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Scientific Journal of
Veterinary Advances
ISSN 2322-1879
Sjournals Publishing Company | www.sjournals.com

Scientific Journal of Veterinary Advance (2022) 10(1) 316-324
ISSN 2322-1879
doi: 10.14196/sjva.v10i1.1717

CODEN (USA): SJVAAS

Contents lists available at Sjournals

Scientific Journal of **Veterinary Advances**

Journal homepage: www.Sjournals.com

Original article

Nutritional and anticoccidial properties of papaya (*Carica papaya*) leaf meal on growing rabbits

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A R T I C L E I N F O

Article history,
Received 02 August 2022
Accepted 30 August 2022
Available online 06 September 2022
iThenticate screening 04 August 2022
English editing 28 August 2022
Quality control 05 September 2022

Keywords,
Papaya leave, Growth performance
Anticoccidial, Rabbits, Diet

A B S T R A C T

The present study aims to evaluate the anticoccidial properties of papaya leaves meal (PLM) and its effect on the productive growing performance of rabbits. The effect on productive performance was investigated using forty-eight growing rabbits divided into 4 groups of 12 rabbits; and the anti-parasitic effect evaluation trial was conducted on separate forty eight growing rabbits rabbits divided into another 4 groups (12 each). The experimental diets (R0, R2, R4 and R8) contained different levels of PLM (0%, 2%, 4% and 8% respectively). For each trial, the experiment lasted for 8 weeks. The measured parameters included daily weight gain, feed intake, feed convertion ratio (FCR), and faecal egg count. Secondary metabolites in PLM were quantified using its hydroalcoholic and aqueous extracts. The results show a presence of phenols, tannins and flavonoids with high contents in the aqueous extract (47.55±12.40 mgeqGA/g, 19.03±0.03 µgeqCAT/mg, 7.45±0.16 mgeqQ/g respectively). No significant difference in feed intake and significant difference in daily weight gain ($p < 0.05$) and FCR ($p < 0.01$) occurred among the treatments, despite the progressive numerical increase in weight gain with the raising PLM in the diets. The use of PLM in the diets significantly decreased ($p < 0,001$) oocyst's abundance in the treatments compared to the control (diet without PLM). Thus, the PLM represents a good alternative for improving the feeding and health status of growing rabbits.

1. Introduction

The increasing cost of feed ingredients, especially protein sources, has resulted in declining profitability of rabbit production (Lebas, 2018; Gidenne et al., 2019). It is therefore very important to use feed ingredients of lower cost and biological values that can replace the expensive conventional protein sources and improve productions profits. Leaf protein, especially from *Carica papaya*, is a promising approach among protein sources that is getting importance because of its relatively low cost, availability and abundance (Jiwuba, 2018; Dotto and Abihudi, 2021). Plant leaves also provide minerals, vitamins and secondary metabolites such as alkaloids, tannins, steroids and quinones useful in treating animal diseases (Juárez-Rojop et al., 2014; Oche et al., 2017).

Carica papaya Linn is an herbaceous plant which belongs to the Caricaceae family. It is a popular plant in the tropics and subtropics because of its easy cultivation, rapid growth, quick economic returns and easy adaptation to diverse soils and climates (Amadinze et al., 2016). *Carica papaya* leaves (pawpaw) are a rich source of protein (Adiwimarta et al., 2010) and a rich source of vitamin C, vitamin A, and vitamin E, the mineral magnesium, calcium and potassium, and the B vitamin, panthothemic acid and foliate fibre (Oche et al., 2017). Pawpaw leaves in the diets showed significantly improvement on productive performance of rabbits, broiler and *Archachatina marginata* (Jiwuba, 2018; Onyimonyi and Onu, 2009; Ahaotu et al., 2018; Nwogor, 2015). Different organs of *Carica papaya* have been used in ethnomedecine to treat several disorders such as ulcers, infections by intestinal parasites, indigestion, diarrhoea (Saran and Choudhary, 2013; Pinto et al., 2015). The seeds have antiparasitic activity (Dakpogan et al., 2019) and the leaves show anti-coccidial effect (AL-Fifi, 2007). Coccidiosis is indeed the most expensive and common diseases of rabbit farming that mainly affects young rabbits (Henneb and Aissi, 2013). However, few studies have investigated the influence of *C. papaya* leaves on the growth parameters and its antiparasitic effects on rabbits. This study sets to fill this gap and increase our scientific knowledge. This study aims to evaluate the effect of the incorporation of *C. papaya* leaves on the growth parameters and their antiparasitic action in rabbits.

2. Materials and methods

2.1. Sourcing and processing of diet ingredients

Mature and disease free *C. papaya* leaves were collected from pawpaw trees in the Southern Benin. The leaves were separated from the stalk, dried at room temperature for two days, then dry in oven at 60°C for 72 hours. Then after, the dry leaves were hammer milled and kept in an airtight container for further use in in ration formulation with other ingredients sources from local feed miller.

2.2. Plant extracts preparation

Fifty (50) grams of C. papaya leaf flour were soaked separately in an alcohol solution (70% ethanol) and in distilled water. After filtration, the filtrates were concentrated using a rotary evaporator and the residues were air-dried in oven at 50°C. The obtained extracts were stored in the refrigerator at 4°C until use.

2.3. Quantification of secondary metabolites

The total phenols content, total flavonoids content and condensed tannins content were quantified using the methods described by Maiga et al. (2020).

2.4. Total Phenols Content

Polyphenol concentrations are expressed as mg gallic acid equivalent (GA) per gram of dry extract according to the expression:

T(mgGAeq/g) = (C .Vr)/(Vp .Cp)

T = Content of the compounds; C = Concentration obtained from the calibration curve Vr = Reaction volume; Vp = Volume of extract taken; Cp = Concentration of the extract solution taken.

2.5. Total Flavonoids Content

Flavonoid contents are calculated from the regression line of the standard (quercetin) and are expressed as mg quercetin equivalent per gram of dry extract according to the expression:

T(mgQeq/g) = (C .Vr)/(Vp .Cp)

T = Content of the compounds; C = Concentration obtained from the calibration curve Vr = Reaction volume; Vp = Volume of extract taken; Cp = Concentration of the extract solution taken.

2.6. Condensed Tannins Content

Tannin contents are expressed in µg catechin equivalent (CAT) per milligram of dry extract according to the expression:

T(µgCATeq/mg) = (C .Vr)/(Vp .Cp)

T = Content of compounds; C = Concentration obtained from the calibration curve Vr = Reaction volume; Vp = Volume of extract taken; Cp = Concentration of the extract solution taken.

2.7. Experimental design and animal management

Table 1
Composition of the diets (R0-R8) as function of the ingredients and estimated chemical composition of the diets during the experiment.

Ingredients (kg)	R0 (0% PLM)	R2 (2% PLM)	R4 (4% PLM)	R8 (8% PLM)
Maize	38.5	38.5	38.5	38.5
Roasted soy	10	08	06	02
Palm kernel meal	20	20	20	20
Rice bran	05	05	05	05
Wheat bran	22	22	22	22
Premix	02	02	02	02
Oyster shell	02	02	02	02
C. papaya leaf meal	00	02	04	08
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100
Calculated composition				
% Crude protein	17.06	17.8	18.805	19.4
% Ether extract	5.7	5.75	5.4	5.75
% Crude fiber	7.83	7.25	7.05	7.99
% Ash	11.85	11.4	13.6	11.1
% Dry matter	88	88.55	87.8	88.3
% Moisture content	12	11.45	12.2	11.7
Metabolisable energy (Kcal/kg)	2715.6	2774.4	2662,05	2752,28

A total of ninety-six rabbits were used in this study. The same experimental design was used to evaluate the influence of *C. papaya* leaves on the growth parameters and of their anti-parasitic effects. For each experiment, forty eight growing local breed rabbits with weigh of 945 g ± 17.28 (average ± standard deviation) were equally and randomly divided into four groups (12 in each one) with four rabbits used as replicate. Four rabbits were housed per hutch (measuring 80 cm x 50 cm x 40 cm and raised 100 cm above the ground level), so each group includes three hutches. Each animal was vaccinated against prevalent disease. The rabbits used for the nutritional value of PLM experiments were dewormed before the experiment; and those for the antiparasitic activity of PLM were naturally infested. Diets were offered twice each day in pellets form ad libitum; and fresh clean drinking water was daily available for the animals. Four experimental diets were formulated (R0, R2, R4 and R8 containing (PLM) at 0%, 2%, 4% and 8% papaya leaf meal, respectively (Table 1).

2.8. Zootechnical data collection

Data were collected daily for feed intake, and weekly for weight gain. Feed intake was calculated as the difference between feed supplied and left over feed the next day. Live body weight gain was computed using weekly weigh-back mechanism by subtracting the present week's weight from the previous week weight. Feed conversion ratio was determined by dividing the average total feed intake by the average total live body weight gain.

2.9. Parasitological data collection

The faecal samples were collected from individual animals from their droppings. Afterwards, all samples were immediately sealed and tied in plastic rectal sleeves, stored in a refrigerator (4°C), and analyzed the same day. The coproscopic were analyzed Per Cringoli et al. (2017) using mini-FLOTAC technique. The number of eggs per gram (EPG) of faeces was determined using the formula (Eq. 1):

$EPG = (n_1+n_2) \times 5$ (Eq. 1)

Were, n_1 = number of oocysts counted in cell 1; n_2 = number of oocysts counted in cell 2

The reduction rate of egg excretion in faeces was calculated using the formula (Eq. 2):

Reduction= $(EPG\ initial - EPG\ final) \times 100 / EPG\ initial$ (Eq. 2)

2.10. Statistical analysis

Ryan Joiner normality test was performed to verify normality and to assess the effect of feeding on zootechnical performance. We carried out analysis of variance (ANOVA) test in case of normality; otherwise, we performed Kruskal Wallis non-parametric test. When the probability was significant ($p < 0.05$), an average was structured using the SNK function of the "agricolae" package (de Mendiburu, 2021). Growth rate curves were drawn using the "Hmisc" package (Franck et al., 2021). Differences between intensity of parasitism, i.e. the arithmetic means of EPG, were analyzed using two-ways ANOVA. When significant variations were noted, Tukey–Kramer HSD post hoc test was performed. All analyzes were performed with R 3.5.1 software (R Core Team, 2018).

3. Results and discussion

The variation in phytochemical content of Carica papaya leaves depended on the extraction solvent. Phenols, tannins and flavonoids were present in two extracts, but with higher contents in the aqueous extract (Table 2).

Table 2
Secondary metabolites contents of *Carica papaya* leaves extracts.

Extracts	Phenols (mgeqGA/g)	Tannins (µgeqCAT/mg)	Flavonoids (mgeqQ/g)
Hydroalcoholic	26.12±0.85	11.27±0.03	4.80±0.23
Aqueous	47.55±12.40	19.03±0.03	7.45±0.16

3.1. Effect of PLM on growth performance of growing rabbits

The effect of PLM addition in diets on rabbits is presented in Table 3. The diets containing PLM improved the daily weight gain of the rabbits ($p < 0.05$) and FCR ($p < 0.01$) compared to the control, with the best results found for the R8 diet. The daily weight gains were 12.65 ± 0.79 g, 18.62 ± 2.68 g, 18.53 ± 1.11 g and 21.10 ± 0.87 g for R0, R2, R4, R8, respectively. The feed intake was not significantly different between groups (p > 0.05).

Table 3
Growth performance traits of rabbits fed diets with graded levels of PLM.

	Diets				
Parameters	**R0**	**R2**	**R4**	**R8**	**p**
Initial live body weight (g)	972.80±20.10	965.60±23.40	931.40±15.60	913.40±10.00	0.101
Final live body weight (g)	1681.10^{b}±34.10	2009.00^{a}±147.00	1969.30^{a}±57.80	2094.90^{a}±41.60	0.016
Feed intake (g/day)	61.77±0.44	62.24±1.66	65.29±0.94	63.62±0.28	0.091
Daily weight gain (g/day)	12.65^{b}±0.79	18.62^{a}±2.68	18.53^{a}±1.11	21.10^{a}±0.87	0.010
FCR	4.97^{a}±0.34	3.64^{b}±0.54	3.57^{b}±0.18	3.04^{b}±0.12	0.006

Letters on the same row compare the results of different treatments. Different letters show significant difference (p < 0.05).

The growth of the rabbits was progressive in all the groups with higher rates for the groups that received PLM (Figure 1). Thus, the live body weights increased from 972.80 ± 20.10 g to 1681.10 g ± 34.10 g for R0 group and from 913.40 g ±10.00 g to 2094.90 g ± 41.60 g for group R8.

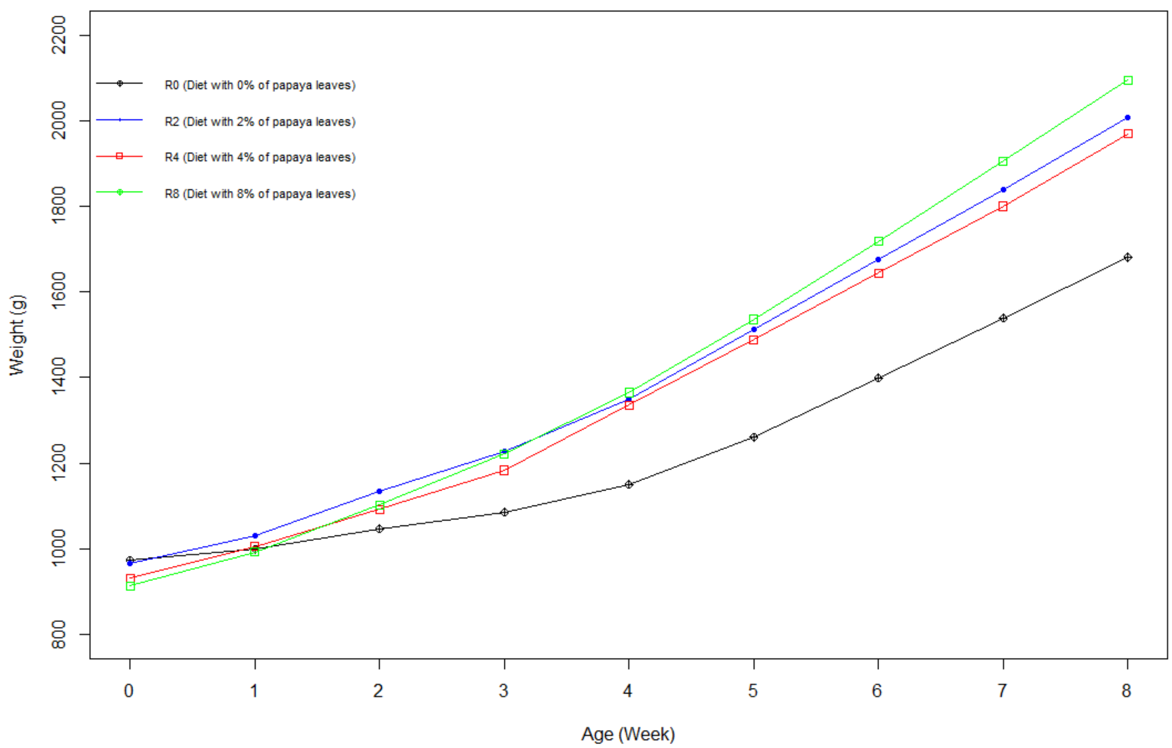

Fig. 1. Weight growth of rabbits fed with rations with different PLM incorporation rates.

3.2. Effects of PLM incorporation in diets on parasite load in growing rabbits

At the beginning of the experiment, coprological analysis revealed the presence of oocysts of *Eimeria spp*, eggs of *Passalurus ambiguus*, eggs of *Giardia spp*. *Eimeria spp* oocysts dominated (p < 0.001) in the samples (11892 ± 2055.70 EPG) over *Passalurus ambiguus* (15 ± 12.24 EPG) and *Giardia spp* (05 ± 04.08 EPG) eggs. Thus, only the faecal excretion of *Eimeria spp* eggs was considered for coprological analysis. The incorporation of PLM into rabbit diets significantly decreased (p < 0.001) the faecal excretion of *Eimeria spp* oocysts in rabbits compared to the control group (Figure 2).

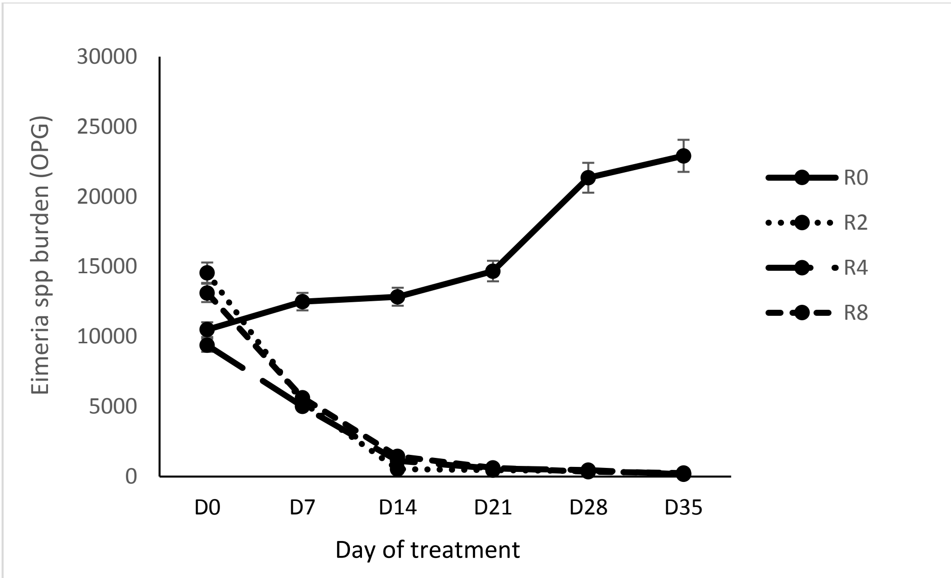

Fig. 2. *Eimeria spp.* count of rabbit fed diet containing varying levels of *C. papaya* leaf meal.

Previous studies have reported the presence of several phytochemical molecules in papaya leaves (Swati and Shivamv, 2018; Abdel-Halim et al., 2021). The dosage of phenols, tannins and flavonoids in this study revealed that the phytochemical molecules content in the leaves varied according to the extraction solvent. This result is in agreement with the study conducted by Abdel‑Halim et al. (2021) which revealed that the method of extraction of plant material has great effects on the yield of extracts and their content of bioactive compounds which guarantee the biological potential.

The contents of the aqueous extract of papaya leaves in phenols and flavonoids (47.55±12.40 mgeqGA/g and 7.45±0.16 mgeqQ/g respectively) were higher than those reported by Swati and Shivam (2018) who obtained 14.532 mg GAE/gm and 5.469 mg quercetin equivalent/gm (for phenols and flavonoids respectively) for papaya leaves collected in India. Abdel-Halim et al. (2021) however have reported higher values 43.33 mg GAE/g total phenolic and 13.28 mg QE/g flavonoid contents, compared to those of the present study, for hydroalcoholic extract of papaya leaves obtained with conventional maceration extraction. Phytochemical levels in plants can be affected by the development and growth conditions, maturity, packaging, storage conditions and extraction methods (Zang and Hamauru, 2003). Concerning tannins, Nwamarah et al. (2019) found in the methanolic extract of *C. papaya* leaves a tannin content of 310.50 ± 11.51 mg/100g, which is lower than that determined in the present study. Overall, these phytochemicals would be the source of pharmacological properties recognized in *C. papaya*. Dèdéhou et al. (2018) showed that tannins and polyphenols are largely involved in the anthelminthic effect of *Parkia biglobosa*. Tannins are known for their antimicrobial activities through iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). They are also used in the treatment of ulcerated or inflamed tissue (Li *et al.,* 2003). Flavonoids have anti-inflammatory (Pelzer et al., 1998) and immuno-modulatory (Damre et al., 2003) properties. They also show antibacterial (Boutlelis Djahra et al., 2012) and antiviral (Gonçalves et al., 2001) activities. According to Mpondo et al. (2012), flavonoids are powerful antioxidants and radicals and contribute significantly to digestion.

Several authors have confirmed the improvement in the feed efficiency of rations after incorporating PLM or PLM extract in rabbits and broilers (Nusrat et al., 2015; Sorwar et al., 2016; Jiwuba, 2018; Abd-ELGhany et al., 2021). The best weight performance (live body weight and daily weight gain) obtained during this experiment in rabbits fed with diets containing PLM could be explained by their higher content of nutrients but also the presence of enzymes such as papain in papaya leaves; which is a proteolytic enzyme that breaks down proteins into their building block amino acids, easily absorbed by the intestinal membrane (Vuorinen et al., 2021).

The value of the daily weight gain obtained during this study in the control group (12.65 ± 0.79 g/day) was lower than those reported by Jiwuba (2018) and Abd-ELGhany et al. (2021) (19.02g/day and 17.85g/day respectively). This could be because the rabbits used in the studies don't have the same initial weights, and the control diets have not the same nutritional values. For the treated groups, the daily weight gains in this study were close to those indicated by the same authors.

Except for the control group, the feed conversion ratios obtained in this study were similar to those from Abd-ELGhany et al. (2021), and lower than those reported by Jiwuba (2018). Coccidiosis is a most important primary causes of digestive disease in fattening rabbits which has a direct impact on performance and acts in synergy with epizootic rabbit enteropathy (Vancraeynest et al., 2008). According to Duszynski and Couch (2013), 17 *Eimeria* species infect rabbits.

The inclusion of papaya leaves in the diet of rabbits significantly reduced the excretion of eggs of *Eimeria spp*. This antiparasitic action would be due to the secondary metabolites present in the leaves of this plant. Previous studies have reported the anticoccidial effects of C. papaya leaves (Dakpogan et al., 2018; Banjoko et al., 2020). However, the mechanism of action of *C. papaya* on *Eimeria spp* is not yet elucidated. According to Dakpogan et al. (2018), the oocysts reduction induced by *C. papaya* crude juice treatment might be ascribed on the one hand to the direct *E. tenella* protozoits digestion by a synergistic action of pancreas chymotrypsin and papaine; and on the other hand to the anti-inflammatory property of the *C. papaya* concentrated vitamin A, which might act in caecal epithelium cell protection, detrimental to the coccidia reproductive activities.

4. Conclusion

This study showed a good performance and parasitological status of growing rabbits fed papaya leaf meal containing diets. The incorporation of PLM at 2%, 4% or 8% in rabbit feed improved weight gain and reduced the shedding of *Eimeria spp* oocysts. It can therefore be concluded that PLM incorporation in diets of growing rabbits could be recommended for improving rabbit production and health.

Acknowledgments

The authors thank the rabbit breeders of "DJAKOLE Elevage Lapin le soleil" farm located in the commune of Dogbo, Department of Couffo for their collaboration.

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How to cite this article: Dèdéhou, V.F.G.N., Alowanou, G.G., Nonviho, G., Zinsou, F., Houngbèmè, G.A., Béhingan, B.M., Agbassou, F., Aboudou, H.K., Hounzangbé-Adoté, S.M., 2022. Nutritional and anticoccidial properties of papaya (*Carica papaya*) leaf meal on growing rabbits. Scientific Journal of Veterinary Advance, 10(1), 316-324.