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Research Article

Phytochemical Study and Effects of Anticoccidian Treatment of *Carica Papaya* L. Seed on Biochemical Parameters and Carcass in Sasso Broilers

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Abstract

Coccidiosis is one of the dreaded pathologies that ruin poultry, causing enormous economic losses for poultry farmers. Chemotherapy control has some challenges, including bioresistance developed by coccidia and the presence of harmful residues in egg products. There is the question to know whether there is an effective, natural-originated, and less expensive substitute to replace synthetic anticoccidial products. The current study proposed to evaluate the anticoccidial effects of papaya seed powder on biochemical parameters in poultry. Six hundred (600) day-old chicks, of Sasso breed and broiler type, were divided into four batches noted: untreated batch, T-; treated batch with amprolium (20%), T+; then P1 and P2 batches, treated with 5% of papaya seed powder, incorporated into the feed, respectively for one and two days per month. The secondary metabolites contained in papaya seed powder have been identified by applying general methods described in the literature. Biochemical parameter assays in serum, recovered by centrifugation of blood collected from birds, were performed using enzymatic colorimetry methods with Mindray BS/China biochemical analyzer system. The results revealed the presence of tannins, flavonoids and alkaloids, which are the main secondary metabolites known for their proven anticoccidial properties. In addition, values of number of eggs per gram (EPG) reduction rate compared to T- batch are 70.60%, 76.92%, 88.16%, respectively in batches P1, P2 and T+. Shortly, papaya seed powder incorporation into the feed leads to lower levels of urea and AST, but an increase in albumin in Sasso broilers. Anticoccidial treatment with *Carica papaya* seed powder (5%) has almost the same anticoccidial efficiency as amprolium (20%) and had no adverse effect on poultry. However, the effect of the seed on organoleptic properties of the meats can be investigated to determine their degree of acceptability among consumers.

Keywords

Sasso Chickens, Papaya Seed, Secondary Metabolites, Anticoccidial Activity, Biochemical Parameters

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1. Introduction

Skyrocketing population growth, coupled with improved living conditions for people around the world, has led to a constant increase in the need for proteins, especially of animal origin. In response to food needs, there is an increase in livestock farming, especially that of short-cycle species. Among these species, poultry occupies a prominent place. In recent decades, poultry farming has become one of the fastest growing sectors thanks to genetic and technical progress [1] and it contributes significantly to the world's food resources [2]. Unfortunately, poultry farming in its momentum is confronted with many constraints, including the pathological one. Indeed, the intensive production system, characterized by high densities with large numbers of birds, has led to increased levels of stress and an intensification in avian pathologies [3, 4]. Indeed, by intrusion of the digestive tract of poultry, some microbes affect the weight growth, health and meat quality of birds [5]. One of the most serious diseases affecting poultry productivity is coccidiosis, an intestinal pathology caused by protozoa of the genus *Eimeria*, also known as coccidia. Coccidiosis is identified as the most common parasitic disease in intensively farmed poultry and remains highly contagious, despite the biosecurity measures often respected [6, 7].

By affecting the digestive tract, coccidia cause enteritis, nutrient malabsorption, reduced production performance, litter degradation, decreased bird welfare, morbidity, and even a high mortality rate in poultry [8, 9]. According to Zhang et al. (2013) [10], coccidia may also promote the spread of other diseases such as mycoplasmosis, necrotic enteritis and colibacillosis. In severe cases, coccidia cause high mortality rates. There are 9 species of coccidia in chickens, 5 of them are considered as the most important because of their degree of virulence and their economic impact. These include: *E. brunetti*, *E. acervulina*, *E. maxima*, *E. necatrix* and *E. Tenella* [11, 12]. In farming conditions, multiple infection is very common and aggravates the disease.

The fight against coccidiosis involves hygiene practices and especially chemoprophylaxis. Intensive or abusive and prolonged use of synthetic anticoccidial chemicals has resulted in the emergence of resistance in coccidia. In addition, the presence of drug residues in poultry meat and egg products is a major health risk to consumers. In this context, alternative actions such as vaccines have been developed. As a preventive strategy, vaccination confers protective immunity on poultry by reducing the replication of coccidia as well as the clinical signs of the disease [13]. This immunity helps to prevent and to attenuate coccidiosis [14, 15]. However, no single vaccine is effective against all of the 5 coccidia species known to be more pathogenic and the antigenicity of coccidian strains may vary depending on the geographical location [16]. In addition, prolonged storage of live vaccines can compromise their effectiveness [17]. Despite the effectiveness of synthetic anticoccidial chemicals and vaccines, coccidiosis remains a significant economic threat to the poultry sector, especially in low- and middle- income countries, which are the most vulnerable [6]. Faced with the limitations of synthetic chemical anticoccidials and vaccines, and the need for healthy products for consumers, it is essential to find alternatives that are less expensive, natural and without major risks to the health of both animals and consumers [18]. Challenges related to coccidia resistance, limitations of chemoprophylaxis, and difficulties in accessing vaccines have led to the exploration of herbal products as an alternative for coccidiosis control [1].

Several studies with promising results have been conducted on the ability of plants to improve the production performance of birds as well as on their anticoccidial properties [19-21]. Among these plants, Carica *papaya* stands out. Indeed, *Carica papaya* is a perennial plant of *Caricaceae* family, native to America [22], but which is now abundant in tropical areas. The use of *Carica papaya* for therapeutic purposes has no adverse effect on the environment. Most studies on the anticoccidial properties of *Carica papaya* have been done using extracts of its organs. These extracts do not generally contain all bioactive molecules of the plant, whereas phytoconstituents sometimes have a synergy of action. In addition, plant extracts are difficult to access and to use by poultry farmers, while *Carica papaya* seeds, which are very little exploited, remain accessible and cheap. Papaya leaves and seeds are a good source of vitamins, minerals, antioxidants and enzymes that aid in the digestion of carbohydrates, proteins and fats and have antimicrobial properties including anticoccidial properties [23]. However, despite the effectiveness of phytotherapy, some plants or their biomolecules can be toxic in high doses, or even in low doses over a long period of use. Therefore, it is necessary to study the effects of these plants on biochemical and haematological parameters, and even on the carcasses of birds, in order to establish their effects on poultry health.

Under these circumstances, the current study is carried out to evaluate the anticoccidial effects of *Carica papaya* seeds on the biochemical parameters and on the internal organs of Sasso broilers, an improved slow-growing strain, in breeding conditions, in order to guide the actors of the poultry sector.

2. Materials and Methods

2.1. Scope and Duration of the Study

This study was carried out in Togo, a sub-Saharan country, more precisely on the technical platform of the Higher School of Agronomy named ESA, in the University of Lomé. Biochemical parameters were measured at the Laboratory of Microbiology and Quality Control of Foodstuffs (LAMICODA), of the Higher School of Biological and Food Techniques called ESTBA, in the University of Lomé, Togo. The phytochemical screening was carried out at the Laboratory of Process and Natural Engineering Resources known as LAGEPREN at the Faculty of Sciences (FDS), in the University of Lomé, Togo. The chicks were raised at the farm called "Ferme ALBERT" near Tsevié, a town situated about 30 km away from Lomé.

2.2. Biological Material

Animal Material

The animal material used for the implementation of the current work were constituted of 600-day-old chicks, of Sasso breed and broiler type. These birds were acquired from “MDL Maison Diop” farm, located in Agbavi, a village situated about 25 km southeast of Lomé.

Plant material

Powder of papaya seeds of Solo variety, collected from papaya resellers in Lomé, were used as plant material in the present study.

2.3. Breeding Equipment

The chicken coop consisted of two open buildings with varying temperature and humidity. Each building was 10.5 m long by 4 m wide and partitioned with fences. The buildings as well as the feeders and watering troughs have been washed and disinfected. The buildings were then subjected to a crawl space for two weeks. Two days before the chicks were installed, the floor was completely covered with shavings and the disinfection of the building was resumed.

2.4. Methods

2.4.1. Livestock Management

The breeding period, which lasted twelve weeks, consisted of three phases. A three-week start-up phase, during which the 600-day-old chicks were initially weighed, then raised together in a brood house. The density during the first week was 50 chicks/m^2, then those of the 2nd and 3rd weeks, 20 chicks/m^2. The chicks were fed during the first phase with the starter feed and water, *ad libitum*. Then, they were vaccinated against New Castle disease, Gumboro disease and infectious bronchitis. During the six-week growth phase and the three-week finishing phase, bird densities were 12 chickens/m^2 and 8 chickens/m^2, respectively. Water and food were served twice a day during these last two phases.

2.4.2. Batch Constitution

The 600 chicks were raised together for 4 weeks considered to be the time needed for a natural infection with coccidia to be occurred. Then, they were then divided into 4 batches, each containing 5 replications of 30 chicks. These are: the untreated batch or negative control batch (T-); the batch treated with amprolium 20% or positive control batch (T+); finally, batches P1 and P2 that were treated with papaya seed powder (5%) incorporated into the feed, respectively for one day and two consecutive days per month.

2.4.3. Parameter Evaluation

(i). Phytochemical Screening

Phytochemical screening was carried out on four extracts obtained by gradual extraction technique of maceration. For this purpose, the seeds of *Carica papaya* Solo were dried in the shade for a week, then ground with Thomas Scientific Laboratory Mill/Model 4, USA; equipped with a sieve with a porosity diameter of 1 mm. A mass of 400 g of papaya seed powder was delipidated for 48 h in 1.5 L of petroleum ether. After the mixture filtration, the recovered residue was also macerated for 48 h in dichloromethane, then in 95% ethanol (vol.), and finally in distilled water [24]. After maceration, each extraction solution was refiltered using a Whatman N°1 filter paper. The extracts were recovered dry by evaporation of the solvents at a temperature of 45 °C using Büchi-type rotary Evaporator system.

The dry extracts were introduced into tinted vials, hermetically sealed and then stored in the freezer at -21 °C for subsequent phytochemical analyses.

Phytoconstituents have been identified, in particular: alkaloids, tannins, flavonoids, and saponin, applying the methods described in the literature [25-27]. The reagents used for the identification of these types of phytoconstituents are: Mayer and Dragendorff reagents for alkaloids; $(CH_3CO_2)_2Pb$ (10%: v/v) in basic aqueous solution, and $CuSO_4$, for tannins; NaOH (1%) and $FeCl_3$ (1%), for flavonoids; while the foam test was specifically used for saponin identification.

(ii). Determination of the Number of EPG Reduction Rate by Papaya Seed Powder

The anticoccidial efficacy of papaya seed powder was evaluated as stated by McMaster method and the coproscopy technique, based on the flotation principle. Batches T+, P1 and P2 underwent anticoccidial treatments at the 4th, 6th, 9th and 12th weeks of age. Fresh faeces were collected from each batch before and after each treatment, for the determinations of EPG number and the rate of reduction (RR). To do this, an aqueous solution of NaCl (50%) was used. Then, 5 g of faeces were crushed in 70 mL of NaCl (50%), and the resulting mixture was filtered. The collected filtrate was then used to carefully fill the cells of McMaster slide without causing the bubbles to form. After 5 min to allow the eggs to float, a microscope observation was made for the determination of EPG number from which the reduction rate (RR) is calculated in conformity with formula (1) [28].

$$\mathrm{RR}\ (\%) = \frac{(\text{EPG before treatment} - \text{EPG after treatment}) \times 100}{\text{EPG before traitement}} \quad (1)$$

(iii). Biochemical Parameter Analyses

After each treatment, three replication subjects were isolated and then 7 mL of blood was drawn from each animal from the wing vein in a homolyze tube. The tube contents were centrifuged for 15 min with rotation speed of 3,000 rpm, then the resulting serum was recovered and stored in a freezer at -20 °C for subsequent biochemical analyses.

Eventually, these analyses have focused on total proteins and albumins assays, to estimate the nutritional status of the animals; then triglyceride and cholesterol assays (HDL, LDL and total) in order to assess their lipid profile. In addition, uric acid, urea and creatinine assays, to assess the effects of anticoccidial treatments on renal function. Finally, transaminases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) assays were also performed to assess the impact of treatments on liver function. Overall, all these various biochemical parameters were determined based on enzymatic colorimetry methods performed using Mindray BS/China biochemical analyzer system.

(iv). Determination of Effects of Anticoccidial Therapy on Internal Organs

For each batch, fifteen animals were sacrificed. Then, their immune organs such as spleen, bursa of Fabricius and thymus plus some organs of the carcass such as: the liver, gizzard and heart, were removed and weighed in order to determine their relative weight (RW) in line with formula (2) [29].

$$RW = \frac{\text{Organ weight x 100}}{\text{Live weight}} \quad (2)$$

2.4.4. Statistical Analyses

The data obtained in this work were processed by "Graph Pad Prism 5" software and were subjected to one way ANOVA analysis of variance, followed by TUKEY post-test to make the comparison between the different batches. The probability $P < 0.05$ was considered to be the significance threshold. The results were presented in the form of means plus or minus the standard error on the mean (M ± S. E.M.).

3. Results

3.1. Effects of Treatments on Coccidia

The values of EPG number and RR corresponding to the four batches studied in this work are recorded in Table 1. It can be seen that at the fourth week of age, before the start of the various anticoccidial treatments, there was no significant difference between EPG number of the different batches. However, following the anticoccidial treatments carried out during this period, a significant reduction in EPG number in P1, P2 and T+ batches was observed compared to T- batch. Between the 6th and 9th weeks of age, EPG number remained significantly low ($p<0.05$) for each treated batch compared to T- batch for which EPG number continuously increased.

In sum, RR of EPG number were 70.60%, 76.92% and 88.16% respectively in batches P1, P2 and T+. In contrast, the T- batch showed an increase in EPG number, characterized by a negative RR (Table 1).

3.2. Phytochemical Screening

Qualitative phytochemical tests showed a variation in the results, depending on the solvent but also on the reagents used (Table 2).

The qualitative phytochemical tests carried out with aqueous and ethanolic extracts revealed the presence of alkaloids, flavonoids and tannins in the seeds of Solo *Carica papaya* variety, while with the dichloromethane extract, the tests revealed only the presence of flavonoids and tannins. However, none of the moss test extracts showed the presence of saponin in these seeds.

Table 1. *EPG number reduction rates in agreeing with treatments.*

Treatments					
Age (weeks)		**P1**	**P2**	**T+**	**T-**
W4	EPG before treatment	86.00 ± 21056^{a}	83.00 ± 14,33^{a}	91.00 ± 11.30^{a}	79.00 ± 11.62^{a}
	EPG after treatment	16.00 ± 3.37^{b}	8.00 ± 1.47^{b}	2.00 ± 1.23^{b}	103.00 ± 10.21^{a}
Rate of reduction W4 (%)		81.39	90.36	97.80	-30.38
W6	EPG before treatment	185.00 ± 8.94^{a}	42.00 ± 7.56^{b}	168.00 ± 25.94^{a}	228.00 ± 15.84^{a}
	EPG after treatment	38.00 ± 4.18^{b}	6.00 ± 1.47^{b}	55.00 ± 17.74^{b}	458.00 ± 61.22^{a}
Rate of reduction W6 (%)		79.46	85.71	67.26	-100.88
W9	EPG before treatment	295.00 ± 23.56^{b}	358.00 ± 21.28^{b}	347.00 ± 13.16^{b}	648 ± 71.89^{a}
	EPG after treatment	84.00 ± 18.98^{b}	73.00 ± 12.96^{b}	17±4,65^{b}	455 ± 73.66^{a}
Rate of reduction W9 (%)		71.52	79.61	95.10	29.78
W12	EPG before treatment	30.00 ± 8.91^{b}	25.00 ± 6.42^{b}	54 ± 14.26^{a}	98 ± 19.83^{a}
	EPG after treatment	15.00 ± 3.94^{a}	12.00 ± 4.76^{a}	4 ± 2.18^{a}	28 ± 10.65^{a}

Treatments				
Age (weeks)	**P1**	**P2**	**T+**	**T-**
Reduction Rate W12 (%)	50.00	52.00	92.60	71.43
Average reduction rate (%)	70.60	76.92	88.19	-7.51

Values on the same line that do not have the same superscript letters are significantly different (P< 0.05).

***Table 2.** Qualitative phytochemical composition of aqueous, ethanolic and dichloromethane extracts of papaya seed.*

Phytochemical compounds analysed	**Reagents used**	**Results obtained**		
		A-Ext	**E-Ext**	**D-Ext**
Alkaloids	Mayer	+	-	-
	Dragendorff	+	+	-
Flavonoids	NaOH (1%)	+	+	-
	$FeCl_3$ (1%)	+	+	+
Tannins	$FeCl_3$ (1%)	+	+	+
	$(CH_3COO)_2Pb$ (10%)	+	+	-
	$CuSO_4$	+	+	-
Saponins	Mousse	-	-	-

+: means that the corresponding test is positive; - means that the test is negative; A-Ext: Aqueous extract; E-Ext: Ethanolic extract; and D-Ext: Dichloromethane extract.

***Table 3.** Biochemical parameters measured at 6 weeks of age of birds.*

Treatments					
Parameters	**P1**	**P2**	**T+**	**T-**	**P value**
Proteins (g/L)	35.50 ±4.49^{a}	33.00 ±3.00^{a}	38.50 ±2.84^{a}	40.00 ±3.65^{a}	0.5315
Albumins (g/L)	10.60 ±0.91^{a}	10.35 ±0.10^{a}	11.75 ±0.74^{a}	10.40 ±0.86^{a}	0.6343
Uric acid (mg/dL)	5.86 ±0.37^{a}	5.58 ±0.57^{a}	5.79 ±0.41^{a}	5.54 ±0.21^{a}	0.9184
Urea (g/L)	0.030 ±0.004^{a}	0.040 ±0.006^{a}	0.040 ±0.004^{a}	0.040 ±0.006^{a}	0.2170
Creatinine (mg/dL)	0.13 ±0.02^{a}	0.10 ±0.00^{a}	0.10 ±0.00^{a}	0.15 ±0.03^{a}	0.4789
Triglycerides (g/L)	0.51 ±0.03^{a}	0.35 ±0.06^{a}	0.41 ±0.07^{a}	0.53 ±0.05^{a}	0.1476
Cholesterol (g/L)	1.34 ±0.10^{a}	1.36 ±0.08^{a}	1.29 ±0.04^{a}	1.49 ±0.03^{a}	0.2742
HDL-Cholesterol(g/L)	1.01 ±0.20^{a}	1.17 ±0.03^{a}	1.24 ±0.04^{a}	1.02 ±0.08^{a}	0.8287
LDL-Cholesterol(g/L)	0.15 ±0.03^{a}	0.15 ±0.03^{a}	0.18 ±0.03^{a}	0.17 ±0.02^{a}	0.8480
ALT (IU/L)	2.00 ±0.58^{a}	1.25 ±0.25^{a}	1.50 ±0.29^{a}	2.75 ±0.48^{a}	0.1119
AST (IU/L)	283.50 ±14.43^{a}	227.8 ±13.17^{b}	287.50 ±10.93^{a}	291.5 ±16.611^{a}	0.0222

Values on the same line not bearing the same letters (a and b) are significantly different (P< 0.05)

Table 4. Biochemical parameters measured at 9 weeks of age of birds.

	Treatments				
Parameters	**P1**	**P2**	**T+**	**T-**	**P value**
Proteins (g/L)	38.75 ±1.03[a]	36.25 ±1.49[a]	35.25 ±1.48[a]	37.00 ±0.81[a]	0.2877
Albumin (g/L)	14.38 ±0.19[a]	12.58 ±0.35[ab]	12.33 ±0.80[ab]	12.10 ±0.40[b]	0.0247
Uric acid (g/L)	5.09 ±0.90[a]	4.04 ±0.79[a]	4.65 ±0.82[a]	3.60 ±0.28[a]	0.5250
Urea (g/L)	0.040 ±0.004[a]	0.040 ±0.002[a]	0.030 ±0.002[a]	0.050 ±0.008[a]	0.1564
Creatinine (g/L)	0.08 ±0.03[a]	0.03 ±0.03[a]	0.08 ±0.03[a]	0.08 ±0.03[a]	0.4262
Triglycerides (g/L)	0.22 ±0.02[a]	0.24 ±0.01[a]	0.23 ±0.01[a]	0.26 ±0.04[a]	0.8023
Cholesterol (g/L)	1.32 ±0.02[a]	1.25 ±0.13[a]	1.32 ±0.08[a]	1.34 ±0.08[a]	0.8933
HDL-Cholesterol (g/L)	0.95 ±0.02[a]	0.94 ±0.04[a]	0.96 ±0.03[a]	0.90 ±0.02[a]	0.5549
LDL-Cholesterol (g/L)	0.40 ±0.04[a]	0.38 ±0.04[a]	0.38 ±0.05[a]	0.40 ±0.04[a]	0.9554
ALT (IU/L)	1.25 ±0.25[a]	2.00 ±0.41[a]	1.00 ±0.41[a]	2.25 ±0.48[a]	0.1330
AST (IU/L)	300.8±15.12[a]	253.50[a] ±7.19[b]	281.30 ±11.66[a]	307.5 ±13.90[a]	0.0386

Values on the same line not bearing the same letters (a and b) are significantly different (P< 0.05)

Table 5. Biochemical parameters measured at 12 weeks of age of birds.

Treatments					
Parameters	**P1**	**P2**	**T+**	**T-**	**P value**
Proteins (g/L)	38.25 ±1.03[ab]	43.50 ±1.89[a]	36.00 ±2.16[b]	33.50 ±0.86[b]	0.0050
albumin(g/L)	10.65 ±1.49[a]	12.05 ±0.88[a]	10.58 ±0.48[a]	10.60 ±1.51[a]	0.7708
Uric acid (g/L)	4.61 ±0.58[a]	4.53 ±0.37[a]	3.10 ±0.41a[b]	2.79 ±0.12[b]	0.0134
Urea (g/L)	0.030±0.003[ab]	0.020 ±0.003[b]	0.020 ±0.002[b]	0.030 ±0.004[a]	0.0134
Creatinine (g/L)	0.08 ±0.02[a]	0.08 ±0.02[a]	0.08 ±0.02[a]	0.13 ±0.01[a]	0.3753
Triglycerides (g/L)	0.19 ±0.03[a]	0.19 ±0.07[a]	0.15 ±0.02[a]	0.23 ±0.04[a]	0.6216
Cholesterol (g/L)	1.11 ±0.19[a]	0.97 ±0.10[a]	0.77 ±0.07[a]	1.21 ±0.01[a]	0.0855
HDL-Cholesterol (g/L)	0.69 ±0.05[a]	0.60 ±0.07[a]	0.55 ±0.05[a]	0.67 ±0.07[a]	0.4227
LDL-Cholesterol (g/L)	0.38 ±0.03[a]	0.40 ±0.05[a]	0.18 ±0.03[b]	0.40 ±0.04[a]	0.,0024
ALT (IU/L)	0.25 ±0.25[a]	0.25 ±0.25[a]	0.25 ±0.25[a]	0.75 ±0.25[a]	0.4262
AST (IU/L)	180,00 ±10.07[b]	229.00 ±13.39[ab]	182.80 ±11.23[b]	237.80 ±14.64[a]	0.0152

Values on the same line not bearing the same letters (a and b) are significantly different (P< 0.05)

Table 6. *Effects of treatment on internal organs at the age of 9 weeks.*

RW (%)	P1	P2	T+	T-	P value
Spleen	0.16 ± 0.02[a]	0.15 ± 0.02[a]	0.14 ± 0.01[a]	0.16 ± 0.02[a]	0.5572
Thymus	0.48 ± 0.03[a]	0.48 ± 0.04[a]	0.,47 ± 0.03[a]	0,44 ± 0.04[a]	0.8631
Bourse	0.16 ± 0.03[a]	0.12 ± 0.03[a]	0.10 ± 0.01[a]	0.11 ± 0.01[a]	0.5658
Heart	0.49 ± 0.04[a]	0.51 ± 0.03[a]	0.54 ± 0.05[a]	0.45 ± 0.04[a]	0.5011
Liver	1.85 ± 0.18[a]	1.84 ± 0.04[a]	1.87 ± 0.06[a]	1.82 ± 0.18[a]	0.9928
Gizzard	3.71 ± 0.29[a]	4.02 ± 0.14[a]	4.26 ± 0.21[a]	3.89 ± 0.31[a]	0.4764

Values on the same line not bearing the same superscript letters (a and b) are significantly different (P< 0.05); RW: Relative weights

Table 7. *Treatment Effects on internal organs at 12 weeks of age.*

RW (%)	P1	P2	T+	T-	P value
Spleen	0.23±0.04[a]	0.22±0.04[a]	0.17±0.03[a]	0.17±0.03[a]	0.4748
Thymus	0.44±0.06[a]	0.42±0.07[a]	0.37±0.04[a]	0.35±0.04[a]	0.6779
Bourse	0.10±0.03[a]	0.08±0.02[a]	0.06±0.02[a]	0.06±0.02[a]	0.5020
Heart	0.44±0.04[a]	0.52±0.01[a]	0.47±0.01[a]	0.45±0.03[a]	0.2327
Liver	1.74±0.11[a]	2.13±0.12[a]	1.60±0.11[a]	1.65±0.18[a]	0.2669
Gizzard	3.61±0.46[a]	3.41±0.32[a]	2.99±0.25[a]	3.55±0.32[a]	0.5957

Values on the same line not bearing the same superscript letters (a and b) are significantly different (P< 0.05); RW: Relative weights

3.3. Anticoccidial Effects on Biochemical Parameters in Birds

The biochemical parameters of Sasso chicks subjected to the anticoccidial treatments mentioned above in this survey are reported in Table 3. Based on the results in Table 3, treatment with papaya seed powder of the Solo variety at week 6 induced a significant reduction ($p < 0.05$) in the AST level in the P2 batch that had undergone two consecutive days of treatment with 5% papaya seed powder compared to the other batches.

Table 3: Biochemical parameters measured at 6 weeks of age of birds.

Similarly, at the 9th week of age, after treatment, there was still a significant decrease ($p < 0.05$) in the AST level in the P2 batch compared to the T- batch, while a significant increase ($p < 0.05$) in the albumin level was observed in the P1 batch compared to the T- batch which had not undergone any anticoccidial treatment (Table 4).

Compared to T- batch, treatment with Solo papaya seed powder variety, at the 12th week of age (Table 5), significantly increased ($p<0.05$) the amount of total protein in P2, then uric acid in P1 and P2; on the other hand, it significantly decreased ($p<0.05$) urea rate in P2 and AST level in P1 and T+ batches.

Table 5: Biochemical parameters measured at 12 weeks of age of birds.

3.4. Evaluation of the Effects of Anticoccidial Treatments on Internal Organs

Anticoccidial treatments did not have a significant effect on the relative weights of internal organs assessed at 9 and 12 weeks of age in the birds (Tables 6 and 7)

4. Discussion

Anticoccidial treatment based on papaya seed powder induced a significant reduction in OPG number in a manner similar to that of the synthetic anticoccidial, amprolium 20% (Table 1). These results are similar to those obtained by Dakpogan et al., (2018) [30], and Agboola et al. (2018) [31], as well as by Toah et al., (2021) [32], who treated chickens experimentally infected with Eimeria from plant extracts.

In this current, the mean OPG number reduction rates were 70.60% in P1 batch, 76.92% in P2 batch, in contrast to 53% obtained by Nghonjuyi et al. (2015) [33] with Carica papaya leaf extracts.

EPG number reduction rates obtained in this work are significantly higher than the results obtained with chickens which received per month treatment of papaya seed powder

(5%) over two, three and four consecutive days. These treatments resulted in RR average of 66.77%; 58.15% and 43.43%, while the RR of positive control (T+) batch that received anticoccidial treatment with amprolium (20%) used monthly for four consecutive days in the chickens' drinking water was 51.93% [28].

In this work, qualitative phytochemical analysis of Solo Carica papaya seed extracts revealed the presence of tannins, flavonoids and alkaloids (Table 2). These results are similar to those obtained by Dada et al. (2016) [34] and Etame et al. (2017) [35] who showed that papaya seeds contain not only steroids, polyphenols, terpenoids, coumarins, reducing compounds, proteins, but also saponins. These results also corroborate those obtained by Wampah et al. (2024) [28] with hydroethanolic extracts (50%-50%: v/v) of the same seed.

The anticoccidial activity of plants is explained by biomolecule types they contain. Indeed, during their growth, plants develop biologically active molecules and preserve them in their various vegetative organs [36, 37]. The phytoconstituents revealed by the phytochemical screening in papaya seed extracts, including alkaloids, flavonoids and tannins, have countless biological properties. Accordant with Abdelli et al. (2021) [38], these plant-synthesized biomolecules preserve the gut microbiota, improve bird immunity to coccidia, and reduce the mortality rate related to coccidiosis. As reported by El-Shall et al., (2022) [11] and Saeed and Alkheraije (2023) [39], the anticoccidial property of plants lies in the antioxidant and anti-inflammatory activities of their biomolecules with the advantage of having a lower risk of developing the phenomenon of resistance in coccidia. In addition, some phytoconstituents exert either a direct proteolytic action on sporozoites [40], or a direct interruption of their life cycle, or a disruption of the formation of the oocyst wall, or an inhibition of endogenous enzymes, responsible for sporulation [41].

In addition to anticoccidial activity exerted by the phytoconstituents contained in papaya seed powder, they can also exert other effects on birds, particularly on their biochemical parameters. Indeed, after two consecutive days of treatment with papaya seed powder (5%) introduced into the diet, P2 batch experienced a significant reduction in AST levels at the 6th and 9th weeks of age (Tables 3 and 4). Similarly, reductions were noted in P1 batch at the 12th week of age (Table 5). These results are similar to those of [42]. As attested by the work carried out by the latter authors, the seed of *Carica papaya* is therefore not hepatotoxic to chickens at this dose. P1 batch experienced a significant increase in albumin levels at the 9th week of age (Table 4). These results are similar to those of [43]. As recorded by these authors, the increase in albumin levels implies adequate maintenance of the integrity of the liver and other extra-hepatic tissues involved in protein synthesis. Indeed, in accordance with Tungland and Meyer (2002) [44], then [44] Onyimonyi and Onu (2009) [45], *Carica papaya* is a real natural source rich in papain, the enzyme that facilitates the digestion of proteins by releasing amino acids necessary for proteosynthesis and growth. Furthermore, vitamin A contained in papaya seeds contributes to the inhibition of inflammation of the intestinal mucosa, promoting increased absorption of nutrients through the intestinal wall of farmed birds. Therefore, treatment with papaya seed powder likely induced a significant decrease in urea in the chickens in P2 batch compared to the T- batch (Table 5) with the highest creatinine level in T- batch. These results are analogous to those of Gotep et al. (2016) [42]. In fact, in their work they admitted that this decrease indicates that with this dose, papaya seed is not nephrotoxic. This confirms its safety for the kidneys, even after two consecutive days of treatment. Overall, anticoccidial treatment based on Solo papaya seed powder has no significant impact on the lipid profile of chickens compared to untreated T- subjects. These results are similar to those of Bashir et al. (2020) [23].

In the current work, there was no significant effect on the relative weight (RW) of the internal organs of the sacrificed birds (Tables 6 and 7). These results are in agreement with those of Bolu et al. (2009) [46] and Oloruntola (2019) [29] who respectively incorporated different contents of seeds and leaves of *Carica papaya* into broiler feed. For Oloruntola (2019) [29], this stability of relative organ weights indicates that chicken health has not been affected by the incorporation of papaya seed powder into their feed. Indeed, in accord with: the work of Ayodele et al. (2016) [47], a change in the relative weight of internal organs is an indicator of the animals' response to toxins in the diet. When treated broilers with ethanolic extracts of *Carica papaya* leaves, some authors also found no significant difference between the relative weights of the internal organs of treated and untreated subjects, with the exception of the relative weight of the heart, which was significantly decreased in subjects treated with *Carica papaya* leaf extract [32]. In contrast, Toah et al. (2021) noted a significant increase in relative liver and heart weights in subjects treated with ethanolic extracts of *Conyza aegyptiaca*, especially at high doses of extracts compared to untreated subjects [31].

5. Conclusions

Solo papaya seed powder incorporated at 5% in the feed is quite as effective as amprolium (20%) in the fight against coccidia. This intake has no reported toxicity against the liver and kidneys. *Carica papaya* seed powder is therefore an effective bio-based alternative in anticoccidial phytotherapy. Thus, it can be used by poultry farmers to improve the productivity of their poultry at a lower cost and in turn, their income.

However, it is necessary as a precaution to carry out additional studies on the organoleptic characteristics of the products obtained in order to know their rate of acceptability by consumers.

Abbreviations

ALT Alamin Aminotransferase

AST	Aspartate Aminotransferase
EPG	Eggs Per Gram
ESA	École Supérieure d'Agronomie
ESTBA	Ecole Supérieure des Techniques Biologiques et Alimentaires
FDS	Faculté Des Sciences
g	Gram
h	Hour
HDL	High Density Lipoprotein
IU	International Unit
L	Liter
LAGEPREN	Laboratoire de Génie des Procédés et des Ressources Naturelles
LAMICODA	Laboratoire de Microbiologie et de Contrôle de Qualité de Denrées Alimentaires
LaRASE	Laboratoire de Recherche sur les Agro Ressources et la Sante Environnementale
LDL	Low Density Lipoprotein
M	Means
rpm	Rotations per minute
RR	Reduction Rate
S.E.M	Standard Error of the Mean
USA	United States of America
W	Week

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Author Contributions

Komlan Mawouli Wampah: Data curation, Formal Analysis, Methodology, Software, Writing – original draft

Kosi Mawuéna Novidzro: Investigation, Project administration, Visualization, Writing – review & editing

Mamatchi Melila: Methodology, Resources, Validation, Visualization

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Conflicts of Interest

The authors declare no conflicts of interest.

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