World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Impact of anticoccidial treatment with Solo papaya seed powder on haematological parameters in Sasso broilers

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World Journal of Advanced Research and Reviews, 2024, 24(02), 409–419

Publication history: Received on 16 September 2024; revised on 28 October 2024; accepted on 31 October 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.24.2.3307

Abstract

Coccidiosis is a highly contagious and damaging poultry disease. Despite its effectiveness against coccidiosis, chemotherapy has not been able to overcome certain difficulties, in particular the phenomenon of bioresistance acquired by the parasites, the rising price of anticoccidials, and the contamination of poultry products by toxic residues that are dangerous for consumers. As an alternative solution, *Carica papaya* is emerging as an interesting and less costly natural substance for eradicating coccidiosis.

Our study aimed to evaluate the anticoccidial efficacy of the seed of Solo papaya and its effects on haematological parameters in Sasso broilers.

Six-hundred-day-old chicks of Sasso breed, broiler type, were divided into four batches with five replicates per batch: untreated batch (T-), batch treated with amprolium 20% (T+), and two batches treated with papaya seed powder (5%), incorporated into the feed for one and two consecutive days per month, noted P1 and P2 respectively. Determination of number of eggs per gram (EPG) was based on Mc Master technique. Haematological parameters were determined from blood collected in tubes containing EDTA using Mindray BC-3000/China automated haematological analyser.

Papaya seed powder Treatment showed anticoccidial efficacy, expressed by a significant drop in EPG in the treated batches. In addition, haematological parameters improved, notably an increase in haematocrit and a decrease in the rate of various leucocytes.

Anticoccidial treatment with papaya seed powder (5%) resulted in an appreciable reduction in parasite load and an improvement in the haematological profile.

The result application could serve as a sustainable alternative to synthetic chemical anticoccidial drugs.

Keywords: Sasso Chickens; Papaya Seed; Anticoccidial Properties; Haematological Parameters.

1. Introduction

Coccidiosis is a disease caused by an apicomplexan protozoan belonging to the subclass Coccidiasina, family Eimeriidae, and genus Eimeria [1]. Contamination occurs through ingestion of sporulated oocysts, which can lead to clinical or subclinical coccidiosis. Coccidia develop in specific locations in the digestive tract of birds [2]. In fact, around nine

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species of coccidia have been identified in chickens. Among these species, *Eimeria tenella* is found in the cecum, while others colonize the intestinal tract. Coccidia cause tissue lesions, diarrhoeal haemorrhage, poor feed conversion, reduced production, increased susceptibility to other pathogens and, in severe cases, mortality in poultry [3, 4]. Simultaneous infection in the field with two or more species of Eimeria usually occurs, aggravating the disease. This sickness can decimate an entire breeder's farm. Thus, according to Toah et *al*. (2021) [5], the high mortality rates caused by coccidiosis epidemics represent the greatest constraint to poultry development, particularly broilers and ordinary village chickens, which provide quality protein and income to small-scale farmers throughout the world. The economic impact of coccidiosis is significant for the poultry industry and particularly for poultry farmers with limited resources.

Prevention and treatment of this disease are commonly carried out using synthetic anticoccidial chemicals [6]. Nevertheless, chemoprevention continues to show its limitations due to the high cost of anticoccidial chemicals and also stock-outs. Furthermore, the indiscriminate and prolonged use of numerous synthetic anticoccidial drugs has led not only to the emergence of drug resistance in Eimeria species, but also to the presence of toxic drug residues in poultry meat and egg products. This signifies a real public health and food safety problem for consumers [7]. As alternative measures, vaccines have been developed, most of which are live. These vaccines gradually build up solid immunity in poultry. However, a single vaccine is not effective against all species of coccidia. In addition, prolonged storage of vaccines reduces their efficacy [8]. In addition, vaccine production is expensive and the antigenicity of coccidian strains varies according to geographical location [9].

The shortcomings of chemoprevention and coccidian vaccines, as well as the need to provide consumers with healthy egg products, have led to the search for new, more natural, less expensive methods capable of reducing infection and boosting host defences. In this respect, plant-based products appear to be a credible alternative in the fight against coccidia, with a lower risk of developing resistance [6, 10]. In poor countries, plant-based remedies are often used in poultry diets because of their natural stimulating effects on the birds' growth and immune systems [3]. Among these plants or parts of plants is the papaya seed. *Carica papaya* (Linn.) species commonly known as the pawpaw, is a plant native to America[11] belonging to the *Caricaceae* family but is widespread throughout tropical Africa [12. It is a perennial medicinal plant that is easy to grow and adapts to a variety of soils [13]. Its use as an anticoccidial alternative is likely to have no adverse effect on the environment [14]. *Carica papaya* seeds are traditionally used by poultry farmers for pest control. This plant has been shown to have several properties [15], including anthelmintic (Nidéou et *al*., 2017) and anticoccidial [16]. These various properties are thought to be attributable to the phytochemical compounds inherent in this species [17]. Phytochemical test carried out on *Carica papaya* leaves revealed the presence of phytoconstituents such as tannins, flavonoids, saponosides, alkaloids, anthraquinones, cardiac glycosides, steroids, reducing sugars and phenolic compounds [18].

Carica papaya seeds have most often been used as a protein supplement. It was noted that incorporating *Carica papaya* seed powder (5%) into broiler chicken feed had a positive effect on zootechnical performance and improved haematological parameters [19]. It has also been found that supplementing the feed with papaya seed powder reduced lymphocyte levels in chickens [20]. However, despite the positive effects of plant-derived substances, some of them can be toxic to animals and humans [21]. Thus, studying the effects of medicinal plants on haematological and biochemical parameters becomes necessary to establish their acceptability [16].

Most previous studies on the anticoccidial properties of *Carica papaya* have generally focused on the action of leaf, fruit or seed extracts on chickens artificially infected with a single species of coccidia. They have also looked at the effects of the extracts on zootechnical parameters. However, according to [22], the different solvents do not extract the same types of natural compounds with the same concentration. As a result, the extracted compounds do not show the same biological activities with the same potential. Extracts therefore do not generally contain all the plant's active molecules. However, the active compounds present in the plant sometimes act synergistically. In addition, plant extracts are difficult to access and use for poultry farmers whose flocks are contaminated by several species of Eimeria simultaneously. Not only multiple infections aggravate coccidiosis, but papaya seed, which is not widely used, is still easily accessible as a means of eliminating coccidiosis. It is against this backdrop that the present study is being carried out, with the aim of evaluating, under rearing conditions and in a tropical zone, the anticoccidial efficacy of papaya seed in naturally infected Sasso broilers and the effects on haematological parameters, with a view to providing guidance to actors in the poultry sector.

2. Material and methods

2.1. Setting and duration of the study

The study was conducted at High School of Agronomy (ESA) of the University of Lomé, Togo. Haematological parameters were measured at the Laboratory of Microbiology and Quality Control of Foodstuffs (LAMICODA) in the High School of Biological and Food Technology (ESTBA) of the University of Lomé, Togo.

The animals were reared on a site close to the town of Tsévié, known as "FERME ALBERT", located approximately 30 km from north of Lomé, for around 12 weeks. The poultry house is an open building with variable temperature and humidity.

2.2. Biological material

2.2.1. Animal material

A total of 600-day-old Sasso and meat-type chicks were used in the current study. The chicks were purchased from the MDL Maison Diop farm, located in Agbavi, an area which is about 25 km from south-east of Lomé.

2.2.2. Plant material

Seeds of Solo papaya variety were collected from papaya sellers in Lomé, then dried in the Animal Physiology Laboratory of the University of Lomé under cover of sunlight, and ground to powder before being used as plant material in this work.

2.2.3. Breeding material

The start-up phase of chick rearing was carried out in a hatchery. The chicks were then reared in two rectangular buildings, each had 10.5 m long and 4 m wide, partitioned by fences. Separately building was washed, disinfected and subjected to a sanitary vacuum for a fortnight. Two days before the chicks were installed, the floor was completely covered with wood shavings and disinfection resumed.

2.3. Laboratory materials and equipment

This included test tubes, volumetric flasks, beakers, spatulas, porcelain mortar, chemical reagents, Pasteur pipettes, electronic scales and a Mindray BC-3000/China automated haematological analyser.

2.4. Computer hardware

Excel and Graph Pad Prime 5 were used to collect and process the data collected in this work.

2.5. Methods

2.5.1. Batching of chicks and scheduling of anticoccidial treatments

Population of 600 chicks were reared together over 4 weeks required to induce natural coccidian infections. Initially, all chicks were vaccinated against New Castle disease, Gumboro disease and infectious bronchitis. During this step, water and feed were served *ad libitum* to the birds.

In the fourth week, all 600 chicks were divided into 4 batches, each containing 5 replicates of 30 chicks. These were: the negative control batch (T-) which received no anticoccidial treatment throughout the study; the positive control batch (T+) which was treated with amprolium (20%) according to the manufacturer's instructions; and finally, batches P1 and P2 which were treated monthly with Solo papaya seed powder (5%) incorporated into the feed, for one day, and two consecutive days, respectively. Anticoccidial treatments of batches T+, P1 and P2 were carried out at 4, 6, 9, and 12 weeks of age. Water and food were served twice a day after the chicks were divided into batches.

2.5.2. Evaluation of parameters

Evaluation of anticoccidial efficacy of papaya seed powder

Anticoccidial efficacy of Solo papaya seed powder was evaluated using the coproscopy technique, based on flotation principle. Mc Master method was implemented for this technique application. Fresh faeces were collected from each batch before and after each treatment to determine the number of EPG. 5 g of faeces were triturated and dissolved in 70 mL of NaCl aqueous solution (50%) and the mixture obtained was then filtered. The filtrate collected was then used to fill the cells of Mc Master slide using a Pasteur pipette. Microscopic observation was used to determine the EPG.

2.5.3. Determination of haematological parameters

Three subjects per replication were isolated after each treatment, then 3 mL of blood was taken from each bird at the level of the wing vein in a haemolysis tube containing the anticoagulant EDTA (Ethylene diamine tetra-acetic Acid) to be used for subsequent haematological analyses.

The haematological parameters measured included: red blood cells (RBC), hematocrit (HCT), mean cell volume (MCV), hemoglobin level (HB), mean cell hemoglobin concentration (MCHC), platelet count (PLT), as well as white blood cells (WBC) with proportions of lymphocytes (LYM), monocytes (MO), eosinophils (EO), basophils (BA) and neutrophils (NEU). A fully automated Mindray BC-3000 haematological analyser from Mindray Buiding, Keji 12th Road South, High-tech Industrial Park, Nanshan, Shenzhen 518057, P.R. China was used to determine these haematological parameters.

2.6. Statistical analysis

The data obtained in the current study were processed by 'Graph Pad Prism 5' software and subjected to ANOVA (One way of variance), followed by TUKEY post-test for comparison of the different batches. The probability $P < 0.05$ was considered as the significance threshold. Excel was used to construct the curves. The results were presented as means plus or minus the standard error on the means (M± S.E.M).

3. Results

3.1. Effectiveness of treatments on coccidia

The effects of the various anticoccidial treatments on the evolution of oocyst secretion as a function of rearing duration are illustrated in Figure 1.

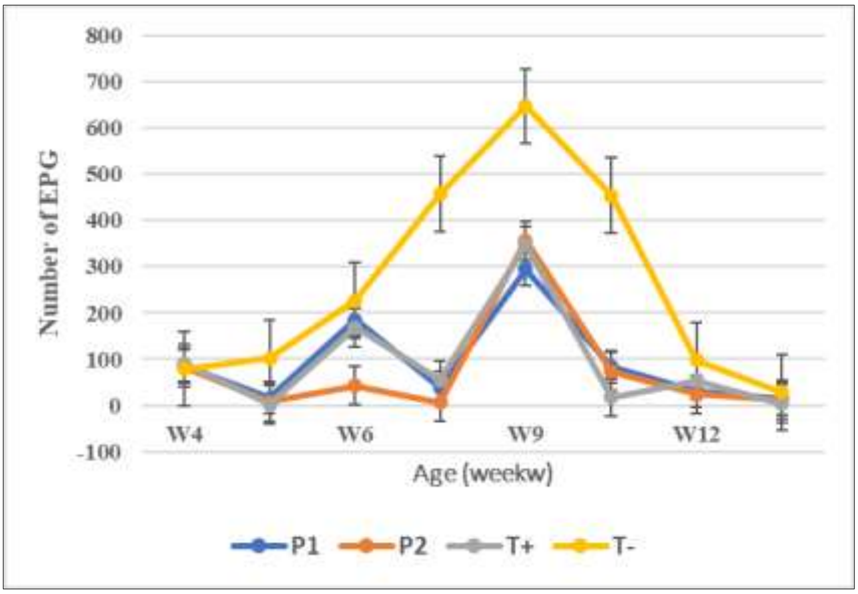

Figure 1 Variation in the number of EPG in different batches as a function of age

Considering the data in Figure 1, it can be seen that at 4 weeks of age (W4), there was no significant difference between the number of EPG in the different batches (treated or untreated). However, following the anticoccidial treatments carried out at 6 weeks (W6) of age, there was a significant reduction in the number of EPG in the treated batches compared with batch T-, and this reduction was greater in batch P2. Up to week 9 (W9), the reductions in the number of EPG in batches P1 and P2, which were almost comparable to those in batch T+, were significantly greater than in batch T-. After week W9, there was a reduction in the number of EPG in all batches.

3.2. Effects of anticoccidial treatment on haematological parameters

At 6 weeks of age, the anticoccidial treatments did not induce any significant changes ($p > 0.05$) in the red blood cell count and the various haematological indices evaluated (Table 1).

Table 1 Red blood cell content and differentials measured at 6 weeks of age in birds

	Treatments				
Parameters	P1	P2	T+	T-	P value
RBC (10^6µL)	2.55 ± 0.08^a	2.64 ± 0.10^a	2.57 ± 0.06^a	2.55 ± 0.02^a	0.8257
HB (g/dL)	6.75 ± 0.32^a	6.82 ± 0.21^a	6.80 ± 0.24^a	6.75 ± 0.15^a	0.9943
HCT (%)	31.50 ± 0.40^a	33.10 ± 0.85^a	33.40 ± 0.98^a	31.40 ± 0.46^a	0.3320
MCV/VGM (FL)	123.5 ± 1.54^a	123.70 ± 1.92^a	124.50 ± 1.98^a	121.30 ± 1.78^a	0.6341
MCH/TCMH (pg)	26.45 ± 0.43^a	26.65 ± 0.82^a	25.65 ± 0.89^a	25.45 ± 0.97^a	0.6687
MCHC/CCMH (g/dL)	21.60 ± 0.12^a	20.65 ± 0.29^a	20.85 ± 0.32^a	20.53 ± 0.36^a	0.0821
PLT (10^3/µL)	6.00 ± 0.40^a	5.55 ± 0.29^a	5.75 ± 0.48^a	6.25 ± 0.48^a	0.6343

RBC: Red blood cells, HB: Haemoglobin, HCT: Haematocrit, MCV: Mean cell volume, MCH/TCMH: mean corpuscular haemoglobin content, MCHC: Mean haemoglobin cell concentration, PLT: Platelet count. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

In contrast, at 6 weeks of age (Table 2), anticoccidial treatments induced a significant decrease ($p < 0.05$) in the overall leucocyte count and the numbers of lymphocytes, eosinophils, monocytes and neutrophils compared to T-. However, there was no significant change ($p > 0.05$) in basophil counts between treated and untreated batches.

Table 2 White blood cell levels and differentials measured at 6 weeks of age in birds

	Treatments				
Parameters	P1	P2	T+	T-	P value
WBC (10^3 µL)	34.14 ± 2.38^b	30.63 ± 1.65^b	33.41 ± 2.28^b	46.40 ± 3.91^a	0.0067
LYM (10^3µL)	13.27 ± 2.02^b	14.23 ± 1.57^b	13.54 ± 2.41^b	27.40 ± 3.36^a	0.0037
MO (10^3 µL)	1.81 ± 0.11^b	2.06 ± 0.06^b	1.76 ± 0.23^b	3.29 ± 0.51^a	0.0083
NEU (10^3 µL)	17.13 ± 1.04^a	9.30 ± 1.14^b	12.00 ± 2.12^b	18.96± 1.84^a	0.0038
BA (10^3 µL)	1.19 ± 0.10^a	1.72 ± 0.16^a	1.74 ± 0.06^a	1.75 ± 0.26^a	0.0866
EO (10^3 µL)	0.015 ± 0.015^b	0.010 ± 0.010^b	0.010 ± 0.010^b	0.065 ± 0.045^a	0.0493

WBC: White blood cells, LYM: lymphocytes, MO: monocytes, NEU: neutrophils, BA: basophils, EO: eosinophils. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

At 9 weeks of age, following the different treatments, there was a significant ($p < 0.05$) increase in red blood cell content, hemoglobin level and haematocrit in the treated batches compared to batch T-, with no significant difference between all the treated batches. However, treatment with Solo papaya seed powder induced no significant difference ($p > 0.05$) in other haematological indices compared to untreated batches (Table 3).

Table 3 Red blood cell levels and differentials measured at 9 weeks of age in birds

	Treatments				
Parameters	P1	P2	T+	T-	P value
RBC (10^6µL)	2.51 ± 0.03^a	2.63 ± 0.05^a	2.62 ± 0.02^a	2.43 ± 0.02^b	0.0082
HB (g/dL)	6.80 ± 0.08^a	7.10 ± 0.14^a	7.20 ± 0.14^a	6.60 ± 0.04^b	0.0105
HCT (%)	31.40 ± 0.40^a	33.10 ± 0.68^a	33.83 ± 0.70^a	29.47 ± 0.30^b	0.0017

MCV/VGM (FL)	127.20 ± 1.22[ab]	126.20 ± 1.64[ab]	129.70 ± 1.94[a]	121.80 ± 1.64[b]	0.0306
MCH/TCMH (pg)	27.60 ± 0.66[a]	27.05 ± 0.45[a]	27.50 ± 1.18[a]	26.68 ± 0.57[a]	0.8182
MCHC/CCMH (g/dL)	21.55 ± 0.49[a]	21.70 ± 0.11[a]	21.93 ± 0.51[a]	21.15 ± 0.38[a]	0.5975
PLT (10^3/µL)	2.25 ± 0.48[a]	3.50 ± 0.50[a]	2.50 ± 0.41[a]	3.50 ± 0.48[a]	0.3096

RBC: Red blood cells, HB: Haemoglobin, HCT: Haematocrit, MCV: Mean cell volume, MCH/TCMH: mean corpuscular haemoglobin content, MCHC: Mean haemoglobin cell concentration, PLT: Platelet count. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

At 9 weeks of age, following the anticoccidial treatments carried out, there was a significant decrease ($p < 0.05$) in the overall leucocyte counted and the numbers of lymphocytes, and neutrophils in P1 compared to the other batches. Monocyte counts were significantly higher ($p < 0.05$) in P2 compared with the other batches (Table 4).

Table 4 White blood cell levels and differentials measured at 9 weeks of age in birds

	Treatments				
Parameters	P1	P2	T+	T-	P value
WBC (10^3 µL)	9.50 ± 0.40[b]	16.14 ± 0.86[a]	14.03 ± 0.80[a]	16.15 ± 1.54[a]	0.0014
LYM (10^3 µL)	4.96 ± 0.33[b]	6.26 ± 0.27[a]	6.40 ± 0.13[a]	6.65 ± 0.31[a]	0.0042
MO (10^3 µL)	0.43 ± 0.03[b]	0.67 ± 0.07[a]	0.54 ± 0.04[ab]	0.47 ± 0.02[b]	0.0152
NEU (10^3 µL)	2.91 ± 0.25[b]	6.92 ± 0.79[a]	5.27 ± 0.26[a]	7.39 ± 0.69[a]	0.0004
BA (10^3 µL)	1.35 ± 0.14[ab]	1.71 ± 0.19[a]	1.81 ± 0.09[a]	0.94 ± 0.14[b]	0.0047
EO (10^3 µL)	0.010 ± 0.006[a]	0.010 ± 0.006[a]	0.010 ± 0.006[a]	0.005 ± 0.005[a]	0.8944

WBC: White blood cells, LYM: lymphocytes, MO: monocytes, NEU: neutrophils, BA: basophils, EO: eosinophils. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

Table 5 Red blood cell levels and differentials measured at 12 weeks of age in birds

	Treatments				
Parameters	P1	P2	T+	T-	P value
RBC (106µL)	2.42 ± 0.10[a]	2.47 ± 0.12[a]	2.37 ± 0.07[a]	2.25 ± 0.05[a]	0.4098
HB (g/dL)	6.92 ± 0.05[a]	6.53 ± 0.27[a]	6.82 ± 0.19[a]	6.60 ± 0.23[a]	0.5005
HCT (%)	31.65 ± 1.13[a]	33.28 ± 1.27[a]	31.18 ± 1.30[a]	29.95 ± 0.86[a]	0.0667
MCV/VGM (FL)	131.2 ± 1.91[a]	133.1 ± 2.80[a]	132.4 ± 1.80[a]	130.6 ± 1.25[a]	0.8123
MCH/TCMH (pg)	27.55 ± 1.06[a]	28.20 ± 0.43[a]	29.30 ± 0.41[a]	28.63 ± 0.23[a]	0.2899
MCHC/CCMH (g/dL)	22.22 ± 0.69[a]	21.68 ± 0.42[a]	22.13 ± 0.30[a]	21.95 ± 0.35[a]	0.8602
PLT (10^3/µL)	2.25 ± 0.25[a]	3.50 ± 0.65[a]	2.50 ± 0.29[a]	3.50 ± 0.87[a]	0.3143

RBC: Red blood cells, HB: Haemoglobin, HCT: Haematocrit, MCV: Mean cell volume, MCH/TCMH: mean corpuscular haemoglobin content, MCHC: Mean haemoglobin cell concentration, PLT: Platelet count. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

At 12 weeks of age, the different anticoccidial treatments carried out did not induce any significant change in haematological indices ($p > 0.05$) between the different treated and untreated batches (Table 5).

At 12 weeks of age, there was no significant difference ($p > 0.05$) in leucocyte count between treated and untreated batches, with the exception of monocyte count which remained significantly higher ($p < 0.05$) in P2 compared to the other batches (Table 6).

Table 6 White blood cell levels and differentials measured at 12 weeks of age in birds

Treatments

Parameters	P1	P2	T+	T-	P value
WBC (10^3 µL)	11.12 ± 0.58[a]	12.83 ± 1.30[a]	15.63 ± 1.92[a]	12.25 ± 1.67[a]	0.2141
LYM (10^3 µL)	5.32 ± 0.45[a]	6.98 ± 79[a]	6.93 ± 0.27[a]	5.40 ± 0.53[a]	0.0794
MO (10^3 µL)	0.51 ± 0.05[b]	0.98 ± 0.10[a]	0.56 ± 0.13[b]	0.51 ± 0.05[b]	0.0069
NEU (10^3 µL)	4.36 ± 0.53[a]	2.21 ± 0.24[a]	6.48 ± 1.94[a]	4.94 ± 1.06[a]	0.1185
BA (10^3 µL)	1.54 ± 0.62[a]	1.91 ± 0.31[a]	1.59 ± 0.21[a]	1.18 ± 0.23[a]	0.6220
EO (10^3 µL)	0.006 ± 0.005[a]	0.010 ± 0.004[a]	0.00 ± 0.00[a]	0.003 ± 0.12[a]	0.3123

WBC: White blood cells, LYM: lymphocytes, MO: monocytes, NEU: neutrophils, BA: basophils, EO: eosinophils. Values on the same line assigned the same letter (a, b), are not significantly different (P> 0.05).

4. Discussion

At 6 weeks of age, a dose-dependent reduction in the number of oocysts was observed with Solo papaya seed powder (Figure 1). Similar results were obtained by [23], [16], [24], and [5], when chickens infected with Eimeria were treated with different extracts of *Azadirachta indica* and *Khaya senegalensis*, *Carica papaya* and *Talinium triangular*, *Carica papaya* and *Vernonia amygdalina* and *Conyza aegyptiaca*, respectively. According to the work realized by [23], these results can be explained by the presence of bioactive compounds in the plants. *Carica papaya* seeds contain some phytoconstituents such as: alkaloids, tannins, steroids, flavonoids, polyphenols, and saponosides [11, 25] which have proven anticoccidial properties [26]. This is because phenolic compounds, and condensed tannins in particular, infiltrate the oocyst wall and inhibit the endogenous enzymes responsible for the sporulation process with the appearance of abnormal sporocysts [27]. In addition, condensed tannins produce reactive oxygen species (ROS) via the degradation of the peroxide complex [28]. Proanthocyanidins also reduce the oxidative stress generated by coccidia [29]. Saponosides, for their part, bind to the sterol in the membrane of oocysts and cause their lysis [30]. Phenolic compounds such as flavonoids present in plant extracts are well recognised as having antiradical activities [31]. Consequently, the presence of these phenolic compounds should also confer on *Carica papaya* (Linn.) seeds an impressive antioxidant power to enhance the physiological state of poultry. The presence of these different biomolecular constituents with various antimicrobial properties in the seeds would be a favourable asset for effectively combating coccidia without causing the phenomenon of resistance. *Carica papaya* seeds also contain papain and vitamin A, which are capable of inducing proteolytic destruction of Eimeria and suppression of inflammation of the intestinal mucosa respectively [32]. Indeed, according to [24] the reduction in the number of oocysts induced by the treatment of raw juice from *Carica papaya* leaves could be attributed to the direct digestion of Eimeria protozoa by a synergistic action of chymotrypsin from the pancreas and papain from *Carica papaya* leaves. In addition, [14] obtained *in vitro* inhibition of *Eimeria tenella* oocyst sporulation from isolated or combined extracts of *Carica papaya* leaves and *Allium sativum* pods, confirming the direct action of *Carica papaya* on coccidia oocysts. From another point of view, the work of [24] indicated that the vitamin A present in *Carica papaya* organs, thanks to its anti-inflammatory properties, strengthens the integrity of the intestinal mucosa to the detriment of coccidian reproduction. Increased levels of zinc and vitamins A and E would therefore boost the immune response of chickens to coccidiosis [33].

The decrease in the number of EPG in batch T-, beyond the 9th week, seems to corroborate [34] observation that severe infections would cause a resistance phenomenon to Eimeria, leading to natural self-deworming. Furthermore, [35] mentioned that natural immunity is acquired by the animal after several cycles of coccidian infection.

The parasitic load is not without consequences for the haematological profile of the subjects. Indeed, according to this study, the anticoccidial treatment based on papaya seed powder, at the 6th week of age, did not induce any significant changes in the haematological profile (red blood cells, hemoglobin levels, hematocrit, etc.) between treated and untreated subjects (Table 1).

Similarly, at the 9th week of age, there is no significant difference in the haematological profile between treated subjects (Table 3). On the other hand, a significant decrease ($p < 0.05$) in red blood cell count, hemoglobin levels, and hematocrit was noted in the T- subjects compared to the treated subjects. These results seem to reinforce the findings of [36] and [5]. Indeed, the latter observed a significant dose-dependent increase in red blood cell count and hematocrit in treated subjects compared to infected subjects who had not undergone any anticoccidial treatment, as well as a significant increase in hemoglobin levels at high doses. According to these authors, these results could be explained by the erythropoietic capacity of the plant extracts administered to the treated subjects. By incorporating Solo papaya seed powder (5%) into the diet of broiler chickens, [19] also observed an increase in red blood cell count and hemoglobin levels in these chickens.

Aside from the erythropoietic property of papaya seeds, the results of the actual work could also be explained by the high parasitic load in the negative control subjects (T-), as coccidia in the intestinal mucosa create severe hemorrhage that can lead to anemia [23]. Moreover, according to [24], *Carica papaya* contains vitamin A, which has anti-inflammatory properties that protect the intestinal mucosal cells.

Additionally, [33] noted that elevated levels of zinc, vitamin A, and vitamin E would enhance the immune response of chickens to coccidiosis. Furthermore, papaya seeds contain other antioxidants such as vitamin C, vitamin E, and β-carotene, which can strengthen the immune response to coccidiosis. This may explain the higher red blood cell count and the elevated levels of hemoglobin and hematocrit observed in treated subjects compared to untreated ones.

At the 12^{th} week of age, there is also no significant difference in the haematological profile between treated and untreated subjects, thanks to the natural resistance developed by the untreated subjects (Table 5). However, [37] found a decrease in red blood cell count and hematocrit in treated groups compared to the untreated group using extracts from *Salvadora persica*, *Zingiber officinale*, and *Curcuma longa*. An increase in white blood cell count indicates health problems. Consequently, systemic inflammation and subclinical diseases can manifest as an increase in white blood cell counts [38]. There was a significant decrease at the 6^{th} week in the treated groups and at the 9^{th} week in the P1 group. By incorporating papaya seed powder at different doses, both [19] and [20] observed no effect on the total white blood cell count and differential counts. Thus, the variations observed between the treated subjects and those untreated can be attributed to differences in parasitic load.

At six weeks of age, the significant elevation in total white blood cell count, as well as the counts of lymphocytes, eosinophils, monocytes, and neutrophils in the T- group (Table 2), can be attributed to the high parasitic load. This results in a significant increase in eosinophil counts in T- subjects, which are immune cells endowed with antiparasitic properties. In contrast, anticoccidial treatments induced a decrease in white blood cell counts, particularly in lymphocytes, at six weeks of age in the T+, P1, and P2 groups. These results align with those reported by [23] and [5], who also noted a significant decrease in granulocyte and platelet counts. According to [23], the reduction in white blood cell count is related to decreased parasitic load, which regulates immune system activity, leading to reduced inflammation.

By the ninth week of age, the reduction in parasitic load was more pronounced in the P1 group (Figure 1). This observation explains the greater decrease in both total white blood cell counts and differential count in this group. The increase in neutrophil numbers in the other groups can be explained by the higher parasitic load causing inflammation. This inflammation triggers an influx of neutrophils, which are abundant phagocytic cells with a short lifespan, dying after eliminating the pathogen through either apoptosis or necrosis [38, 39]. This explains the absence of differences in neutrophil counts among the various groups at twelve weeks of age. The increase in monocyte counts in P2 persisted until the twelfth week, likely due to the accumulation of anti-nutritional factors that induce inflammation, leading to the production of more persistent monocytes [39].

At twelve weeks, there was no significant difference in white blood cell counts or differential counts between treated and untreated subjects (Table 6). This could be explained in untreated subjects by not only the development of natural resistance but also the decrease in the virulence of coccidia [38], as coccidia virulence is more pronounced in younger subjects. This results in a reduction in parasitic load beyond the ninth week of age. [33] indicated that subclinical infections lead to the development of strong, specific natural immunity without apparent disease. This decrease in parasitic load reduces inflammation, leading to a blood profile trending toward normal [23]. The powder from *Carica papaya* seeds shows significant effectiveness in anticoccidial treatment.

5. Conclusion

The incorporation of Solo papaya seed powder (5%) into poultry food shows beneficial effects against coccidia, as evidenced by improvements in the haematological profile of the chickens. The seeds of *Carica papaya* can thus serve as an effective biological alternative for poultry farmers in anticoccidial phytotherapy, enabling them to enhance poultry productivity at a lower cost and improve their income.

Despite the efficiency of phytochemical substances, some of them can be toxic at high doses and even at low doses when used over prolonged periods. Therefore, it is necessary to study the effects of *Carica papaya* seeds on internal organs to assess their impact on bird health. Furthermore, additional research is needed on organoleptic characteristics to determine the acceptability rate of products by consumers.

Compliance with ethical standards

Acknowledgements

The authors gratefully acknowledge the support of the Authorities of the University of Lomé, Togo".

Disclosure of Conflict of interests

All authors declare that there are no conflicts of interests regarding this paper publication.

Statement of ethical approval

All procedures used in this work were approved by the scientific ethics committee of of the University of Lomé, Togo.

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