 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 done using a hematology analyzer (Model KT 6180 S/N 701106101557) and the target parameters were red blood cell, leukocyte and platelet indices.

Statistical analysis: The data obtained was subjected to the Analysis of Variance (ANOVA) at 2 factors and the Duncan's Test was used to separate the means where there was a significant difference. The p-value calculation was performed using the student t-Test. The p-value of less than 0.05 was considered significant. The normality of data was tested by the Shapiro-Wilk Test. The relationships between different parameters were highlighted by the correlation coefficient of Bravais-Pearson[16]. The SPSS 22.0 (Statistical Package for Social Sciences) software was used for the analysis.

RESULTS

The phytochemical screening of *D. edulis* and of *M. indica* leaves powders are summarized in Table 2. The results revealed in the powders of *Dacryodes edulis* and *Mangifera indica* leaves powder the presence of flavonoids, phenols, alkaloids, saponins and steroids (Table 2). However, terpenoid tests were negative for mango leaves powder.

Effects of *D. edulis* or *M. indica* leaves powder in feed on live body weight, relative carcass weight, lymphoid organ weights and their volume: The effects of *D. edulis* and *M. indica* leaves powders on live body weight, relative carcass weight, relative lymphoid organ weights and volume were summarized in Table 3. It shows that the type of powder and their inclusion level in hen feed did not significantly ($p<0.05$) affect the relative weight of the Carcass yields 1. The live body weight of the hens fed with the ration containing the leaf powder of *D. edulis* or *M. indica* at different concentrations except for 0.5% of *D. edulis* leaf powder significantly ($p<0.05$) increased compared to that recorded in the hens receiving the control ration. The powder leaves of *D. edulis* or *M. indica* whatever its rate in the feed induced an increase in the carcass yields 2 but this effect was significant ($p<0.05$) only with 0.75% of *M. indica* leaf powder. The live body weight was positively and not significantly correlated with the African carcass weight ($\rho = +0.62$ and $p>0.05$).

The incorporation of *D. edulis* leaf powder into hen feed had no significant effect on the relative weight of the bursa of Fabricius. On the other hand, the powder leaves of *M. indica* in the feed of the hens induced an increase in the relative weights of the bursa of Fabricius in reference to that of the hens receiving the control ration. However, this increase was significant ($p>0.05$) only with 0.25%. In addition, the relative weight of the spleen of the hens fed with the food containing the powders leaves of the two plants was comparable with that of the control ration, with the exception of the hens fed with the feed containing 0.25% *M. indica* leaf powder which was significantly lower compared to the value noted in the control group. Whatever the type and the rate of powder in the feed of the hens, the volumes of the spleen decreased but that effect was significant only with 0.50 and 0.75%. The volumes of the Fabricius bursa recorded in the hens fed with the food containing the powder leaves of *D. edulis* or *M. indica* whatever the rate in the feed were comparable with that of the hens of the control group.

Effect of feed supplemented with *D. edulis* and *M. indica* leaves powder on some hematologic parameters in Brahma birds: The effect of feed containing *D. edulis* or *M. indica* leaf powder on the hematologic parameters in the Brahma hen was summarized in Table 4. It appears that white blood cells and lymphocyte numbers in the Brahma hens fed with rations containing *D. edulis* or *M. indica* leaves powder were comparable to those of the hens fed the control ration, with the exception of the values of these characteristics recorded with the *M. indica* powder at 0.75% in the food which have significantly ($p<0.05$) decreased. Furthermore, the blood granulocyte content decreased significantly with the incorporation of the powders in the food whatever the type and the concentration when compared to the value recorded with the control. The powders whatever the type and their concentrations in the food did not significantly ($p<0.05$) influence the blood content in red blood cells.

Powder leaves of *D. edulis* and *M. indica* except at 0.75% in the birds' feed induced blood hemoglobin levels which were comparable to that of a control group. With the exception of *M. indica* at 0.25% in hens feed which significantly ($p<0.05$) increased the hematocrit, the leaves powders of *D. edulis* and *M. indica* whatever the rate in the ratio of the hens induced the percentages of hematocrit comparable with that recorded with the control ration. The different concentrations of *D. edulis* or *M. indica* leaves powder with the exception of 0.75% of *D. edulis* and 0.50% of *M. indica* resulted in significantly ($p<0.05$) lower blood platelet counts compared to the control diet.

Influence of feed containing powder leaves of *D. edulis* or of *M. indica* on serum proteins: Table 5 shows the influence of feeds containing *D. edulis* or *M. indica* leaf powders on serum proteins in Brahma hens. It results that the leaves powder of *D. edulis* and *M. indica* in Brahma feed except 0.75% of the *D. edulis* and 0.25% of *M. indica* leaves powders which, respectively significantly ($p<0.05$) decreased total

Table 2: Phytochemical screening: powders of *D. edulis* and of *M. indica* leaves

Tests	Secondary metabolites	*Dacryodes edulis*	*Mangifera indica*
Shinoda	Flavonoids	+	+
	Tannin	+	+
Ferric chloride	Phenols	+	+
Dragendorff	Alkaloids	+	+
Foam index	Saponins	+	+
Liebermann-burchard	Steroids	+	+
	Terpenoids	+	-

-: Absent and +: Present

Table 3: Effects of feed containing *D. edulis* or of *M. indica* leaves powder on live body weight, relative weight of carcass, relative weight of organs lymphoid and their volumes

		Treatments						
		Levels of *D. edulis* powder leaves (%)			Levels of *M. indica* powder leaves (%)			
Characteristics	Control group	0.25	0.5	0.75	0.25	0.50	0.75	p-value
Live body weight (kg)	1.33±0.15^{c}	1.47±0.11^{ab}	1.40±0.11^{bc}	1.510±0.00^{ab}	1.47±0.06^{b}	1.58±0.11^{a}	1.51±0.05^{b}	0.00
Carcass yields 1 (g)	64.27±2.92	65.01±2.55	66.08±1.50	65.70±2.47	65.02±2.90	66.04±3.64	67.21±5.77	0.75
Carcass yields 2 (g)	76.08±2.73^{b}	77.83±3.17^{ab}	78.12±2,47^{ab}	78.19±2.50^{ab}	77.99±3.20^{ab}	77.87±5.23^{ab}	80.40±2.50^{a}	0.02
Bursa of Fabricius (g)	0.05±0.02^{b}	0.05±0.01^{b}	0.06±0.01^{b}	0.05±0.03^{b}	0.09±0.02^{a}	0.07±0.03^{ab}	0.07±0.02^{ab}	0.04
Spleen (g)	0.15±0.02^{a}	0.14±0.02^{a}	0.14±0.01^{a}	0.13±0.02^{ab}	0.11±0.01^{b}	0.14±0.03^{a}	0.14±0.02^{a}	0.04
Volume of organs (mL)								
Bursa of Fabricius	1.13±0.28^{ab}	1.45±0.046^{a}	1.01±0.04^{b}	1.00±0.00^{b}	1.02±0.13^{b}	1.27±0.41^{ab}	1.11±0.23^{ab}	0.04
Spleen	2.29±0.40^{a}	2.21±0.55^{ab}	2.23±0.74^{ab}	1.91±0.66^{ab}	2.26±0.46^{ab}	1.75±0.51^{b}	1.54±0.86^{b}	0.03

^{abc}On the same line and values having the same letter do not differ significantly (p>0.05)

Table 4: Effects of *D. edulis* or *M. indica* leaves powder in feed on some hematologic parameters in Brahma birds

		Treatments						
		Levels of *D. edulis* powder leaves (%)			Levels of *M. indica* powder leaves (%)			
Characteristics	Control group	0.25	0.5	0.75	0.25	0.50	0.75	p-value
WBC (×10^{3} µL^{-1})	154.85±9.48^{a}	161.30±2.83^{a}	159.72±5.51^{a}	150.20±11.30^{a}	154.30±0.35^{a}	161.54±3.01^{a}	133.87±5.78^{b}	0.00
LYM (×10^{3} µL^{-1})	20.67±2.11^{a}	19.50±0.42^{ab}	21.10±1.94^{a}	19.40±0.28^{ab}	21.70±0.70^{a}	19.64±1.28^{ab}	15.80±5.23^{b}	0.01
GRAN (×10^{3} µL^{-1})	142.00±5.23^{a}	126.63±1.89^{b}	122.66±3.86^{bc}	126.61±3.35^{b}	117.87±1.10^{c}	127.18±6.19^{b}	117.67±3.05^{c}	0.00
RBC (×10^{6} µL^{-1})	2.43±0.16	2.31±0.17	2.38±0.07	2.06±0.40	2.44±0.25	2.31±0.29	2.47±0.41	0.48
HGB (g dL^{-1})	12.15±0.43^{a}	11.25±0.64^{abc}	11.75±0.48^{ab}	10.37±0.83^{c}	11.40±0.84^{abc}	11.33±0.95^{abc}	10.82±0.57^{bc}	0.03
HTC (%)	25.55±4.41^{b}	30.55±3.27^{ab}	30.95±5.27^{ab}	28.20±9.47^{ab}	33.82±2.84^{a}	32.46±2.33^{ab}	31.67±1.91^{ab}	0.04
PLT (×10^{3} µL^{-1})	201.25±44.1^{b}	126.50±14.00^{c}	151.00±34.20^{c}	258.00±22.60^{a}	119.00±11.90^{c}	137.67±25.70^{c}	147.00±25.35^{c}	0.00
PCT (%)	0.08±0.04^{a}	0.05± 0.17^{c}	0.06±0.03^{b}	0.08±0.07^{a}	0.05±0.01^{c}	0.08±0.03^{a}	0.06±0.03^{b}	0.0

^{abc}On the same line, values having the same letter do not differ significantly (p>0.05), WBC: White Blood Cell, Lymph: Lymphocyte, GRAN: Granulocytes, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelet and PCT: Plateletcrit

Table 5: Influence of feed supplemented with *D. edulis* and *M. indica* leaves powder on serum proteins on Brahma birds

		Treatments						
		Levels of *D. edulis* powder leaves (%)			Levels of *M. indica* powder leaves (%)			
Characteristics	Control group	0.25	0.5	0.75	0.25	0.50	0.75	p-value
Total protein (g dL^{-1})	4.04±0.99^{a}	3.97±0.38^{a}	4.02±0.22^{a}	3.51±0.00^{b}	3.27±0.00^{b}	3.87±0.31^{a}	3.88±0.24^{a}	0.00
Albumin (g dL^{-1})	1.70±0.12	1.77±0.07	1.71±0.28	1.69±0.11	1.75±0.11	1.67±0.14	1.69±0.14	0.86
Globulin (g dL^{-1})	2.53±0.62^{a}	2.21± 0.41^{a}	2.34±0.20^{a}	1.82±0.11^{bc}	1.52±0.29^{c}	2.18±0.29^{ab}	2.18±0.23^{ab}	0.00

^{abc}On the same line, values with the same letter are not significantly different (p>0.05)

protein and globulin levels, induced the serum levels of total proteins and globulins comparable to that recorded in the hens fed with the control ration. On the other hand, the powders whatever the type and the rate, did not significantly ($p<0.05$) affect the serum albumin content. There was a positive and non-significant correlation between the serum globulin level and the number of lymphocytes ($\rho = +0.26$ and $p>0.05$) (Table 6).

Effects of powders of *D. edulis* and of *M. indica* leaves on the structure of bursa of Fabricius: Figure 2a and b show the histological structure of the bursa of Fabricius in a Brahma hen fed with a food containing the leaves powder of *D. edulis* and *M. indica.* It is resulted that the histological structure of the bursa of Fabricius recorded in the hens fed with the control ration shows a slight degradation of the follicle and the interfollicular. Furthermore, the inclusion *D. edulis* or *M. indica* leaves powder in control ration corrected the alterations noted with the control ration. However, these corrections were more marked in the bursa of Fabricius of birds fed on 0.50% *D. edulis* or *M. indica* fed Brahma.

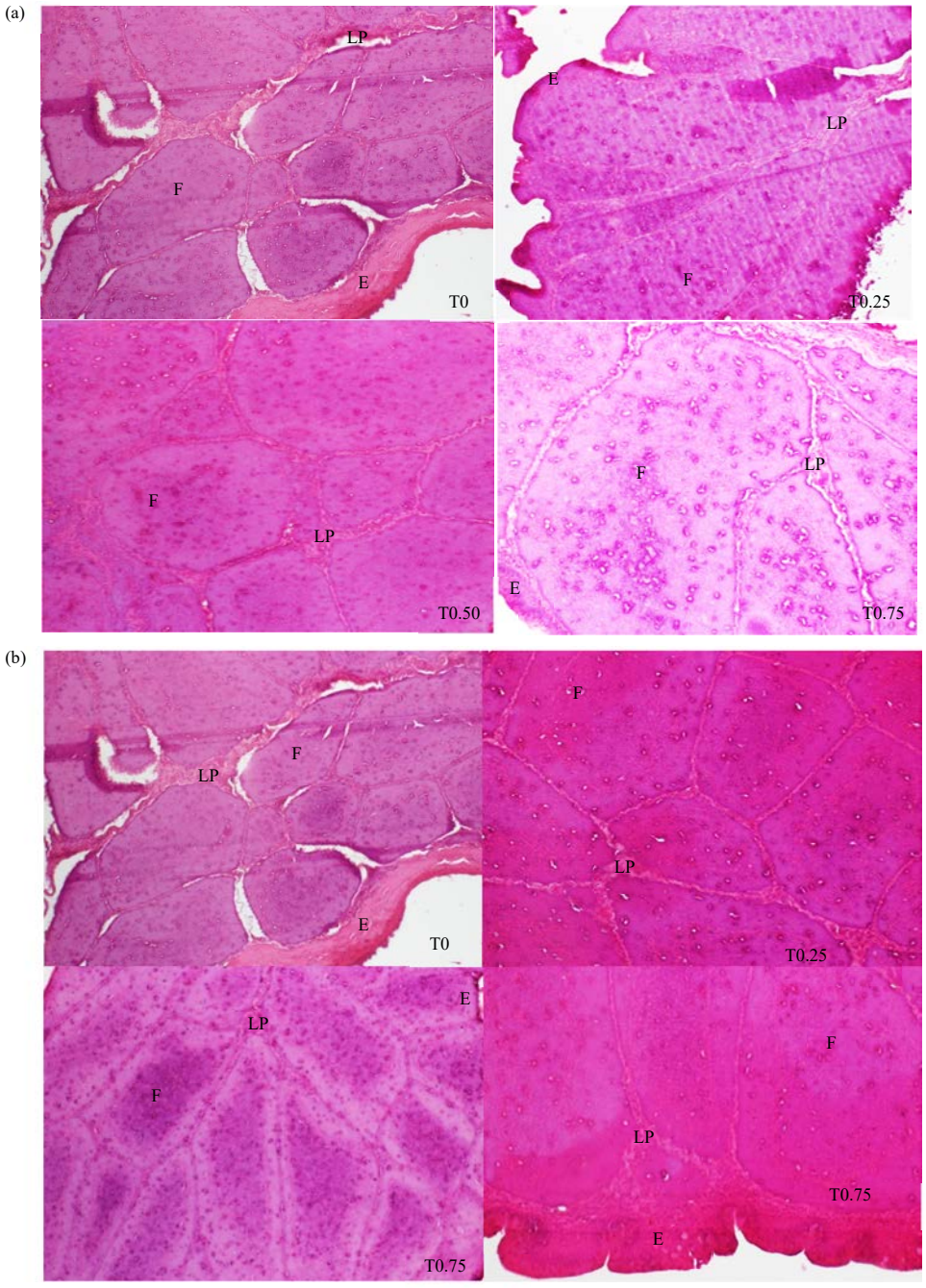

Fig. 2(a-b): Effects of powder of (a) *Dacryodes edulis* and (b) *Mangifera indica* on the structure of the bursa of Fabricius

E: Epithelium, LP: Lamina propria, F: Follicle, T0: Control ration, T0.25: Control ration+0.25% of *D. edulis* leaves powder, T0.50: Control ration+0.50% of *D. edulis* leaves powder and T0.75: Control ration+0.75% of *D. edulis* leaves powder

Table 6: Correlation between the different parameters studied

Parameter	Relative weight of bursa	Relative weight of spleen	Carcass yield 2	Carcass yield 1	Live weight	White globule	Lymphocyte
Relative weight of spleen	-0.32	1					
Carcass yield 2	0.65	-0.50	1				
Carcass yield 1	0.48	-0.50	0.85	1			
Live weight	0.47	-0.91**	0.62	0.70	1		
White globule	-0.34	-0.22	-0.59	-0.54	-0.10	1	
Lymphocyte	-0.27	0.17	-0.68	-0.74	-0.35	0.79*	1
Platelet	-0.57	0.46	-0.53	-0.15	-0.29	-0.19	-0.16
Total protein	-0.42	0.00	-0.03	0.24	-0.11	0.01	-0.48
Globulin	-0.34	0.88**	-0.63	-0.68	-0.96**	0.06	0.26

*Correlation is significant at the level of 0.05 and **Correlation is significant at the level of 0.01

DISCUSSION

The incorporation of *D. edulis* or *M. indica* leaf powders at different concentrations in the feed of Brahma hens for 60 days resulted in a significant increase in live weight compared to that of hens receiving the control ratio. This increase could be due to the antioxidant properties of the leaves of *D. edulis* or *M. indica* attributed to bioactive molecules such as flavonoids, vitamins A, E, C and D, tannins, phenols and terpenoids[19]. These molecules are reputed to have the ability to limit the attacks of reactive oxygen species on the cells of animal tissue. This effect subsequently improves the thickness of the cell membrane, hence the increase in live body weight. To destroy the pathogenic microorganisms of the digestive tract in favor of the bioavailability of the nutrients necessary for the development and growth of the animal. The powders of the leaves, whatever the type and the concentration in the feed of the hens, induced an increase in the relative weight of the carcasses compared to that of the control group. This result would be a logical consequence of the high live weight recorded in the present study. Indeed, a positive correlation ($\rho = +0.70$) between the live weight and the relative weight of the carcasses, suggests a variation in the same direction of the two variables.

Primary lymphoid organs are the organs in which T and B lymphocytes are made and acquire their differentiations into cells equipped to recognize an antigen[20]. In birds, the bursa of Fabricius, which is a primary lymphoid organ, plays a key role in immunity by ensuring the maturation and differentiation of B lymphocytes. In the present study, the relative weights of the bursa of Fabricius recorded in Brahma hens fed with the feed containing the leaves powder of *M. indica* in their feed were comparable but, high in reference to that of the hens receiving the control ration. These results agreed with those obtained by Abdel-Fattah *et al.*[17] after administration of organic acid supplements in broilers. The increase in the masses of this organ is due to the presence of bioactive molecules such as flavonoids, phenolic acid, steroids and anthocyanins in the leaves of *D. edulis*[21] and *M. indica*[9]. These molecules are likely to stimulate the increase in tissue mass by synthesis and accumulation of tissue proteins. However, proteins are the building blocks of animal tissue cells and therefore allow the increase in body mass. This increase would be correlated with the increase in live weight and the carcass yield obtained.

In addition, a positive correlation ($\rho = +0.47$) was observed between the live weight and the relative weight of the bursa of Fabricius. According to Katanbaf *et al.*[22], increased primary lymphoid organ weight is considered an indication of immune cell development. The increase in the weight of an organ would result not only from the good development of its membrane but also from the good growth of its cytoplasm. The incorporation of powders whatever the type and the concentration in the Brahma ratio reduced the alterations observed on the histology of the bursa of Fabricius of the untreated hens. However, these corrections were more marked in hens receiving 0.50% of *D. edulis* and *M. indica* leaf powder. The effects observed suggest the protective power of these leaves against attacks on the structures of animal organs.

The spleen is a secondary lymphoid organ. It represents the site of passage, accumulation and the antibody-antigen reaction[23]. Relative weight of spleen recorded in hens fed diet containing *D. edulis* or *M. indica* leaf powder decreased but this decrease was only significant with 0.25% leaf powder of *M. indica* in food compared to control. These results are in contradiction with those of Tangomo *et al.*[10], who recorded a non-significant effect in spleen weight with the 0.5 and 1.0% of *D. edulis* leaves powder in broiler chicken feed. The difference between these results would be related to the bird genetic types, the duration of exposure to the additive, also to the bird age difference. However, compounds such as flavonoids and terpenoids contained in the leaves of *D. edulis* and *M. indica* would have exerted their antimicrobial activity to eliminate antigens and therefore limit antibody-antigen reactions within this organ. This effect would have played in favor of the reduction in the weight of this organ. A negative

and significant correlation was recorded between the relative spleen weights and the live body weight of the hens. This suggested that the increase in spleen weight was linked to an intense activity of the immune cells in response to foreign agents. This effect reduces the energy that can be used the increase animal body weight.

Proteins are very important macromolecules for living organisms, playing a vital role in building the body and enabling protection and defense through immunoglobulins or antibodies produced by lymphocytes[24,25]. In this study, the powders of the leaves of *D. edulis* and *M. indica* at 0.75 and 0.25%, respectively in the feed of the hens induced a significant decrease in the serum level of total proteins and globulins compared to that of the control. These results are in agreement with those obtained by Nahed *et al.*[26], who recorded a significant decrease in serum total protein and globulin content at doses of 200 and 300 mg kg^{-1} ginger powder in broiler hens. Moreover, they are in contradiction with those of the works carried out by Tchoffo *et al.*[27] with the oral administration of essential oil from ginger rhizomes in Japanese quail. The difference between the results of these two works would be due to the difference in breed, plants used, the rates used and the experimental conditions. The decrease in serum total protein and globulin levels noted in this study could be related to bioactive compounds such as flavonoids, tannins and saponins contained in the leaves of *D. edulis* and *M. indica*. These molecules would have eliminated the pathogenic agents and consequently, the rate of immunoglobulins (Ig) circulating in the blood would have decreased. Indeed, Igs are produced by lymphocytes during antigenic stimulation. The decrease in the serum Ig level would be the consequence of the decrease in the number of lymphocytes. Moreover, a positive correlation was recorded between the globulin and lymphocyte level ($\rho = +0.26$) and between the number of granulocytes ($\rho = +0.87^*$), suggesting the variation of these variables in the same sense. The number of phagocytic cells (granulocytes) and lymphocytes recorded in hens fed with the food containing the powders leaves of *D. edulis* and *M. indica* were significantly lower at the concentration of 0.75% of *M. indica* leaf powder compared to that of the hens receiving the control ration. The drop in leukocytes with the inclusion of *M. indica* powder at a high concentration in hen feed would result from their ability to protect the body from the attack of foreign particles. The work of Igwé *et al.*[28] showed that Iso-Brown hens infected with Velogenic Newcastle disease virus that have not received a vaccine have high levels of lymphocytes (8.77 and 12.26%, respectively at 3 and 6 weeks) compared to those of infected and vaccinated hens (7.73 and 7.17%) during these same weeks.

Hemoglobin is the protein of red blood cells that is used to transport respiratory gases[29]. According to Marjory *et al.*[30], hemoglobin level is influenced by oxygen demand and erythrocyte count. In the present study, powder leaves of *D. edulis* and *M. indica* except at 0.75% in the birds feed induced blood hemoglobin levels which were significantly lower ($p<0.05$) than that of control group. This result would be due to the antioxidant properties of the flavonoids contained in the leaves of *D. edulis* and *M. indica*. These molecules would have exerted a protective action on the animal organism against attacks by reactive oxygen species and subsequently have reduced the cellular metabolism which needs more hemoglobin for the transport of respiratory gases.

Thrombocytes play an important role in coagulation and have greater phagocytic activity than macrophages and microphages[31]. Powders of *D. edulis* and *M. indica* leaves at 0.75% each in hen feed induced a decrease in the number of blood platelets compared to the control. This decrease could be due to the anti-aggregation and anti-platelet activities mediated by polyphenols, bioactive compounds contained in the leaves of *D. edulis* and *M. indica*. These would therefore have induced in hens the inhibition of the synthesis and aggregation of blood platelets[32].

The implication of this study is to increase bird productivity by using phytoadditives with diverse pharmacological activities and less environmental impact. *D. edulis* and *M. indica* leaves which are available and accessible in Cameroon could be an excellent alternative to antibiotics banned in animal production. When formulating diets for their birds, the poultry producers are advised to use *D. edulis* and *M. indica* leaves powders at 0.5% for better health and growth.

CONCLUSION

Dacryodes edulis and *Mangifera indica* leaf powder at 0.05 and 0.75% included in the feed of Brahma hens for 60 days consolidate his immune system and stimulate an increase in live body weight and carcass yield. Pending additional studies are required to further confirm these results, the poultry producers can incorporate *D. edulis* and *M. indica* leave powders at 0.5% in the feed of birds to neutralize the environmental effect and subsequently increase productivity.

SIGNIFICANCE STATEMENT

This study discovered that the incorporation of Safou (*Dacryodes edulis*) and mango (*Mangifera indica*) leaf powders in the feed of a hen can fortify his immune system, protect cells from reactive oxygen species attacks and subsequently

promote growth. These are potential alternatives for antibiotics. In bird feed, producers can use *D. edulis* and *M. indica* leave powders at 0.5% to neutralize the effects of exogenous factors that reduce production performance. These photo additives have shown positive effects on hen growth performance but it is necessary to define the quantity of each bioactive molecule present for rational use.

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