Research Article

Efficacy of Ethanolic Extract of Carica papaya leaves as a Substitute of Sulphanomide for the Control of Coccidiosis in KABIR Chickens in Cameroon

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Abstract | Coccidiosis remains one of the main diseases affecting performance in poultry. This work was aimed at evaluating the efficacy of an ethanolic leaf extract of *Carica papaya* with regard to growth, parasitological and haematological parameters in *Eimeria*-infected KABIR chickens. Fresh leaves were harvested, dried in gentle heat, ground and sieved powder was used to prepare the ethanolic extract. Chickens were infected with 3200 *Eimeria* oocysts until they began shedding oocysts in their faeces. The ethanolic extract was administered at doses of 0.32g/chicken/day (T1), 0.80 g/chicken/day (T2) and 1.44 g/chicken/day (T3), directly into the chickens' mouths. Chickens of T4 received a standard anti-cocciadial drug, sulfaquinoxalina-LH, those in T5 were infected but not treated while T6 was the neutral group. Red blood cell (RBC) and white blood cell (WBC) counts as well as haemoglobin (Hb) values were determined. Among the trial groups, T3 and T1 had the highest and lowest feed conversion rates of 1.87% and 1.62% respectively. The highest average growth rate was recorded in T1 (7.09%) while the least was registered in T3 (2.74%). Carcass parameters were similar in all groups except for the hearts. In the trial groups chickens in T3 had the highest oocyst reduction rate (97.4%) while those in T1 had the lowest (94.5%), but the differences were not statistically significant. Overall, the highest oocyst reduction count was recorded in chickens of T4 (98.7%) while the lowest was recorded in chickens of T5. RBC was significantly highest ($P \leq 0.043$) in T1 and lowest in T5. WBC was significantly highest ($P \leq 0.031$) in T4 and lowest in T5. Hb values were similar in all groups. Ethanolic leaf extract of *C. papaya* could be as effective as standard anti-coccidial drugs if used before the threshold level of parasitic infection.

Keywords | *In vivo*, *Carica papaya*, Efficacy, Coccidiosis, KABIR chickens

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INTRODUCTION

More than 50 billion chickens are reared annually as a source of food, for the purpose of both meat and eggs. Poultry and poultry products such as poultry meat and eggs are important foods for improving nutritional and health status particularly for children, pregnant women and immunologically weakened persons. In realization of the importance of animal protein, governments of various developing and developed countries have been pursuing programmes at various levels to boost mass production of livestock products to ensure the attainment of Food and Agriculture Organization (FAO) recommendation of 35g/input of animal protein per day (FAO, 2009).

Many of these poultry production farms are hampered by internal parasites, such as coccidia which are the leading internal parasites of poultry, causing death losses of 20 percent or more. Coccidiosis plays an inhibitory role in the growth of the poultry industry and is caused by different species of *Eimeria* which inflict the birds in both clinical and sub-clinical forms. The clinical form of the disease manifests through prominent signs like bloody faeces, diarrhoea, morbidity and mortality and subclinical coccidiosis manifests mainly by poor weight gain and reduced efficiency of feed conversion and gives rise to the highest proportion of the total economic losses (Alfifi, 2007).

Chemotherapy has been the main approach for controlling coccidiosis in chickens. *Eimeria species* are able to complete their life-cycles without large numbers of infective oocysts building up in the environment. Such subclinical infections result in the development of strong, specific natural immunity without overt disease. However, studies have shown that resistance to anticoccidial drugs develops with time (Usman et al., 2011). There is therefore a need to treatment that is effective and cheaper to the farmer.

The use of medicinal plant extracts in the treatment of both human and animal diseases is gaining popularity because it is affordable by farmers in developing countries (Kubkomawa et al., 2013). According to the World Health Organization (WHO), about 80 to 90% of the world's population still relies on traditional medicine for their health care needs (Ajay et al., 2013). Cameroon has a rich biodiversity, with approximately 8,620 plant species, some of which are commonly used in the treatment of several microbial infections and a range of diseases (Ntie-Kang et al., 2013). These plants have been used both for the treatment of human and animal diseases. Since the ultimate goal of a farmer is to maximize profits, many farmers go for the cheapest and easiest means of fighting diseases such as coccidiosis.

Carica papaya has been known to be effective against coccidiosis in some countries, but such studies have not been reported in southwest Cameroon. Therefore the aim of this study was to evaluate the efficacy of an ethanolic leaf extract of *C. papaya* with regard to growth parasitological and haematological parameters in KABIR chicks infected with *Eimeria* oocysts.

MATERIALS AND METHODS

Study Area and Population Descriptions

The study was carried out in the Africa Brazil Marketplace project poultry farm located in Lyongo village, Buea, Southwest, Cameroon. This is one of the villages located at the foot of Mount Cameroon and is very rich in vegetation. The experimental animals consisted of Cameroonian KABIR chickens aged 9 weeks comprising 8 fowls per experimental group and of both sexes. They were of mixed breed, displaying a variety of feathering and plumage colours.

Poultry Farm Management

After leaving the hatchery the experimental chicks were grown under uniform brooder conditions from a day old to experimental ages. The birds were housed in a disinfected deep litter system with wood shavings being the bedding material. Each treatment occupied an area of 2.25 m^2 where feed and water were provided *ad libitum*. Incandescent bulbs provided light and heat day and night during the brooding period.

Study Design

Chickens were divided into 6 chambers (Groups). Groups 1-3 (T1-T3) were given the *C. papaya* ethanolic plant extracts in different concentrations (0.32 g, 0.80 g. and 1.44 g) respectively. Group 4 (T4) was given the synthetic anticoccidial drug (Sulfaquinoxalina-LH). Group 5 (T5) was the negative control group which was infected but not treated while group 6 (T6) was the neutral group which was not infected at all. All the chickens in each group were of equal numbers, had the same feed intake and composition,

under the same environmental conditions with *ad libitum* access to feed and water. They were inoculated at their eighth week with the parasites and treatment started four days after inoculation and went on for seven days. The chickens for each extract were inoculated orally with equal numbers of *Eimeria* oocysts (3200 oocysts per gram, OPG) except for the neutral group (T6). The McMaster technique was used to monitor the oocyst load.

Preparation and Administration of *C. papaya* Ethanolic Leaf Extract

Fresh leaves were harvested, dried in gentle heat, grounded and sieved powder used for ethanol extract. 95% of ethanol was diluted to 70% ethanol using Gay-Lussac's dilution method to form alcoholic water which was used for the preparation of the ethanolic extract. 500g of *C .papaya* leaf powder was put into 1000 ml of 70% alcohol and covered to avoid evaporation. This was macerated and stirred after every 6 hours, for 72 hours after which, the mixture was filtered through filter paper and the liquid was evaporated to dryness using an evaporator at 40°C. The powder is very rich in alkaloids (the main components being carpaine; $C_{14}H_{27}NO_2$) and was given in doses of 0.32 g, 0.80 g and 1.44 g to *Eimeria*-infected chickens of T1, T2 and T3 respectively. T4 was treated with sulfaquinoxalina-LH at a dose of 1 teaspoon full daily in 2.5 L of water and was taken for 4 days. The weights of these birds were taken after every two days using a sensitive balance. The McMaster counting technique was used to quantify the oocysts in the faeces of the chickens.

Haematological and Carcass Parameter Analyses

At the end of the experiment, five of the chickens from each treatment were sacrificed, blood samples were collected from their aortic veins into EDTA tubes for haematological analysis and their organs were removed, observed and weighed. The organs included live weight (LWT), carcass weight (CWT), heart (HT), liver (LV), lungs (LGS), spleen (SPL), fabricius bursa (FB), gizzard (GZ), proventriculus (PV), thymus (TH), caecal weight (CEWT), caecal length (CEL), kidney (KN), empty caecal weight (ECWT) and bile (BL). The blood was transported in ice and carried to the Laboratory of Animal Health (LASAN) of the Department of Animal Production, of the Faculty of Agronomy and Agricultural Sciences, University of Dschang, for analysis. Blood parameters (WBC, RBC and Hb) were analysed using the haematology analyser machine for animals (Beckman Coulter, Coulter A^C.T different Haematology Analyser, BR-13692A).

Statistical Analysis

The data collected from the experiment were entered and analysed using SPSS Package version 20. The data collected were subjected to ANOVA at 95% confidence level ($P<0.05$) to compare treatments, and when there were significant differences between treatments, these were separated using the Duncan multiple range test. The mean oocyst counts that were calculated before and after the administration of the different extracts were used to evaluate percentage in reduction or reduction rate, using the formula below:

Reduction rate = (Initial oocyst counts-final oocyst counts) X 100/ Initial oocyts counts

Also, some production parameters such as the average feed intake (FI) which is the total amount of feed consumed by the chickens from the day of the administration of the plant extract till the last day, average daily weight gain (AWG) between treatments were calculated. These parameters were calculated as follows:

Average daily weight gain (AWG) = Total weight gain per chicken per group/ Total number of days

Feed consumption rate (FCR) = FI/ Total AWG

Where, FI = feed intake and AWG = average weight gain (Unigwe et al., 2014).

RESULTS

Growth Parameters

The Growth parameters of *Eimeria*-infected KABIR chickens treated with ethanolic leaf extract of *C. papaya* leaves are presented in Table 1. Results obtained showed that there was no significant difference in the feed intake between the different groups receiving the ethanolic *C. papaya* leaf extract ($P\geq0.05$). On the other hand, a significant difference ($P < 0.05$) was observed in the average weight gains between groups.

The highest average growth rate was observed in T1

Table 1: Feed intake, cumulative average weight gain, growth rate and feed conversion ratio for KABIR chickens treated with ethanolic *Carica papaya* leaf extract

Treatments	Growth parameters			
	FI (g ±SEM)	AWG (g ±SEM)	AGR (%)	%FCR
T1	1352.25±151.26	833.94±34.20[b]	7.09	1.62
T2	1245.86±221.26	744.65±19.04[a]	3.53	1.67
T3	1395.13±215.59	744.73±16.72[a]	2.74	1.87
T4	1276.00±134.09	835.63±34.01[b]	5.40	1.53
T5	1137.63±55.12	737.66±61.99[a]	5.98	1.54
T6	1332.63±203.55	831.35±25.61[b]	3.11	1.60
Values in a column followed by the same superscripts are not significantly different (P ≤ 0.05)				

SEM = standard error of means, FI = feed intake, AWG = average weight gain, AGR = average growth rate, FCR = feed conversion rate

Table 2: Average weight of some internal organs of *Eimeria*-infected KABIR chickens treated with ethanolic extract of *C. papaya* leaves

Parameters	Treatments					
	T1 (0.32g)	T2 (0.8 g)	T3 (1.44 g)	T4 (Sulfaquinox-alina-LH)	T5 (Positive control)	T6 (Negative control)
Live wt	493.5±72.8	595.7±175	670.8±49.6	763.8±91.2	685.5±107	758.7±79.2
Carcass wt	332±52.7	410±126.1	466.3±37.8	526.7±81.3	457.2±73.1	564.2±65.1
Heart	2.4±0.1[a]	3.3±0.6[c]	3.5±0.1[c]	4.6±0.4[b]	4.2±0.8[b]	5.6±0.7[b]
Liver	15.1±0.9	12.7±6.4	19.8±1.3	19.8±2.5	21.4±1.4	22.5±2.1
Lungs	2.6±0.5	3±0.2	3.8±0.6	4.5±0.6	3.7±0.7	5.3±1.2
Spleen	1.4±0.3	0.8±0.2	1.6±0.5	1.6±0.4	1.3±0.2	1.3±0.2
Fabricus	0.7±0.1	0.6±0.2	0.8±0.1	0.7±0.1	0.7±0.1	1.6±0.3
Gizzard	15.2±1.8	18.3±4.3	20.9±2.7	26±3.5	23.6±3.6	20.1±2.6
Proventriculus	3.4±0.6	3.2±0.4	4±0.4	6.2±1.4	3.6±0.7	3.4±0.3
Thymus	0.7±0.4	0.7±0.2	0.4±0.2	1.4±0.1	2.5±0.5	0.6±0.3
Caecal wt	6±0.5	4.6±0.6	6.4±0.8	7.7±1.2	6.6±1.6	9.1±1.2
Caecal length	11.5±0.8	10.9±0.6	11.9±0.2	12.9±0.1	15.3±2	14.1±0.5
Kidney	3.6±0.1	4.8±0.3	5.5±1.1	5.4±0.2	6.1±1	5.9±0.6
Empty caeca	3.2±0.4	3.0±0.6	3.2±0.2	4±0.2	3±0.5	4.9±1.1
Bile	0.3±0.1	0.4±0.2	0.2±0.1	0.6±0.1	1±0.5	0.5±0.2
Values with different superscripts indicate that differences were statistically significant at p ≤ 0.05 while those without superscripts are not significant to either of the superscripts at the same probability level.						

(7.09%). Moreover, the highest feed conversion ratio was observed in T3 (1.87) which received the highest dose of the plant extract, followed by T2 (1.67) and T1 (1.62).

Carcass Characteristics

The carcass characteristics of *Eimeria*-infected KABIR chickens treated with ethanolic extract of *C. papaya* leaves are presented in Table 2. The results obtained revealed no significant differences in the weights of organs between groups (P = 0.064) except for the heart. The highest heart weight was observed in T6 (5.60±0.68g) which was the neutral group, followed by T4 (4.60±0.40g) which received the standard anticoccidial drug and the difference was statistically significant (P = 0.041).

Faecal Oocyst Counts

The faecal oocyst counts of *Eimeria*-infected KABIR

Table 3: Reduction rate of *Eimeria* oocyst counts in KABIR chickens treated with ethanolic extracts of *Carica papaya* leaves

Treatment (Ts)	Oocysts count±SEM		%Reduction	Status
	Initial	Final		
T1	12375±1550.4	685.7±192[a]	94.5	H-L
T2	17028.6±1880	771.43±219[a]	95.5	H-L
T3	22612.5±516.9	584.9±148.4[a]	97.4	H-L
T4	11987.5±389.11	150±50[a]	98.7	H-L
T5	3500.00±5114.3	19112.5±2532.5[b]	-49.0	M-H

a, b on the same column, the values assigned the same letter are not significantly different (P> 0.05). H-L = high to low, M-H = Medium to high opg

Table 4: Effects of treatment with ethanolic *Carica papaya* leaf extract on the RBC, WBC and Hb of *Eimeria*-infected KABIR chickens

Treatment	RBC(x 10^{12}/L)	WBC(x 10^{9}/L)	Hb(g/dL)
T1	2.8±0.1[a]	50.0±14.7[a]	9.3±0.2
T2	2.6±0.7[a]	33.2±4.5[ab]	9.1±0.6
T3	2.4±0.4[a]	61.8±14.6[a]	10.2±0.6
T4	2.8±0.3[a]	98.8±0.3[a]	9±1.1
T5	0.8±0.3[b]	18.3±11.8[b]	7.0±0.1
T6	1.7±0.4[b]	56.7±17.1[a]	8.2±0.6

a, b on the same column, the values assigned the same letter are not significantly different (P> 0.05

chickens treated with ethanolic extracts of *C. papaya* leaves is presented in Table 3. The final oocyst counts for ethanolic *C. papaya* leaf groups were not significantly different (P ≤ 0.05) between treatment groups. The highest oocyst count reduction rate was observed in T4 (98.7 %) which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was observed in T3 (97.4 %), followed by T2 (95.5 %) and then T1 (94.5%). T5 had the lowest percentage reduction value and negative value because it was infected and not treated, thus the oocyst counts increased.

Haematological Parameters

The haematological parameters for *Eimeria*-infected KABIR chickens treated with ethanolic extracts of *C. papaya* leaves are presented in Table 4. There was a statistically significant difference in RBC and WBC counts at P ≤ 0.043 and P ≤ 0.031 respectively between experimental groups treated with ethanolic *C. papaya* leaf extract and T5. The highest RBC (2.8±0.1 x 10^{12} /L) and WBC (98.8±0.3 x 10^{9}/L) values were recorded by T1 and T4 respectively. T5 showed the lowest RBC, WBC and Hb values.

DISCUSSION

Coccidiosis remains a major health problem in the production of chicken. This study was aimed at assessing the efficacy of ethanolic extract of *C. papaya* leaves on growth, parasitological, and haematological parameters of KABIR chicken infected with *Eimeria* oocysts. The results showed that the feed intake of the chickens between groups was not affected by the plant extracts. It could therefore be assumed that the beneficial effect of the plant extract obtained in this group of infected chickens was not a consequence of its stimulating influence on the chickens' appetite, but as a result of its anticoccidial properties (Arczewska-Włosek and Świątkiewicz, 2012). In contrast to the present findings, Guo et al. (2004), Al-fifi (2007), Durrani et al. (2007) and Windisch et al. (2008) reported significant differences in the feed consumption of broilers fed with *Aloe vera*, *C. papaya* leaves, *Vernonia amygdalina* leaves and other herbal extracts. T1 which received the lowest dose of the ethanolic *C. papaya* leaf extract on the other hand, had the highest percentage average weight gain (7.09%). This could be attributed to the fact that the *C. papaya* leaf extract in low doses might have increased the absorption of nutrients in the chickens' digestive tract thus leading to the weight gain of the chickens in these groups. This extract probably becomes a bit toxic when given in high doses in the chickens' metabolic pathway thereby leading to a low weight gain. The weight gain could also be due to the presence of papain in the *C. papaya* leaf extract which aids protein digestion thus

enhancing the release of free amino acids necessary to enhance growth (Onyimonyi and Onu, 2009). Ogbuokiri et al. (2014) also reported significant effects of *C. papaya* leaves as well as other plant extracts on the weight gain of chickens with respect to the control group. In this case, higher feed conversion ratios were observed in T3 (1.87), T2 (1.67) and T1 (1.62). These higher feed conversion ratios among groups which received the ethanolic *C. papaya* leaf extract could be attributed to the high nutritional value in terms of protein and minerals/vitamins of the *C. papaya* leaf extract, especially when the concentration given to the chickens is increased (Unigwe et al., 2014).

With reference to carcass parameters, the plant extract did not have any significant effects on the majority of the major body organs. Findings of the present study are in agreement with the reports of Amaechi and Iheanetu (2014) who reported absence of significant differences in major body organs.

Results obtained on the final oocyst counts and the oocyst reduction rates showed that all the plant extracts were effective in reducing the initial oocyst loads of the parasite. In this experiment, the highest oocyst reduction rate was observed in T4 (given commercial anticoccidial drug), T3 or T2 (given higher doses of the extracts) and lowest reduction rates in T1 (given the lowest doses of the extracts) meaning that the commercial anticoccidial drug was very effective and the efficacy of the plant extracts also increased with an increase in the dosage. The synthetic coccidiostat was also given only for four days in their drinkers and the marvelous results were obtained.

More so, the anti-inflammatory property of the *C. papaya* leaves with high concentrations of vitamin A might have acted in caecal epithelium cell protection which is detrimental to the coccidial reproductive activities. Vitamin A is essential for the integrity of chicken mucosal surfaces and enforces body resistance to coccidian. Thus, an increase in the levels of Vitamins A, E and Zinc will probably lead to a strong immune response to coccidiosis. Al-fifi (2007) reported that when *C. papaya* leaf powder was given to birds that were challenged with coccidiosis it reportedly reduced the number of oocysts per gram to 53%.

Results obtained also showed that ethanolic *C. papaya* leaf extract had no significant effects on the RBC, WBC and Hb of the chickens between extract treated groups. In line with the present findings, Jatau et al. (2014) did not also record significant differences in the haematological parameters of chickens in an experiment they carried out with experimental fowls. However, the present findings are in line with the findings of Oyagbemi and Adejinmi (2012) and Ogbuokiri et al. (2014) who reported significant differences in the RBCs and WBCs of chickens when they were given dry *C. papaya* leaves and other plant extracts. The latter observed significant differences in WBC and Hb values in chickens fed with *C. papaya* leaf extract.

CONCLUSIONS

Papaya leaves extract has proven to be of significant importance in fighting coccidiosis as shown in reduction of oocyst counts, and in improving RBC and WBC values in the chickens. Thus, these could be used as organic alternative of synthetic chemicals to combat coccidiosis and improve chicken health.

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CONFLICT OF INTEREST

There is no conflict of interest between authors.

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