Efficiency of *Carica papaya* L. Seeds in Anticoccidial Control for Cockerel of Lohmann Brown Strain

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**Abstract:** To combat avian coccidiosis, chemical products are widely used in poultry farming. However, chemotherapy has inconveniences due to the bioreistance of *Coccidia* and the presence of undesirable residues in consumer products. The aim of this study is to investigate the toxicity of papaya seeds as a bio-insecticide against *Coccidia* in Lohmann brown chickens in order to improve their zootechnical parameters. Experimentally, 260 cockerels were divided into five groups among which three experimental groups, marked EX1-3, were treated per month with 5% of the papaya seed powder incorporated in the cockerel feed for two, three, and four consecutive days, "respectively". The 4th group T- which received no treatment, served as a negative control group; while the 5th group T+ was treated once with Amprolium 20%, introduced in the drinking water of the cockerels, served as a positive control group. The one-way ANOVA test was applied to discriminate the mean values calculated statistically, at the 5% probability threshold. The results of laparoscopic analyses, after 15-23 weeks of age, revealed a reduction in the average number of eggs per gram with rates of 66.77; 58.15, and 43.43%, respectively in groups EX1-3. Phytochemical tests carried out on the hydroethanolic extracts (50-50%: v/v) confirmed the presence of biomolecules such as alkaloids, tannins, flavonoids, terpenoids, coumarins, reducing compounds, and proteins in the seeds. The anticoccidial activity revealed in this study would therefore be justified by the presence of the suspected biomolecules in the seeds by the phytochemical tests. Monthly treatment of two consecutive days with 5% of papaya seed powder incorporated in the feed is effective in the fight against cockerel *Coccidia*. This study shows that papaya seed is an interesting natural alternative to synthetic chemical products in the fight against coccidiosis.

**Keywords:** Poultry, Coccidiosis, Chemotherapy, Papaya Seeds, Avian Safety

### Introduction

The consumption of poultry meat and eggs in Sub-Saharan Africa is forecast to increase by up to 200% between 2010 and 2020 (Heise *et al.*, 2015). The relentless need for animal protein in connection with the exacerbated population explosion has led to an intensification of the breeding of short-cycle species; hence the growth of poultry farms around large cities, especially in Africa. Thus, each year, more than 50 billion broilers are raised, representing more than a third of the protein feed for human consumption (Quiroz-Castañeda and Dantán-González, 2015).

Unfortunately, poultry farming is confronted with several zootechnical, economic, health, and pathological constraints (Fall, 2010).

Avian coccidiosis is one of the most frequent and costly diseases for poultry farmers (Dakpogan and Salifou, 2013), causing annual losses that can exceed US$2.4 billion (Quiroz-Castañeda and Dantán-González, 2015). This parasitosis, caused by intestinal protozoa of the genus *Eimeria* (Gilbert *et al.*, 2011) called *Coccidia* is the source of the decline in the zootechnical performance of parasitized birds (Kouakou *et al.*, 2010). The parasites damage the intestines of the birds, causing clinical symptoms such as intestinal lesions, hemorrhage, and
bloody stools resulting in the death of the birds (Muthamilselvan et al., 2016). Mortality rates can be as high as 80-100% of total bird number (Buldgen et al., 1996). In Africa, the conditions for the development of Coccidial oocysts are most favourable south of the Sahara. The most commonly used conventional approach to coccidiosis prevention for chickens is the incorporation of synthetic coccidiostats into their diet during the first weeks of life (Barbour et al., 2015). However, chemoprophylaxis continues to show its shortcomings, due to the high cost of chemicals, stock-outs, and bioresistance developed by the Coccidia themselves (Bichet et al., 2003). As a result, undesirable residues are found in consumer products of avian origin (Chapman et al., 2010), thus constituting a significant public health problem. As an alternative to chemotherapy, vaccines have been developed for most live species, allowing chickens to gradually build up robust immunity (Williams, 1994). About nine species of Coccidia are distinguished in chickens (Joyner and Long, 2008). However, simultaneous infection in the environment with several species of Eimeria is very common. Unfortunately, the same vaccine is not effective against all species of Coccidia. Therefore, there is a risk of introducing into the poultry farming environment the Eimeria species present in living vaccines. Consequently, the search for new, more natural, less expensive methods able to reduce infection and strengthen the immune system of the host is necessary (Kouakou et al., 2010).

In this line of thought, medicinal plants that possess biomolecules with high antiparasitic potential (Bauri et al., 2015) offer very good prospects. Indeed, extracts of some plants such as Aloe vera, Azadirachta indica, Artemisia annua, and Pinus radiata have been shown to reduce oocyst secretion and or intestinal lesions (Yim et al., 2011). Traditional pharmacopoeia is now emerging as a record model for Coccidia control (Muthamilselvan et al., 2016; Dakpogan et al., 2018).

The papaya plant “Carica papaya” is a perennial medicinal plant that is easy to grow and adapts to various soils (Agboola et al., 2018). Many studies have shown that the plant exhibits various antimicrobial properties (Abd Elgadir et al., 2014) including anthelmintic (Soedji et al., 2017), anticoccidial (Hema et al., 2015; Dakpogan et al., 2018) and immune system enhancing (Muazu and Aliyu-Paiko, 2020). Most studies on the use of Carica papaya seeds in poultry farming have focused mainly on their anthelmintic effects. Therefore, the originality of our study lies in the use of papaya seed as an alternative to synthetic chemical insecticides against avian coccidiosis.

In this context, the current study was undertaken to evaluate the anticoccidial properties of papaya seeds in cockerels, with a view to improving zootechnical parameters and then to mention the phytoconstituents that would be responsible for the seeds’ anticoccidial activities.

Materials and Methods

Study Frameworks and Period

All the works related to the current study were carried out in Togo, from September 2018 up to August 2019, precisely on a breeding site close to Badja village, called ”Ayedole” farm. This site is characterized by a humid tropical climate with annual ambient temperatures ranging from 25°-32°C and an average humidity level of 75%. The poultry house is an open building with variable temperature and humidity.

The coproscopic analyses were carried out at the Centre d’Excellence Regional sur les Sciences Aviaires (CERSA) of the Université de Lomé.

Phytochemical tests were carried out at the "Laboratoire de Génie des Procédés et des Ressources Naturelles (LAGEPREN) of the Université de Lomé ".

Plant and Animal Materials

Papaya seeds, variety ‘solo’ (Fig. 1), were used as plant material to combat the Coccidial effects of cockerels.

The seeds, purchased from papaya sellers in Lomé-Togo, were dried under air conditioning at 20°C in the CERSA laboratory, and then crushed in a mill. The powder obtained was stored, protected from sunlight, for later use.

To ensure that the total number of birds studied was representative and in the interest of obtaining statistically significant results, the animal material consisted of two hundred and sixty (260) Lohmann brown Cockerels, which had been reared together for eight weeks. During this period, the birds were vaccinated against Newcastle disease, infectious bronchitis, Gumboro disease, and smallpox and treated with Amprolium 20%.

Fig. 1: Photos of Carica papaya L. “Solo” (A = Fruiting plant and B = Fruit and seeds contained in fruit)
Experimental Methodologies

In accordance with current CERSA regulations, all ethical rules concerning the use of animals in experiments have been respected.

Disinfection of the Breeding Site and Groups' Constitution

Before installing the 260 cockerels on the site, the building reserved for the bioassay was washed, disinfected, and subjected to a sanitary vacuum for a fortnight, and then disinfection was repeated two days before the cockerels were installed. In order to protect the welfare of the chickens, regulations limit the number of chickens per m² to 17 cockerels in the standard poultry house.

After three weeks of acclimatization, the 260 cockerels were initially weighed before being randomly divided into five groups, each containing 52 cockerels. The five groups were numbered as follows:

- T-: Group that received no anticoccidial treatment throughout the experiment, served as a negative control group
- T+: The group that received a monthly anticoccidial treatment with Amprolium 20% for four consecutive days in the drinking water, served as a positive control group
- EX1: The experimental group that received a monthly treatment of 5% papaya seeds on two consecutive days
- EX2: The experimental group that received a monthly treatment of 5% papaya seed for three consecutive days
- EX3: The experimental group that received a monthly treatment of 5% papaya seed on four consecutive days

The cockerels were grown on floor bedding at a density of 17 cockerels/m², from start-up through to the growth phase. The experiments started on 23rd September 2018 and ended on 25th February 2019, for a duration of 20 weeks.

Throughout the trials, water and feed were provided Ad libitum to the cockerels.

Coproscopic Examination

This analysis is based on the Mc master method of Eimeria egg counting. According to the experimental protocol, 2 g of feces from each replication was taken with a spatula and crushed in a mortar with a pestle in 60 mL of a saturated solution of NaCl (50%). The mixture was filtered through a sieve to remove large particles. Using a Pasteur pipette, both cells of the Mc master slide were filled with the filtrate while avoiding the formation of air bubbles. After 5 min of waiting, the Number of Eggs Per Gram (NEPG: OPG) was determined by observing the oocysts under a light microscope. The Reduction Rate (RR) of the NEPG was calculated using the following formula:

\[
RR = \frac{\text{NEPG before treatment} - \text{NEPG after treatment}}{\text{NEPG before treatment}} \times 100\%
\]

Oocyst Extraction and Coccidia Identification

Hemorrhagic areas were checked for along the digestive tract by means of autopsy. Extraction of the intestinal contents allowed us to identify the species of Coccidia that affected the subjects. For this purpose, two subjects per replication were sacrificed and their digestive tracts were isolated. For each digestive tract, the intestine was then isolated and sectioned into its four different constituent portions, namely: Duodenum, jejunum, ileum, and caecum. Each portion was incised and a smear was taken and observed under the microscope. The remaining intestinal contents were blended in tap water and filtered through a sieve to remove large particles. The filtrate was left to settle for 2 h and the supernatant was removed. The pellet was centrifuged at 3000 rpm for 7 min. The supernatant was again removed and the pellet was suspended in salt water (50%) and centrifuged at 2000 rpm. The supernatant finally recovered was diluted in tap water and the mixture was centrifuged at 3000 rpm. A few drops of the pellet containing the oocysts were mounted between the slide and the coverslip and observed under a microscope at a magnification of 60. The remaining pellet was allowed to sporulate at room temperature for three days and observed again under the microscope.

Assessment of Zootecchnical Parameters

Birds and feed were weighed with a CAMRY/EK 3250 scale to determine the following zootecchnical parameters.

Daily Feed Consumption (DFC): This was calculated using the following formula:

\[
DFC = \frac{\text{Amount of feed distributed (g)} - \text{Amount of feed remaining (g)}}{\text{Length of time} \times \text{number of subjects}}
\]

Consumption Index (CI): This was determined using the following formula:

\[
CI = \frac{\text{Amount of food consumed over time (g)}}{\text{Gain in weight during the time (g)}}
\]

Cockerel weight progression: The weekly weight measurements of the birds were used to determine the Live Weight (LW) and to calculate the Average Daily Gain (ADG) using the following formulae:
Phytochemical Screening of Papaya Seeds

Carica papaya L. seed powder was extracted with a hydroethanolic mixture (50-50%: v/v) using the soxhlet extraction method. The total extracts collected were used for qualitative and quantitative phytochemical analysis for the identification of secondary metabolites, mainly present in the seeds.

Qualitative Phytochemical Analyzes

Dragendorff, Wagner, and Mayer reagents were used for the search for alkaloids (Eke et al., 2014; Yadav et al., 2014). The foam test was used in the search for saponosides (Eke et al., 2014). Tannins were detected by using the 1% ferric chloride, 10% lead acetate, and ammoniacal copper sulphate reagent tests. Flavonoids were tested with the 1% sodium hydroxide reagent. The tripterpenes were detected with sulphuric acid and the coumarins with sodium hydroxide solution. The identification of proteins was performed by the xanthoprotein test and that of reducing compounds by the Fehling test (Eke et al., 2014; Yadav et al., 2014).

Quantitative Phytochemical Assays

Quantification of Total Phenols by Calibration with Gallic Acid

In each test tube were introduced 200 µL of the sample solution, either gallic acid or extract (1 mg/mL), and 500 µL of Folin Ciocalteu’s reagent (diluted to ½ in distilled water). After a reaction time of 5 min, 500 µL of sodium carbonate (20 g/L) was added to the previous mixture and then supplemented with 4 mL of distilled water. After homogenization, the solutions thus prepared were incubated for 30 min at laboratory temperature and in the dark. The absorbance of each solution was read at 760 nm with a UV-visible/METASH spectrophotometer (UV-5200 PC) against a blank. The calibration curve (Fig. 2) was established with a gallic acid solution (200 mg/L). The result was expressed as mg Gallic Acid Equivalent per gram (GAE/g) of dry extract.

Assay of Proanthocyanidins or Condensed Tannins

In a test tube, 0.2 mL of an Ammoniacal Iron Sulphate solution (NH₄Fe(SO₄)₂: 20 g/L), 7 mL of a mixture of BuOH/H₂O (95-5%: v/v), and 0.2 mL of an aqueous extract solution (30 mg/mL) were successively introduced. After incubation of the mixture in a water bath at a temperature of around 95°C for 40 min, a pink or red coloration was obtained. The proanthocyanidin concentration of an unknown sample was estimated by reading the Optical Density (OD) of the solution at 550 nm with a UV-visible/METASH spectrophotometer (UV-5200 PC). The Content of proanthocyanidins (Cₚₐ) in a sample, expressed as mg Catechin Equivalent (CE)/g of extract, was calculated using the relationship established by Aksamit-Stachurska et al. (2008) as follows:

\[ c_{\text{ce}} = \frac{DO}{0.280} \]

With: \( OD = 0.280 \) which is equivalent to 1% Catechin Equivalent (CE) and using catechin as the standard molecule.

Assessment of the Antioxidant Activity of Total Extracts

A volume of 1 mL of total extract (30 mg/mL) was added to 9 mL of phosphomolybdate reagent. The previous solution was heated in a water bath at 95°C for 90 min and then allowed to cool to room temperature. Optical densities were measured at 695 nm with a UV-visible/METASH spectrophotometer (UV-5200 PC). The calibration curve (Fig. 3) was established with ascorbic acid solution and the result was expressed as mg Ascorbic Acid Equivalent (EAA) per gram of dry extract.
Statistical Analysis of Data

The data collected on animal weight, feed consumption, and NEPG were statistically processed using Graph Pad Prism 5 software. The one-way ANOVA test was applied to discriminate the mean values calculated statistically, at the 5% probability threshold. The results, presented as means plus or minus Standard Errors of Means (SEM), were compared with each other by applying the TUKEY test.

Results

Zootechnical Parameters of the Cockerels

Feed Consumption

The Daily Feed Consumption (DFC) for cockerels is shown in Fig. 4. No significant differences are observed for the different groups treated. However, the values observed in groups T- and EX3 appear to be slightly higher than those of the other groups.

Cockerel Weight Progression

Live Weight

Figure 5 shows the Live Weight (LW) of the cockerels. The LW values of all of the groups presented a similar evolution between 12th and 17th weeks. However, from the 18th week onwards, group EX1 had the highest growth rate towards the end of the experiment, i.e., around week 22.

Average Daily Gain (ADG)

Figure 6 shows the ADG values of the cockerels. The value obtained by the group EX1 is significantly higher than that of the group T-. However, this value was not significantly different from those of the other groups (EX2, EX3, and T+).

Index of Consumption (IC)

The data displayed in Fig. 7 clearly indicate the IC of the cockerels. The cumulative IC value of the group T- during the trial is the highest, but between groups T- and EX3, the difference is not so significant.

Parasitic Load Evolution

Number of Eggs Per Gram (NEPG) Variation

As a result of the papaya seed treatment, groups EX1 and EX2 showed remarkably lower NEPG than that of group T-. The parasite load of group EX3, although appearing to be higher than that of group T+, remained significantly lower than that of group T- (Fig. 8).

NEPG Reduction Rate

The data presented in Table 1 shows that there was a reduction of NEPG between the cockerels before and after their treatments. The different treatments with papaya seed powder resulted in an average reduction NEPG equal to: 66.77, 58.15 and 43.43%, respectively for the groups EX1, EX2, EX3, and 51.93% for the T+. Generally, the best rate of reduction in NEPG was obtained in group EX1 after two days of treatment.
Table 1: NEPG variation before and after treatment

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Deadline and discount rate</th>
<th>EX1</th>
<th>EX2</th>
<th>EX3</th>
<th>T-</th>
<th>T+</th>
</tr>
</thead>
<tbody>
<tr>
<td>S15</td>
<td>First treatment NEPG before treatment</td>
<td>J0</td>
<td>425</td>
<td>2025</td>
<td>1500</td>
<td>21750</td>
</tr>
<tr>
<td></td>
<td>NEPG after treatment</td>
<td>J2</td>
<td>175</td>
<td>900</td>
<td>363</td>
<td>26600</td>
</tr>
<tr>
<td></td>
<td>Corresponding reduction rate (%)</td>
<td>Second treatment</td>
<td>J4</td>
<td>58.82</td>
<td>55.55</td>
<td>75.80</td>
</tr>
<tr>
<td>S19</td>
<td>Second treatment NEPG before treatment</td>
<td>J0</td>
<td>1950</td>
<td>4700</td>
<td>5450</td>
<td>14250</td>
</tr>
<tr>
<td></td>
<td>NEPG after treatment</td>
<td>J2</td>
<td>775</td>
<td>1525</td>
<td>3500</td>
<td>49375</td>
</tr>
<tr>
<td></td>
<td>Corresponding reduction rate (%)</td>
<td>Third treatment</td>
<td>J3</td>
<td>60.25</td>
<td>67.55</td>
<td>37.77</td>
</tr>
<tr>
<td>S23</td>
<td>Third treatment NEPG before treatment</td>
<td>J0</td>
<td>400</td>
<td>925</td>
<td>3475</td>
<td>2825</td>
</tr>
<tr>
<td></td>
<td>NEPG after treatment</td>
<td>J2</td>
<td>75</td>
<td>450</td>
<td>18.70</td>
<td>50.31</td>
</tr>
<tr>
<td></td>
<td>Corresponding reduction rate (%)</td>
<td></td>
<td>J3</td>
<td>81.25</td>
<td>51.35</td>
<td>-</td>
</tr>
<tr>
<td>S15-S23</td>
<td>Average reduction rates (%)</td>
<td></td>
<td>J4</td>
<td>66.77</td>
<td>58.15</td>
<td>43.43</td>
</tr>
</tbody>
</table>

Fig. 8: Variation in NEPG in treated groups according to the age of the cockerels

**Autopsy and Intestinal Smear Results**

The autopsy showed hemorrhagic areas of the intestinal mucosa throughout the digestive tract, indicative of the presence of *Coccidia* (Fig. 9).

The hemorrhagic areas were more intense in group T- compared to the treated groups. This trend was confirmed by the intestinal mucosal smear, which also showed non-spore-forming oocysts in groups T- and EX3 (Fig. 10). In addition, group T- subjects had more bloody stools than group EX3 subjects.

Extraction of intestinal contents indicated the presence of *Eimeria* species such as *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria brunetti*, and *Eimeria tenella* in the cockerel gut (Fig. 11) and the four sites of localization in the intestinal tract were: Duodenum, jejunum, ileum, and caecum (Table 2).

**Results of Phytochemical Analyses**

**Cases of Qualitative Phytochemical Analyses**

Qualitative tests reveal in the hydroethanolic extract (50-50%) of the papaya seeds used in this study the presence of the secondary metabolites presented in Table 3.

**Cases of Quantitative Phytochemical Analysis**

The contents of proanthocyanidins (or condensed tannins) and total phenols and antioxidant activity of the hydroethanolic extracts of papaya seeds are presented in Table 4.

Fig. 9: Autopsy results

Fig. 10: Bowel smear results
Fig. 11: Extracted pictures of Coccidia species

Table 2: Coccidia species identified in the digestive tracts of cockerels

<table>
<thead>
<tr>
<th>No.</th>
<th>Coccidia species</th>
<th>Intestinal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eimeria acervulina</td>
<td>Duodenum</td>
</tr>
<tr>
<td>2</td>
<td>Eimeria maxima</td>
<td>Jejunum</td>
</tr>
<tr>
<td>3</td>
<td>Eimeria necatrix</td>
<td>Jejunum</td>
</tr>
<tr>
<td>4</td>
<td>Eimeria brunetti</td>
<td>Ileon</td>
</tr>
<tr>
<td>5</td>
<td>Eimeria tenella</td>
<td>Caecum</td>
</tr>
</tbody>
</table>

Table 3: Results of qualitative phytochemical analyses

<table>
<thead>
<tr>
<th>Investigated phytochemical compounds</th>
<th>Analytical methods</th>
<th>Achieved results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing compounds</td>
<td>Fehling reagents</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner reagent</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride 1%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate 10%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ammoniacal copper sulphate</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Sodium hydroxide (1%)</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Sodium hydroxide (10%)</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes and phytosterols</td>
<td>Sulphuric acid (1 M)</td>
<td>+</td>
</tr>
<tr>
<td>Saponosides</td>
<td>Vigorous shaking of the solution</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>Nitric acid + Ammonia</td>
<td>+</td>
</tr>
</tbody>
</table>

Legend: + = positive test, while - = negative test

Table 4: Results of quantitative phytochemical analyses of hydroethanolic extracts of papaya seeds

<table>
<thead>
<tr>
<th>Kinds of quantitative analysis</th>
<th>Measured values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothocyanidin content (mg CE/g)</td>
<td>12.05</td>
</tr>
<tr>
<td>Total phenol content (µg GAE/g)</td>
<td>45150.01</td>
</tr>
<tr>
<td>Antioxidant capacity (mg AAE/g)</td>
<td>42.33</td>
</tr>
</tbody>
</table>

Discussion

The feed consumption of cockerels of groups EX3 and T- was higher than that of the other groups. It could be explained by the higher parasite load of oocysts, because according to Dossou (2008), the feed consumption of an animal depends on several factors, including its LW.

It was noted that Coccidia are spoliating gastrointestinal parasites like helminths (Brugère-Picoux and Silim, 1992). Therefore, the low weight gain recorded in group T- (Fig. 6) would be attributable to the impacts caused by these parasites.

In the case of coccidiosis, the effect of parasitism may be to maintain or even exacerbate the hunger, in order to compensate for the deficits in nutritional intake caused by the intestinal lesions. The low weight gain observed in groups T- and EX3 could be explained by the high parasite load, as parasites are qualitative spoliators that attack their host’s metabolism and divert essential nutrients such as amino acids, vitamins, and minerals to themselves (Maminaina, 2017).

According to our results, all the groups treated with Carica papaya L. seed powder showed lower CI compared to the group T-. More specifically, groups EX1, EX2, and EX3 showed better feed utilization efficiency compared to group T-. Similar results were also found by Essomba (2003) who, in the course of his investigation, noted that the IC of chickens was higher in Coccidia-infested subjects than in non-Coccidia-infested subjects. In their approach, (Chrysostome et al., 2010) observed that during the growth phase, cockerels in the tropics have an IC value of around 5.12%.

In the current study, Carica papaya seeds induced a decrease in the IC in the cockerels of groups EX1 and EX2, thus showing better use of the feed provided, so the use of Carica papaya L. seed powder significantly reduced oocyst excretion in cockerels. Similar results were also obtained by Mpoame et al. (2003); Dakpogan et al. (2018) in broilers.

In our first treatment, the rate of reduction of NEPG is proportional to the days of treatment with the seeds. However, over time, the effectiveness of the treatment decreases with the days of treatment, thus with the dose of papaya seeds ingested. These results are in contradiction with those of Mpoame et al. (2003) who observed an increase in the rate of reduction with increasing doses of the treatment product in broiler chickens infested by Eimeria tenella. Perhaps, these authors used aqueous extracts of Carica papaya L. seed powder, possibly more concentrated in coccidiostatic active principles against Eimeria tenella.

However, in the second treatment (Mpoame et al., 2003) obtained the highest NEPG with the highest dose, which is then in accordance with our results.

The current study showed that the efficacy of seeds decreases over time with only high doses. This hypothesis could be explained either by the indirect effect of anti-nutritional factors contained in the seeds, or by an accumulation of some phytochemical constituents which, beyond a certain quantity, would present harmful effects on the cockerels.
However, the results presented in the current study are in conformity with those obtained by Mpoame and Essomba (2000) in the treatment with aqueous decoction of Carica papaya L. seeds, against gastrointestinal parasitosis in naturally local infested chickens. These authors observed a decrease in the rate of reduction of the NEPG with the dose of the treating product.

The efficacy of papaya seeds in the fight against Coccidia would vary with the galenic form of use (aqueous extract, ethanolic extract, decoction, or powder); so according to Eke et al. (2014), the antibacterial efficacy of Carica papaya seeds depends on the extraction solvent.

According to the points of view (Chapman, 2014), the immediate action of Coccidia in the intestine is the destruction of enterocytes, accompanied by inflammation, hemorrhages, atrophies of the intestinal villi, abnormal differentiation of epithelial cells, and thickening of the intestine. This leads to a slowing of intestinal transit, increased permeability, and a reduction in the rate of absorption of nutrients. The diversion of nutrients by coccidia therefore causes a deficit in nutritional intake for poultry (N'dri, 2009).

The results presented in this study show that by treating the subjects with Carica papaya seed powder, the average weight gain as well as the efficiency of dietary use is inversely proportional to the powder dose. These results are consistent with the work done by Bolu et al. (2009); Soedji et al. (2017). These authors found that poor feed utilization is observed when the dose of seeds is increased due to the accumulation of anti-nutritional factors contained in the seeds. Indeed, some anti-nutritional compounds in plants interact with the nutrients of birds. The disadvantage is that the ingestion of high levels of anti-nutrients leads to a decrease in the bioavailability of minerals (Reddy et al., 1982), with negative consequences for animal health and productivity. For example, in high doses, tannins can damage intestinal cells, interfere with mineral absorption, and even generate a toxic effect (Butler, 1989). Therefore, a high tannin content in bird feed decreases microbial enzyme activity and cellulose digestion (Aletor, 1999). Flavonoids in turn, at high doses, reduce the availability and digestibility of protein and other nutrients such as fiber and starch (Jeroch et al., 1999). Thus, in the EX3 batch with the highest feed conversion ratio, the accumulation of anti-nutritional factors at an intense proportion could be questioned.

Phytochemical screening of Carica papaya (Linne) seeds revealed the presence of phytochemical compounds such as Alkaloids, tannins, flavonoids, terpenoids, coumarins, reducing compounds, and proteins (Table 3). These results are comparable to those of (Dada et al., 2016; Loe et al., 2017) who also showed that Carica papaya L. seeds contain alkaloids, tannins, flavonoids, steroids, polyphenols, but also saponosides by phytochemical analysis. However, in this study, saponosides were not found in the hydroethanolic extract of the seeds. However, saponosides absence in hydroethanolic extracts of the current work are in agreement with the work done by Singh et al. (2018) on ethanolic and aqueous extracts of Carica papaya leaves and roots. The presence or absence of saponosides in Carica papaya seed extracts could be either related to the nature of the varieties studied or to the polarity of the extraction solvent. Indeed, the work carried out by Eke et al. (2014) thus reported the presence of saponosides in the ethanolic extract while they were absent in the chloroform and benzene extracts of the fruit and seeds.

The anticoccidial activity of Carica papaya L. is linked to the phytochemical constituents synthesized in the seeds of this plant. Indeed, among the various secondary metabolites whose existence has been revealed in our extract, phenolic compounds (N'dri, 2009), alkaloids (Edgar and Flanagan, 1979), condensed tannins (Molan et al., 2009), and flavonoids (Muthamilselvan et al., 2016) have been identified as biomolecules endowed with anticoccidial properties. Therefore, the anticoccidial effect of Carica papaya L. seeds could then be attributed to the chemical compounds they contain, so according to Kaleem et al. (2014), tannins isolated from Emblica officinalis induced humoral immune response in chickens against Coccidia infection. Conferring some previous work, the anticoccidial action of Carica papaya L. seeds can also be explained by the proteolytic action of the papain contained in the seeds (Al-Fifi, 2007; Nghonjuyi et al., 2015).

Conclusion

In fine, the results presented in this study show that the incorporation of Carica papaya L. seed into the feed presented toxic effects against avian coccidiosis in cockerels. Consequently, there is a reduction in the Consumption Index (CI) and an increase in the Average Daily Gain (ADG) of chickens, thanks to the significant reduction in Coccidian load; hence the good performance of the zootechnical parameters of cockerels. The presence of some biomolecules revealed in the seed, such as alkaloids and phenolic compounds, would be the basis of this anticoccidial activity. Overall, the seed treatment at a dose of 5% for two days achieved the best results.

However, to allow chicken breeders to make more reliable use of the results, it would be appropriate to isolate the active ingredients contained in the seeds in order to test their effectiveness on each species of Coccidia; and then determine the mechanism of action of the active principles and finally evaluate the toxicity of the seeds for chickens.
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Author’s Contributions

Komlan Mawouli Wampah: Carried out the various experiments and acquisition of data, interpreted the results and participated in article writing.

Kosi Mawuena Novidzro: Coordinated the experiments and interpretations of data and participated in article written critical revision of the article and final approval of the article.

Namponi Dianam: Participated in the experiment’s realization and data interpretation.

Abalo Essosimna Kulo: Designed the research plan and organized the study.

Ethics

All procedures adopted in the current study were approved by the scientific ethics committee of the Centre d’Excellence Régional sur les Sciences Aviaires (CERSA) of the université de Lomé authorized the defense of the master’s thesis under the number No. 065, publicly supported on 13/09/2019.

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72


