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Phytochemical characterization, antioxidant activity and anthelmintic effect of *Carica papaya* L. (Caricaceae) seeds in Lohman Brown cockerels

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Abstract

Helminths are diseases which exist frequently with the poultry. To cure these parasitosis, breeders use very often synthetic products which are source of food poisoning for the consumers. However, past studies showed that some plants are known to their anthelmintic properties.

For sanitary biosafety, the current study aimed to gauge helminthic capacity of papaya seed and established the relationship between phytochemical composition and anthelminthic activity.

Qualitative and quantitative phytochemical analyses were carried out to identify phytoconstituents endowed with anthelmintic activity. Bioguided analyses were carried out in three months upon 260 Lohmann Brown cockerels which were divided in 5 batches: T-, T+, GP1, GP2, and GP. Cockerels of batches GP1, GP2 and GP3 received in their food papaya seed powder (5%) per month, respectively for one, two and three days. Batch T- did not receive any treatment but cockerels of batch T+ were treated by Levamisole (25%) on day per month.

Phytochemical tests revealed the presence of alkaloids, reducing compounds, flavonoids, tannins, proteins and coumarins as chemical group in the seeds. Bioguided analyses proved that the reduction rates of number of EPG in batches T+, GP1, GP2, et GP3 are respectively: 90.08; 28.00; 53.00, and 90.35, but it was negative in batch T-. Batches T+ and GP3 presented higher average weekly gains, while average cumulative weight gains were identical for batches T+ and GP3. Nevertheless, it was batch GP3 that provided the lowest consumption index.

In fact, cockerel treatment with papaya seed present almost the same anthelminthic efficacy as Levamisole.

Keywords: Cockerels; Synthetic anthelmintic; Food poisoning; Papaya seed; Sanitary biosafety

1. Introduction

In the most of African countries, the poultries diseases remain till now a serious problem of public health [1] to which researchers are constantly coming up with innovative solutions. In poultries breeding, heavy losses are usually caused by infectious diseases such as Newcastle disease, Gumboro, and Marek, with mortality rates of 80-100%, 50-60% and 15%, respectively [2]. Furthermore, other more or less serious damage is caused by parasitic diseases, including intestinal parasitosis caused by parasites that develop in the animal's digestive tract. Intestinal helminthoses is one of the most common parasitic diseases encountered in poultry farming. Helminthoses are caused by the presence and

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development in the digestive tract of poultry of worms belonging to the nematode, cestode and, more rarely, trematode classes. These worms cause great health problems which trouble seriously the poultry production in developing countries [3]. The economic losses engendered, are so considerable because of the establishment of animal's immunity, stunted growth in young birds and a drop in poultry production [4]. For Sven *et al*. (2009) [5], the helminths for example: ascaridiosis, heterakidiosis, and capillariosis, are the most frequently encountered parasites in poultry farming.

In order to reduce losses of various kinds caused by parasitic diseases in poultry, various strategies have been imagined for ages. These include good biosecurity practices, as well as chemoprevention, aimed at periodically eliminating parasites from the poultry's digestive tract. However, the chemicals used to control parasites are not only expensive, but often unavailable in developing countries [3]. Secondly, their use often leads to the emergence of new, more resistant strains of parasites. Finally, their use leaves residues in eggs and meat, making them unfit for human consumption [6]. According to EFSA, 20% to 30% of human cases of campylobacteriosis are directly attributable to chicken meat consumption [7].

To deal with this worrying situation, medicinal plants are increasingly emerging as one of reliable alternatives to bring down some problems related to the use of chemical products in poultry farming. Indeed, the high interest given to medicinal plants is actually due to the fact that they are not only effective, but also accessible, and less costly than synthetic products. Moreover, their use is often related to socio-cultural and historical practices and personal beliefs [8].

The aim of the current study was to contribute to food security through papaya seed valorization in veterinary medicine.

2. Material and methods

2.1. Breeding site of cockerels

"AYODÉLÉ" farm, with geographical coordinates of 6°22'15.65''N and 0°58'07.35'' E, located in Badja village, in Avé district, from 47 km northwest of Lomé city, served as cockerel breeding site. This site's average annual temperatures ranged from 25°C to 32°C, and humidity averaged 75%.

2.2. Animal and rearing equipment

In this work, bioguided experiments were carried out on 260 cockerels of the Lohmann Brown strain (Figure 1), supplied by the "Terre Bénie" agropastoral farm, located in Game, situated near Notsè town, in Togo. The rearing equipment consisted of pens separated into poultry batches by iron fences.

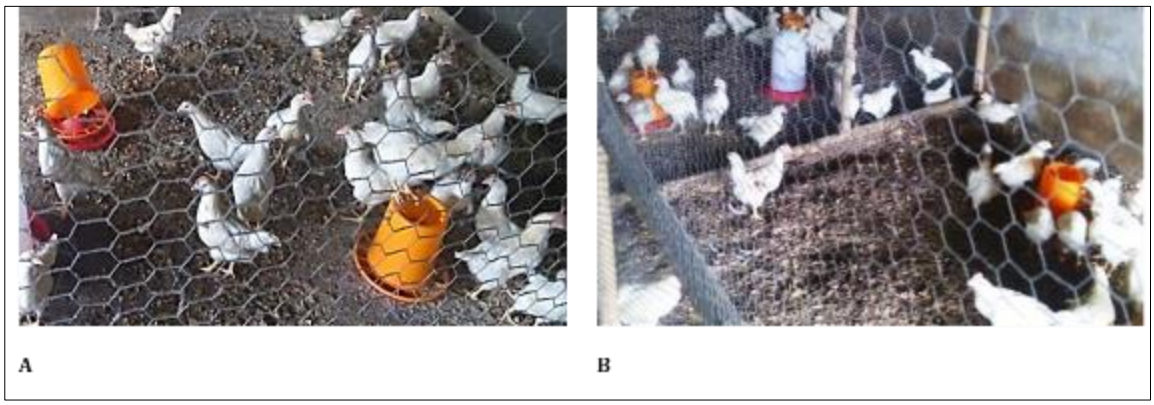


Figure 1 Cockerels distributed in the cages A and B for the experiments

2.3. Plant material

Powder obtained by mechanical grinding of *Carica papaya* L. seeds of Solo variety No. TOGO15448 (Figure 2) was used as plant material to evaluate anthelmintic properties. Fresh seeds, collected from papaya sellers in Lomé, were first dried in the laboratory under air conditioning at 20°C and protected from light before being crushed into powder.

Figure 2 *Carica papaya* L. seeds of Solo variety being dried

2.4. Food ration for cockerels

Table 1 shows the composition of the food ration used to feed the chickens during rearing.

Table 1 Cockerel food ratio composition

Ingredients	**Quantity (kg)**
Maize	52.5
Cubed bran	21.0
Roasted soybeans	13.0
Flesh concentrated	3.0
Fish meal	8.5
Shell	2.0
Total	100.0
Additive (prémix)	0.025
Characteristics	
EM (kcal)	2763.00
PB (%)	18.05
Lysine (%)	0.91
Méthionine (%)	0.40
Méthionine + cystéine (%)	0.61
Calcium (%)	1.23
Phosphore (%)	0.78

2.5. Phytochemical study of papaya seeds

2.5.1. Seed extraction

Carica papaya L. seed powder was extracted by maceration in ethanol (95%) under continuous stirring with a magnetic stirrer for 48 hours. After filtering the solution with filter paper, the filtrate collected was evaporated to dryness using a Büchi rotary evaporator system, with the water bath temperature set at 45°C.

2.5.2. Qualitative phytochemical analysis

Identification of alkaloids

The detection of alkaloids in the ethanolic extract of papaya seeds was carried out qualitatively using three different tests: Dragendorff test, Mayer test and Wagner test [9, 10, 11].

Saponin identification

This identification was carried out using the foam test described by [11] and [10].

Search for reducing compounds

Reducing compounds in the ethanolic extract of papaya seed powder were recognized using the Fehling reagent test described by [11].

Tannin detection

The presence of tannins in the ethanolic extract was highlighted by three different reagents [10] such as: 1% ferric chloride; lead acetate and ammoniacal copper sulfate.

Flavonoid detection

Flavonoids were revealed by mixing the ethanolic extract with a few drops of NaOH (1%). Intense yellow color turning colorless after the addition of a diluted solution of HCl show a positive test [11].

Identification of triterpenic phytosterols

Ethanolic extract of papaya seed was treated with a few drops of H_2SO_4 (1 M), then the mixture was shaken and left to stand. Appearance of a golden-yellow color was used to indicate the presence of triterpene phytosterols in the ethanolic extract [9, 11].

Protein analysis

Proteins were detected in the ethanolic extract of papaya seed using the xanthoprotein test. HNO_3 solution was added to the ethanolic extract introduced into test tubes, followed by adding a few drops of NH_3 solution. Appearance of an orange color was signal used to show the presence of proteins in the extracts.

Testing for coumarins

Two mL of the ethanolic extract solution and 0.5 mL of a NaOH (10%) were introduced into a test tube. The mixture was heated in a water bath until boiling. After cooling down at room temperature, the tube containing the heated solutions was observed under UV light at wavelength of 365 nm. The formation of a fluorescent yellow coloration has been a landmark to accept the presence of coumarins in the extract.

2.5.3. Quantitative phytochemical analysis

Determination of total phenols

UV-Visible spectrophotometric measurement method described by [12] was based on a redox reaction between the hydroxyl group (-OH) of phenols and Folin Ciocalteu reagent. Calibration curve (Figure 3) was established with a gallic acid solution.

Determination of proanthocyanidin or condensed tannins

Condensed tannins or pranthocyanidins were quantified in ethanolic extract by using UV-Visible spectrophotometric analysis method, and mixture of methanol or HCl, as described by [13]. The proanthocyanidin (Pro-C) content of the ethanolic extract was calculated by the *formula 1* as given by [14].

Pro-C = DO/0.280……*Formula 1*

DO = 0,280 is the value equivalent to catechin CT (1%), was used as a standard**.**

Determination of antioxidant activity of extracts

Antioxidant activity of the ethanolic extract papaya seed was assessed *via* the technique of reduction of molybdate ion Mo^{6+} to molybdate Mo^{5+} ion by antioxidant compounds contained in the ethanolic extract [15]. Ascorbic acid was used as a standard to calibrate the standard curve (Figure 4).

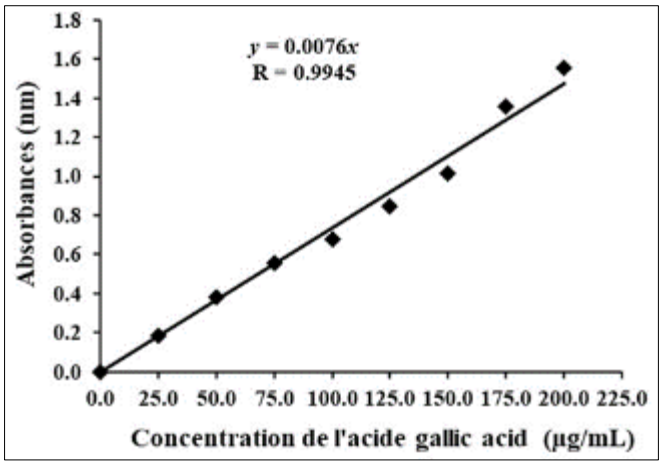


Figure 3 Calibration curve of gallic acid

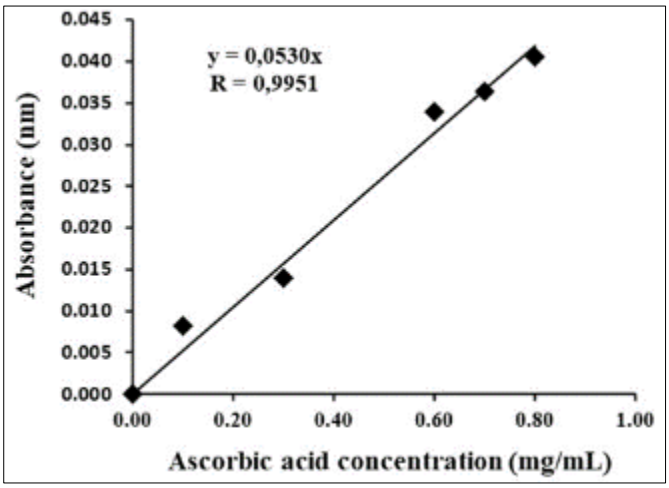


Figure 4 Calibration curve of gallic acid

2.5.4. Bioguided study

Sanitation realization

Prior to the beginning of the bioguided experiment, sanitation actions were applied to the poultry house, including washing, drying and pulverizing the site with insecticides, rat repellents and disinfectants. This was followed by a 15-day sanitation period, with disinfection renewed two days before animals arrived and settled in their poultry houses.

Batching and treatment of cockerels against helminths

A workforce of 260 cockerels of 7 weeks old, were received and installed on the breeding site. The cockerels were divided into 5 batches, numbered: T-, T+, GP1, GP2 and GP3. In each batch, two replicates were carried out, with 26 subjects per replicate. Cockerels in batches GP1, GP2 and GP3 received 5% of papaya seed powder in their food for one, two or three days, respectively. T- was the negative control batch that received no anthelmintic product, and T+ was the positive control batch that received Levamisole at the manufacturer's dosage

Collection of cockerel excrements

Cockerel excrements were collected for analysis before treatment at D_0 and after treatment at D_5, D_{10}, D_{17}, D_{24} and D_{30}. Four droppings' samples per batch were collected in plastic bags and sent to the laboratory for immediate microscopic examination and storage for one to three days, in a refrigerator at +4°C.

Coproscopical analysis

To assess parasite loads in terms of the number of parasite eggs per gram (EPG) and evaluate the effectiveness of different treatment frequencies, two methods were adopted: flotation method (qualitative coprology), and Mac Master technique (quantitative coprology).

Qualitative coprological method

With this method, 2 g of excrements were triturated in a mortar and suspended in 60 mL of a saturated NaCl solution. After sieving out the larger elements, a test tube (10 mL) was filled to the top of the meniscus. Five minutes after placing a coverslip on the surface, the floating eggs stuck to it. The slide was then removed and placed on a microscope slide, and observed under an electronical microscope ×100 [16].

Coprological quantity method

The experimental protocol involved triturating 2 g of excrements, then suspending the residue in 60 mL of a saturated NaCl solution. After sieving out the larger elements, the two cells of the Mac Master slide were filled, avoiding air bubble formation, and left to rest for five minutes, before observation under a microscope (magnification × 100) and counting the parasite elements [16, 17].

Parasite load assessment

Determination of the number of eggs per gram (EPG) in excrements was carried out to assess the intensity of parasitic infestation using the method described by [16]. The number of EPG was determined using *formula 2*.

$$\textbf{Number of EPG} = (N_1+N_2) \times 100 \quad \text{.....(}\textit{Formula 2}\text{)}$$

With: N_1: number of eggs counted in cell 1; and N_2: number of eggs counted in cell 2.

Reduction rate

Reduction rate (RR) of the number of EPG was calculated using *formula 3*.

$$RR = \frac{(\text{Number of EPG before treatment} - \text{Number of EPG after treatmen}) \times 100}{\text{Number of EPG before treatment}} \quad \text{..... (}\textit{Formula 3}\text{)}$$

Parasitological autopsy and worm extraction

The experiment consisted in slaughtering three cockerels per repetition to recover the digestive tract, opened lengthwise with a kitchen knife. The contents of the digestive tract were drained into a tray with a little water, then sieved with mesh sieve of a porosity of 200 µm to recover the worms. The worms were recovered with a needle, then immersed in a liquid fixative consisting of a mixture of 95% alcohol and formalin. Worms were identified with a binocular magnifying glass, using the keys defined by [18, 17].

2.5.5. Determination of zootechnical parameters

Cockerels were weighed on arrival and weekly for the duration of the trial to calculate zootechnical parameters such as: individual daily food consumption, live weight, average daily gain and food consumption ratio.

Average daily food consumption

Average daily food consumption (DFC) per cockerel was calculated using *formula 4.*

$$DFC = \frac{\text{Quantity of feed distributed (g)} - \text{Quantity of feed remaining (g)}}{\text{Period duration x Number of birds}} \quad \textit{(Formule 4)}$$

Live weight

Cockerels live weight (LW) was determined from *formula 5*.

$$\mathbf{LW} = \frac{\textbf{Sum of bird weights}}{\textbf{Total number of birds}} \qquad \textbf{(}\boldsymbol{Formula\ 5}\textbf{)}$$

Average weekly gain

The average weekly gain (AWG) was calculated using *formula 6.*

$$\mathbf{AWG} = \frac{\textbf{Weight gain (g) during a period}}{\textbf{Period duration (days)}} \qquad \textbf{(}\boldsymbol{Formula\ 6}\textbf{)}$$

Consumption index

The consumption index (CI) is defined as consumed food quantity per subject to gain 1kg of live weight. It was calculated using *formula 7.*

$$\mathbf{CI} = \frac{\textbf{Quantity of feed consumed over a period (g)}}{\text{Weight gain during the period (g)}} \qquad \textbf{(}\boldsymbol{Formula\ 7}\textbf{)}$$

2.5.6. Data statistical analyses

The data collected and the calculations performed were analyzed statistically using Graph Pad Prism 5 software. ANOVA One Way test was used to process the results, presented as means plus more or less the standard errors of the means. Results were compared using the TUKEY test. The probability significance level was set at $P < 0.05$.

3. Results

3.1. Qualitative phytochemical composition of papaya seeds

Qualitative phytochemical analyses revealed the presence of the phytoconstituents listed in **table 3** in the ethanolic extract of papaya seed powder.

Table 3 Phytochemical compounds detected in ethanolic seed extract

Phytochemical compounds tested	Results
Alkaloïds	+
Reducing compounds	+
Saponins	-
Tanins	+
Flavonoïds	+
Protéins	+
Triterpenic phytosterols	-
Coumarins	+

Legend: + = presence of tested compounds; and - = absence of tested compounds.

3.2. Total phenols & proanthocyanidins contents and antioxidant seed activity

The contents phenols totals and proanthocyanidins and antioxidant activity of seeds in the extract are presented in Tableau 4.

Table 4 Total phenol and proanthocyanidin content and antioxidant activity of ethanolic seed extract

Phytochemical compound quantified	Values in ethanolic extract (mg/g DE)
Total phenols content (GA Eq)	37.56
Total condensed tannin content (CT Eq)	11.05
Anti-free radical (AA Eq)	26.33

GA Eq: Gallic acid equivalent; CT Eq: Catechin equivalent; AA Eq: Ascorbic acid equivalent; DE: Dry extract.

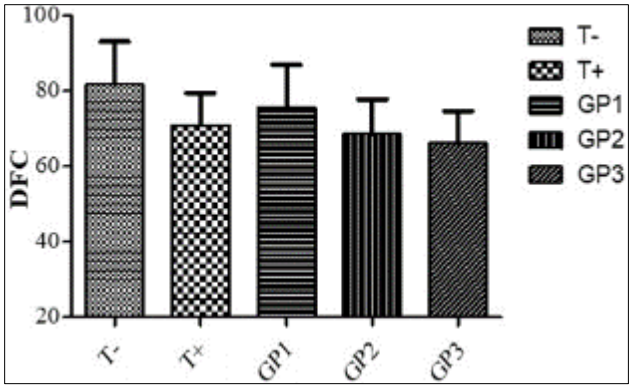
3.3. Cockerels' zootechnical parameters

3.3.1. Average food consumption of chickens

Average daily food consumption (DFC) per cockerel in batches T-; T+; GP1; GP2 and GP3, were respectively: 81.81 ± 11.36; 71.05 ± 8.49; 75.73 ± 11.29; 68.72 ± 9.128; 66.36 ± 8.49 g/d (Figure 5). DFC values showed that, daily food intake average was higher in batch T- than in other batches, while batch GP3 had the lowest food intake. However, statistically there was no significant difference between batches ($P < 0.05$).

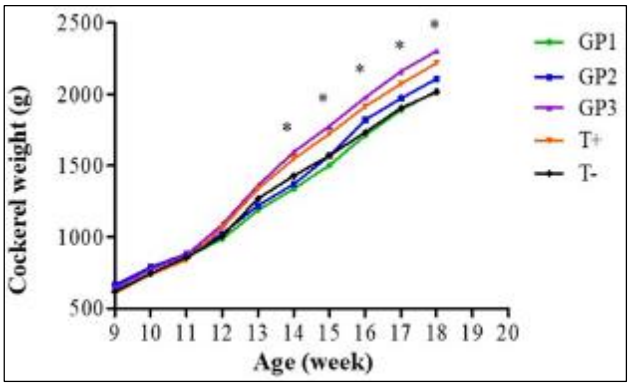
3.3.2. Live weight

The curves in Figure 6 show that, in all batches, the live weight (LW) values of the cockerels increased with age.



* Indicates that the difference is significant between batches GP3 & T+ and batches GP1, GP2 & T- ($p < 0.05$).

Figure 5 Daily food consumption of cockerels



* Indicates that the difference is significant between batches GP3 & T+ and batches GP1, GP2 & T- ($p < 0.05$).

Figure 6 Evolution of LW values of cockerels

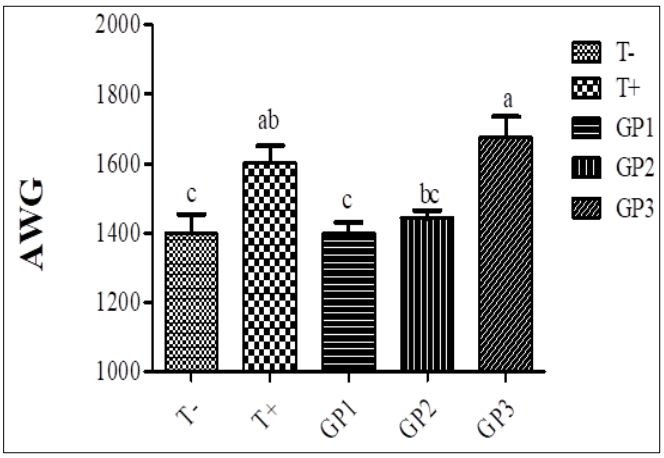
Indeed, at the start of the experiment when the cockerels were 9 weeks old, they all had around 600 g live weight. This value increased slightly from week 9 to week 13. However, from week 14 to week 18, LW values of subjects in GP3 and T+ batches became significantly higher than those of the other three batches ($P < 0.05$). Indeed, around the 18th week of age, LWs of the subjects reached values of: 2016 g, 2218 g, 2024 g, 2109 g and 2304 g, respectively in batch T-; T+; GP1; GP2 and GP3.

3.3.3. Average weekly gain

Cumulatively, the results shown in Figure 7 indicate that the average weekly gain (AWG) values for subjects in batches T+ and GP3, i.e. 1,604 g and 1,675 g respectively, were significantly higher ($P < 0.05$) than those in the other batches (GP1, GP2 and T-). The lowest AWG values were provided by cockerels from batches GP1 and T-, *i.e.* 1398.56 g and 1398.85 g respectively; while that of batch GP2 was 1443.86 g.

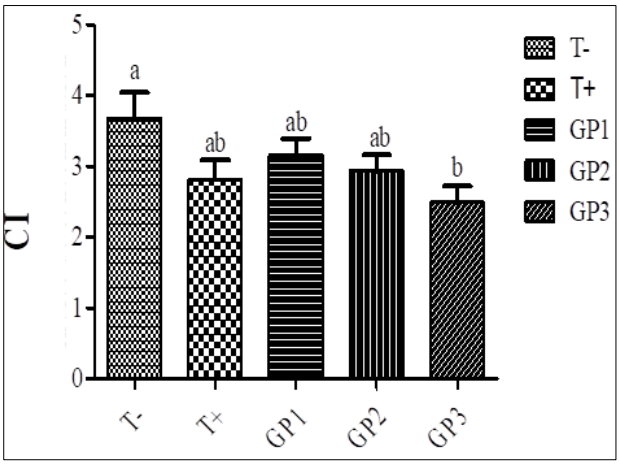
3.3.4. Consumption index

The results presented in Figure 8 show that the consumption index (CI) of the cockerels depends closely on the type of treatment they have received. Indeed, CI value recorded in batch T-, at 3.67, was the highest; that of batch GP3 (2.49) was significantly lower ($P < 0.05$) than the case of batch T-.



Histogram bars not bearing the same letter are significantly different (P <0.05)

Figure 7 Average weekly gain cumulated



Histogram bars not bearing the same letter are significantly different (P <0.05)

Figure 8 Consumption Index of chickens

3.3.5. Weekly food consumption and weekly growth

The curves plotted in Figure 9 (A-E) showed the evolution of average daily food consumption (DFC) and average weekly gain (AWG) of cockerels in the different batches studied in the current work. In general, the curves show WFC values ranging from 318 g to 883 g, and AWG values from 104.15 g to 276.4 g.

From the 12th week of age, the difference between DFC and AWG values in the T- batch was greater than in the other batches (T+, GP2 and GP3). For batches T+ and GP3, there was no significant difference in the evolution of DFC and AWG.

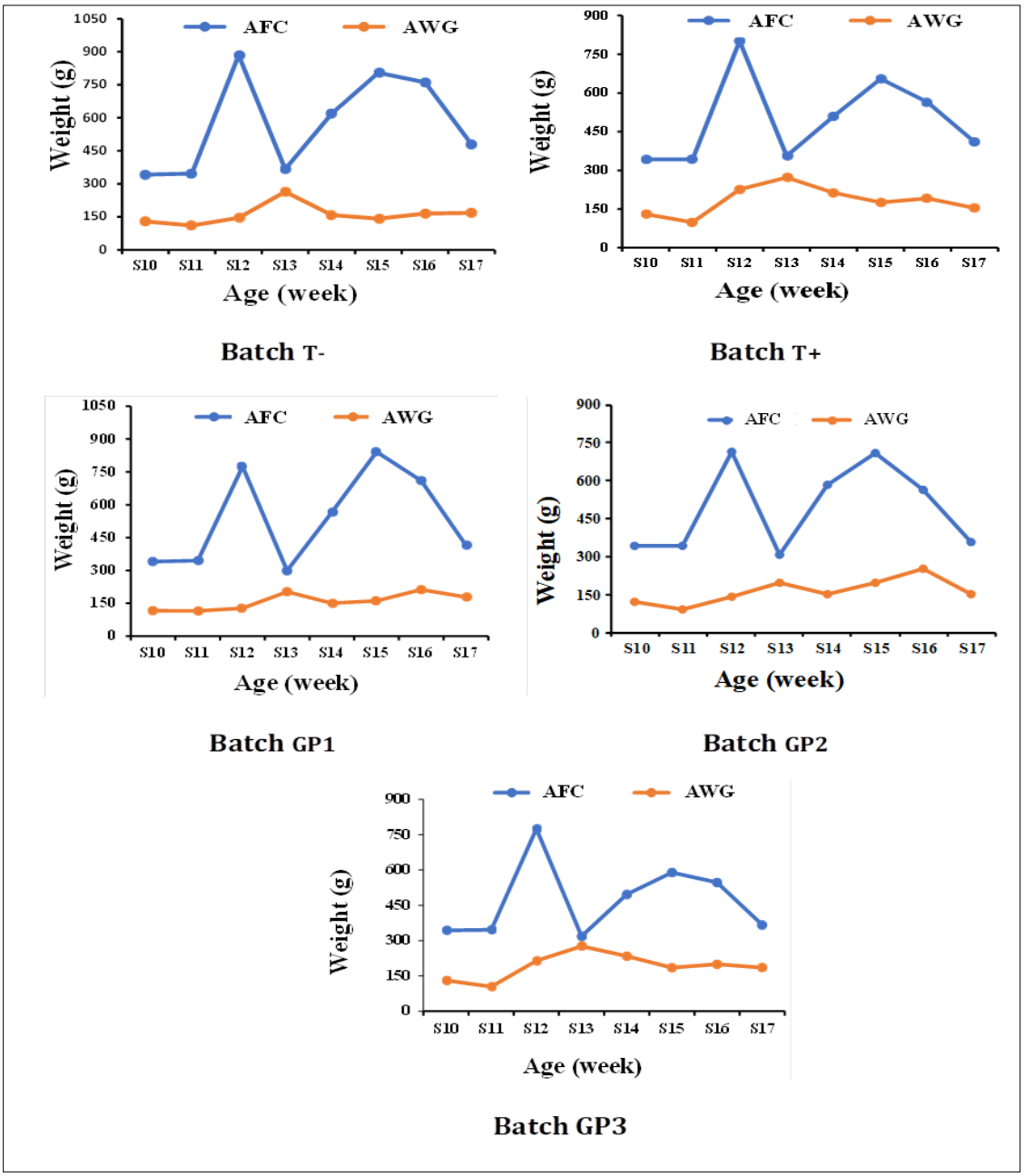


Figure 9 Trends in food consumption and weekly growth of cockerels

3.4. Effect of seeds on parasite load

3.4.1. Variation in the number of eggs per gram of excrements

Figure 10 shows the variation in the number of EPG in each batch studied. The papaya seed powder and Levamisole treatments significantly reduced the parasite load in cockerels. Indeed, in batch T-, there was a clear increase in the number of EPGs from 96 to 50,355 for a duration of 55 days, indicating the increasing parasitic state of the cockerels in this batch. In particular, it was in batch GP3 that the number of EPGs counted reached the highest values.

3.4.2. Reduction rates of EPG

Reduction rates (RR) of the number of EPGs in the different batches during this experiment are shown in Table 5. According to Table 5, RR of EPG numbers after the two treatments carried out over seventeen days increases overall as a function of time in batches GP1, GP2 and GP3.

Table 5 Effect of treatments on the RR of EPG numbers as a function of time

Average EPG number and egg reduction rate (%)					
The day of sampling	**Control batch types**		**Types of experimental batches**		
	T-	**T+**	**GP1**	**GP2**	**GP3**
D0	96.00 ± 10.59	119.00 ± 7.22	123.00 ± 2.41	105.00 ± 9.72	101.00 ± 7.14
First treatment					
D5	143.00 ±7.04^{a}	23.00 ± 4.93^{d}	101.00 ± 11.10^{b}	94.00 ± 6.17^{b}	60.00 ± 3.24^{c}
Reduction rate (%)	-48.95	80.67	17.88	10.47	40.59
D10	327.00 ±7.67^{a}	73.00 ± 4.20^{bc}	99.00 ± 4.81^{b}	70.00 ± 9.12^{bc}	50.00 ± 6.31^{c}
Reduction rate (%)	-240.62	38.65	19.51	33.33	50.49
D17	318.00 ±12.45^{a}	82.00 ± 4.6^{c}	118.00 ± 8.25^{b}	98.00 ± 6.01^{bc}	18.00 ± 4.41^{d}
Reduction rate (%)	-231.25	31.09	4.06	6.66	82.17
D24	223.00 ±13.97^{a}	111.00 ± 2.88^{b}	122.00 ± 5.84^{b}	108.00 ± 6.55^{b}	99.00 ± 7.05^{b}
Reduction rate (%)	-132.29	6.72	0.81	-2.856	1.98
D30	260.00 ± 4.70^{a}	121.00 ± 13.24^{b}	153.00 ± 15.75^{b}	143.00 ± 9.95^{b}	114.00 ± 13.45^{b}
Reduction rate (%)	-170.8	-9.01	-24.39	-36.19	-12.87
Second treatment					
D5	296.00 ± 11.00^{a}	12.00 ± 1.29^{c}	143.00 ± 16.60^{b}	109.00 ± 7.92^{b}	24.00 ± 3.29^{c}
Reduction rate (%)	-13.84	90.08	6.53	23.77	78.94
D10	303.00 ± 22.04^{a}	21.00 ± 2.73^{c}	110.00 ± 22.04^{b}	94.00 ± 9.03^{b}	17.00 ± 3.48^{c}
Reduction rate (%)	-16.53	82.64	28.10	34.26	85.08
D17	461.00 ± 30.88^{a}	25.00 ± 2.67^{c}	128.00 ± 2.58^{b}	66.00 ± 4.74^{c}	11.00 ± 2.94^{c}
Reduction rate (%)	-77.3	79.33	16.33	53.84	90.35
D24	503.00 ± 31.56^{a}	56.00 ± 8.01^{c}	160.00 ± 8.61^{b}	132.00 ± 4.60^{c}	32.00 ± 8.08^{c}
Reduction rate (%)	-93.46	53.71	-4.57	7.69	71.92

D_0: Day of the second treatment; on the same line, values not bearing the same letter are significantly different (P <0.05).

Indeed, in batch GP3, the reduction rate was 82.17% at 1st treatment and 90.35% at 2nd seventeen-day treatment; in batch GP2, it was 6.66% at 1st treatment and 53.83% at 2nd seventeen-day treatment; in batch GP1, the reduction rate at the end of the two treatments was 19.51% at 1st treatment and 28.1% at 2nd ten-day treatment. In batch T+, the best reduction rate after the two treatments was 80.67% and 90.08%, respectively at the 1st and 2nd five-day treatments. On the other hand, in batch T-, the RR remained negative from the beginning to the end of the two treatments.

3.4.3. Helminthological autopsies

The results of the helminthological autopsies (Table 6) show that the number of parasitic worms seen in cockerels in batch T- was significantly higher than in batches T+, GP2 and GP3. However, there was no significant difference between the parasitic load of batch T- and that of batch GP1. Batch GP3 had the lowest parasite load.

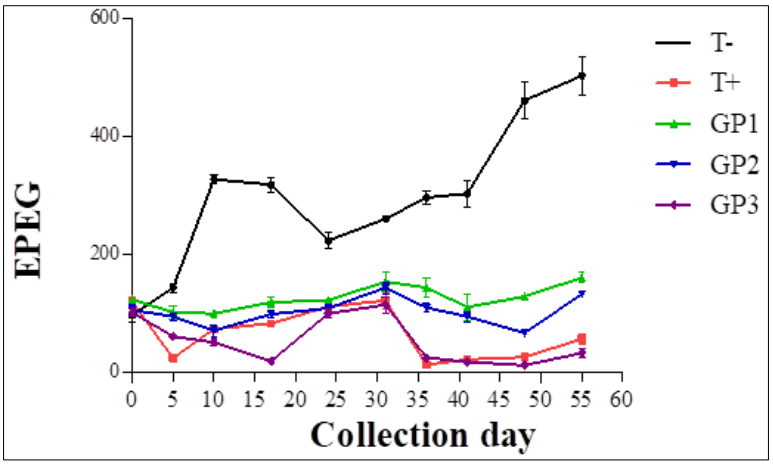
Table 6 Effect of different treatments on average parasite load per subject

Batches	**T-**	**T+**	**GP1**	**GP2**	**GP3**
Parasites load	9.33 ± 1.94^{a}	1.50 ± 0.42	6.50 ± 2.61^{ab}	2.50 ± 0.95^{bc}	0.83 ± 0.54^{c}

Values not bearing the same letter are significantly different (P <0.05).

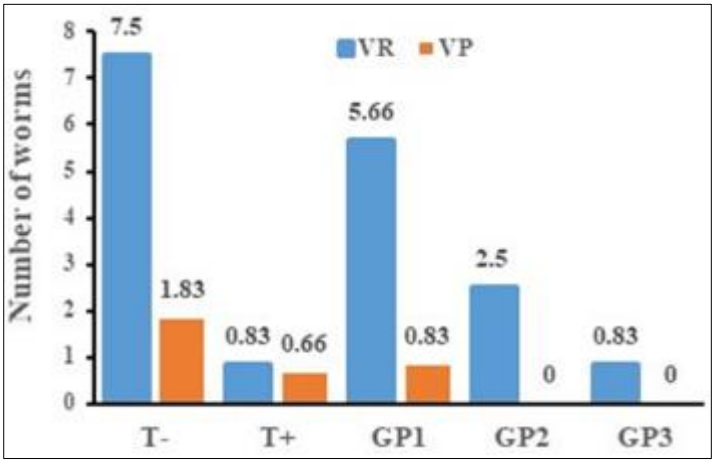
3.4.4. Impacts of worms' treatments

After the autopsy, counts of the various parasites identified in cockerels that had undergone the different types of treatment received during the course of this study yielded the results shown in Figure 11. Batches T+ GP3 had the highest number of roundworms, with 0.83 worms counted per cockerel.



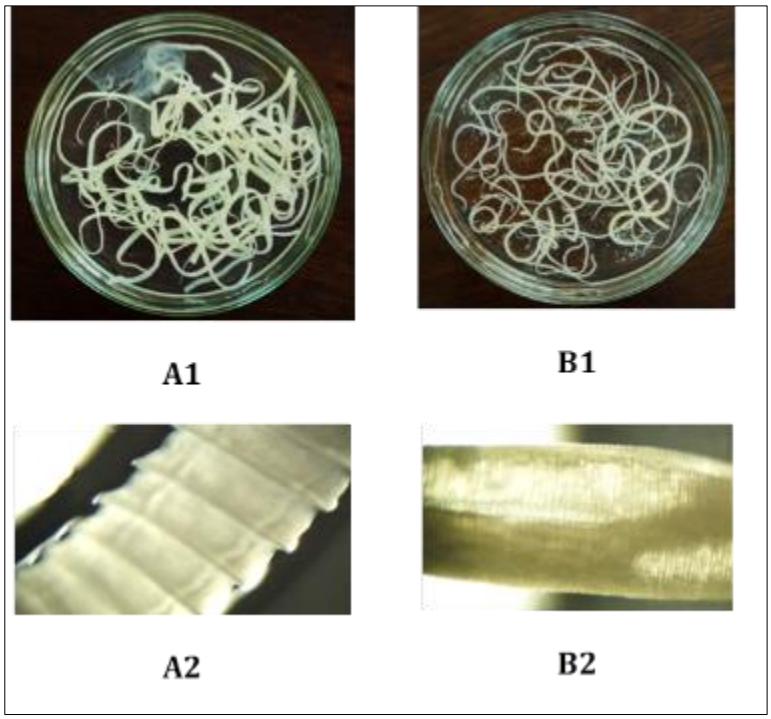
Legend: VR: Roundworms; VP: Flatworms

Figure 10 Variation in the number of EPGs over time with different treatments



Legend: VR: Roundworms; VP: Flatworms

Figure 11 Number of parasitic worms counted at autopsy by type of treatment



A1 = flatworms; B1: roundworms; A2: flatworms enlarged under the microscope and B2: roundworms enlarged under the microscope.

Figure 12 Photographs of some of the worms extracted from the digestive tracts of cockerels

Observation of the worms found in the cockerels' digestive tracts with the glass or microscopy, revealed two types of parasites: roundworms and flatworms, as shown in the photographs in Figure 12. However, no flatworms were found in batches GP2 and GP3. Overall, the number of roundworms found largely exceeded that of flatworms, regardless of the type of treatment applied. It was noted that the parasite load was more pronounced in batches T- and GP1.

4. Discussion

Qualitative phytochemical analyses revealed the presence the following chemical groups: alkaloids, reducing compounds in the ethanolic extract of papaya seeds: flavonoids, tannins, proteins and coumarins (Table 3).

These results are similar to those obtained by [19], who also noted that papaya seeds contain biomolecules such as: alkaloids, tannins, saponins and phenols. Similar results were also obtained by [10], with the exception of the negative test obtained in this search which invalidated saponin presence in the papaya seeds.

With regard to the quantitative phytochemical tests carried out, the results indicate that phenolic compounds and condensed tannins or proanthocyanidins are present in appreciable quantities in the ethanolic extract of Solo papaya seeds. Existence of these phenolic compounds in non-negligible quantities in Solo papaya seeds is in perfect agreement with the antioxidant activity highlighted in the current work [6]. Indeed, a number of works published in the literature have sufficiently demonstrated that phenolic compounds such as flavonoids and tannins possess remarkable antioxidant properties [6]. This can be explained by the existence of conjugated double bonds within the intrinsic structures of these compounds, enabling them to stabilize free radicals by acquiring several resonance-stabilized mesomeric forms [20, 21].

In the current study, anthelmintic treatment of cockerels with Solo papaya seeds improved their zootechnical performance, such as DFC, LW, AWG and CI (Figures 5-9). This improvement in zootechnical performance resulted from the reduction in parasite load in cockerels, thanks to the synergistic toxic effects of the phytoconstituents present in papaya seeds [6]. In fact, the higher DFC in the T- and GP1 batches than in the others would be due to the inconvenience caused by the presence of a more abundant population of gastrointestinal parasites, which would divert to their benefit a large part of the food consumed by the cockerels. This hypothesis is in line with the one put forward by [22] to justify the fact that the detrimental effect of parasitism on poultry can result in the retention or even exacerbation of appetite in order to compensate for deficits in nutrient intake caused by parasites [6].

The results of the current study on the evolution of LW show that cockerels from batches GP3 and T+ were significantly heavier than those from batches T- and GP1, from the 14th week of age (Figure 6). They also had a better AWG value. These results are comparable to those obtained by [3], who reported that zootechnical performance, like AWG, improved in their investigation of the anthelmintic efficacy of ethanolic extracts of papaya seeds administered orally in drinking water against avian ascariasis in broilers.

The better growth observed in cockerels from batch GP3 (Figure 6) is related to the greater reduction in parasite load, while the poor growth recorded in batches GP1 and T- is attributable to the more accentuated negative impacts inflicted by the parasites. Indeed, helminthoses has been shown to depress zootechnical performance, leading to a reduction in growth rate and an increase in food consumption ratio [23, 24], due to the competition between parasite and host for nutrients.

In the current investigation, the CI was remarkably higher in batch T-. Therefore, it seems that a treatment of less than three days with Solo papaya seed powder is still not sufficient to guarantee effective deworming of cockerels for a month. In fact, cockerels from batches GP1 and GP2, which received only one- and two-days' treatment per month respectively, showed a higher CI than those from batch GP3, which received three days' treatment per month.

Many authors have argued that the high DFC and low AWG could be attributable to the effects of parasites, which are qualitative spoliators that attack their host's metabolism and divert essential elements such as amino acids, vitamins and minerals to their own benefit [6, 25, 26].

As coproscopie is considered an inadequate technique, it should be backed up by a parasitological autopsy. The variation in the value of the number of EPG showed a clear increase in batch T-, whereas in the dewormed batches, there was a reduction in the number of EPG after each treatment (Table 5). The methods of deworming with Solo papaya seed powder show some efficacy depending on the number of days of treatment. These results obtained in the current search corroborate those of [27], according to whom there was a significant increase in the number of EPG in the non-dewormed batch T- compared with the experimental batches treated with Solo papaya seeds.

In this work, the treatment frequency of three days per month (batch GP3) resulted in EPG reduction rates of 82.17% and 90.35%, respectively after the 1st and 2nd treatments after 17 days. These results are similar to those of [3] who, in the course of their research, obtained reduction rates of 82.14% and 72.66% after 3 days of treatment with broilers against ascariasis by ethanolic extract of papaya seeds administered orally in their drinking water.

The antiparasitic effect felt in the GP3 batch may be comparable to that of Levamisole, a synthetic chemical which, after just 5 days, reduced the parasite load by 90.08%, thus demonstrating more lightning-fast antiparasitic activity than papaya seeds. However, according to [28], treating gastrointestinal parasites with papaya seeds would also present the same efficacy as veterinary anthelmintics.

The autopsy results presented in this study confirm the degree of anthelmintic efficacy of each type of treatment carried out. Depending on the mechanism of action of the anthelmintic activity of the seeds, it seems that the biomolecules they contain act either by momentarily limiting the excretion of parasite eggs, or by eliminating part of the worm population [29]. For [30] and [31], the destruction of parasitic worms is due to the presence of the benzyl isothiocyanate molecule present in papaya seeds.

However, in our approach, the anthelmintic activity of the seeds could be explained by the synergistic action of the various phytochemical compounds highlighted in this study such as: tannins, polyphenols, alkaloids, coumarins and proteins, as all these substances have been shown to have remarkable antimicrobial properties [11]. For example, [32] demonstrated that plants such as *Mitragyna inermis*, *Combretum glutinosum,* and *Bridelia ferruginea*, which are very rich in condensed tannins and phenolic compounds, possess anthelmintic activities *in vitro* on all three developmental stages of the nematode *Haemonchus contortus*. In particular, the absence of tapeworms in batches GP3 and GP2, whereas they were found in batch T+, would then be due to the fact that Solo papaya seeds display an apparently more effective toxicity against cestodes than Levamisole. This is because the synergistic action of the seeds' secondary metabolites against tapeworms is more effective than the Levamisole molecule as a single active ingredient.

In fine, treatments with *Carica papaya* L. seed powder increased the rate of parasitic load reduction. The intensity of reduction increased with the number of days and frequency of treatment with seed powder. Parasite load reduction resulted in a positive impact on cockerel growth and food consumption.

5. Conclusions

The various investigations carried out in this study showed that *Carica papaya* L. seeds have anthelmintic effects comparable to Levamisole, a veterinary antiparasitic chemical product, commonly used in poultry farming. In fact, treatment with Solo papaya seed powder reduced significantly the parasitic load of cockerels, helping to improve their AWG and CI. From the results of EPG number, RR and the helminthological autopsy, it emerged that the 3-day treatment with Solo papaya seeds achieved the same efficacy as that carried out with Levamisole at a dose of 1 g per liter of water. Consequently, it was suggested that for optimal deworming, 3 consecutive days of treatment at a dose of 5% with papaya seed powder incorporated into the cockerel food would be required.

Phytochemical studies carried out beforehand to justify the anthelmintic effects of the seeds revealed the presence of phytoconstituents such as: alkaloids, carbohydrates, flavonoids, tannins, proteins, and coumarins in the ethanolic extract of papaya seeds. However, the search for saponins and terpene phytosterols in the seeds was unsuccessful. Most of all these phytochemical compounds identified in the seeds have already been recognized as biologically active against worms, viruses and microbes.

However, for a more appropriate use of seeds as a veterinary product in poultry farming, further work should be carried out before to examine the biochemical and hematological parameters of the beds, during and after each treatment; then carry out a histological analysis of subjects' digestive tracts.

Compliance with ethical standards

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Disclosure of conflict of interest

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

Statement of ethical approval

All procedures used in this work were approved by the scientific ethics committee of Regional Center of Excellence for Avian Sciences (CERSA) of the University of Lomé, Togo.

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