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Antiparasitic activity of papaya seed extract (C*arica papaya*) in free-range local breed chicken (*Gallus gallus*) production system in Ketou District.

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1 SUMMARY

A field study with a completely randomized design was carried out to evaluate the antiparasitic activity of dried seed meal of unripe mature papaya fruit on gastrointestinal parasites of the traditional chicken farming system in Ketou district. The targeted parasites were *Ascaridia galli, Eimeria sp., Capillaria sp.* and *Heterakis sp.* Nine chicken flock units of at least 10 local chicken were enrolled in the study which makes three experimental groups of chickens with three replications per treatment. The first group was treated with the Niclosamide-levamisole molecule complex, a conventional chicken antiparasitic drug (*VPV: Vermifuge Polyvalent Volaille*) with one tablet orally given to an individual adult and half a tablet orally given to an individual young chicken. The second group received, in drinking water, dried seed meal of papaya fruit at a dosage of 1 mg per chicken for 5 days. The third group received drinking water as a placebo. A total of 198 faecal samples were analysed in the laboratory with the quantitative flotation method using the McMaster Chamber. The results obtained demonstrated the effectiveness of *Carica papaya* extract treatment on coccidia in the first place and also on *Ascaridia galli* with effects comparable to that of the conventional antiparasitic drugs used in commercial chicken production system. The efficiency time period was 3 weeks for the extract of *Carica papaya*. This means that the treatment must be renewed every 3 weeks to guarantee its effectiveness. However, the most appropriate dose remains to be elucidated and the use of other organs of the plant for better efficacy will be the subjects of further investigations.

2 INTRODUCTION

Chicken is an important protein source because of its short production cycle (Smil, 2002). The local chicken plays an important socio-cultural role in rural community; frequently used for traditional pharmacopoeia, various festive occasions, wedding ceremonies, gift, and meal for important guests (Guèye *et al.,* 1998, Fotsa *et al.,* 2007). The paramount role of traditional poultry farming in Benin both in rural household economy and in global national meat supply (second source of meat after beef) makes of this activity a potential strategic tool for rural poverty alleviation (Sodjinou, 2011). Unfortunately, traditional poultry farming development is hampered by devastating pathologies (Dakpogan *et al.,* 2011) even though it is going through a transitional period in the developing countries, going from a simple activity of subsistence to a more commercial activity. If we agree to incriminate infectious diseases whose control is not for tomorrow in scavenging poultry systems, we must not lose sight of the parasitic diseases that remain, for the most part, insidious in holdings with the effect of lower performance. These diseases, although not often direct causes of mortality in chickens, cause considerable economic losses due, on the one hand, to the decrease in bird resistance to other diseases, and on the other hand, cause reduction in production and productivity. For the farmers, it is therefore essential to find less costly and accessible means of controlling those affections. The use of medicinal plants dates back several centuries and is one of the best profits that man derives from plant resources. At present, treatments based on medicinal plants are little valued because studies to confirm their effectiveness following a scientific approach and analysis remain few (Kasonia and Ansey, 1993). However, in the semi-intensive exotic chicken breed production system, some medicinal plants like *Andrographis paniculata, Sophora flavescens, Allium sativum, Salvia officinalis, Echinacea purpurea, Thymus vulgaris, Origanum vulgare, Artemisia sp., Foeniculum vulgare* have been substantially investigated with promising reports (Youn and Noh, 2001; Sujikara, 2000; Arczewska and Swiatkiewicz, 2010; Shazia *et al.,* 2013; Emilio *et al.,* 2013; Loredana *et al.,* 2015). Dakpogan *et al.* (2018) reported the anticoccidial effect of papaya leaf extract on exotic chicks experimentally infected with *Eimeria tenella*. The current study came up with the effect of dried seed meal of unripe mature *Carica papaya* fruit on *Eimeria sp., Ascaridia galli, Heterkis sp.* and *Capillaria sp.* in free-range local chicken production system in Ketou district, Benin.

3 MATERIALS AND METHODS

3.1 Study design and experimental groups : The study is a two-month longitudinal field study carried out in a completely randomized design, in Ketou district, an East-southern region of Benin (7° 10' and 7° 41' 17 " N 2° 24' 24"and 2° 47'40" E, 1775 km^2, 1.55% of the national territory). The objective was to evaluate the antiparasitic activity of sun-dried seed meal of unripe mature papaya fruit (*Carica papaya*) on chicken gastrointestinal parasites such as *Eimeria sp. Ascaridia galli*, *Heterakis sp.* and *Capillaria sp.* frequently encountered in smallholder free-range poultry production system (Permin and Hansen, 1998). Nine (9) smallholder free-range chicken flocks of local breeds with a minimum size of 10 chickens were enrolled in the study and randomly allocated into three experimental groups of three (3) flocks each (three replications).The first group was treated with conventional antiparasitic drug a complex Niclosamide-levamisole (*VPV: Vermifuge Polyvalent Volaille*). One tablet was orally given to an individual adult and half a tablet orally given to an individual young chicken. The second group received the dried seed meal of papaya fruit in drinking water for 5 days with a dose of 1 mg per chicken. The third group was the control

group where the chickens received simple drinking water for 5 days. The treatment was repeated once with 4 weeks between the two treatments.

3.2 Data Collection: Fresh faecal samples were collected in the morning across each chicken flock unit during the 4 weeks following the starting of the treatment. Five samples were collected during 5 five successive days in the first week following the starting of the treatment and 2 samples on day one and two in the remaining 3 weeks. A total of 198 faecal samples were collected and coccidian oocysts and nematode eggs were detected and counted in the Laboratory of Research in Animal Ecology and Zoogeography of the National Agriculture University through the quantitative simple flotation method using McMaster chamber (Soulsby, 1986). The targeted parasites were *Eimeria sp*. *Ascaridia galli*, *Heterakis sp*. and *Capillaria sp*. Eggs and oocysts were identified using the morphological references developed by Soulsby (1982).

3.3 Statistical analysis: The collected data was stored in Excel 2010 version of Microsoft Corporation and statistical analyses and graphs performed in SPSS software. Cumulative infestation rates of coccidia, *Ascaridia*, *Heterakis* and *Capillaria* were calculated using the frequency procedure and comparisons with the Chisquare test using Logrank's two-by-two comparison method. The efficacy of the treatment over time was evaluated by monitoring the variation of the parasite infestation, in the post-treatment time using the Kaplan Meier survival analysis procedure with Kaplan Meier curves. Means and standard errors of oocysts or eggs per gram of faeces were calculated and compared with the One Way ANOVA procedure and Student t test.

4 RESULTS

Compared to the untreated control flocks infestation rate, *Carica papaya* extract treatment reduced significantly the *Ascaridia galli* and *Capillaria sp*. cumulative infestation rates in treated flocks ($p < 0.05$), results comparable to the conventional antiparasitic drug treatment effectiveness. Also, *Carica papaya* dried seed meal treatment exclusively reduced significantly the *Eimeria sp*. infestation. Indeed, *Heterakis sp*. was not so ever affected by the treatments.

Table 1: Cumulative infestation rates

Experimental groups	*Ascaridia galli* (%)	*Eimeria sp.* (%)	*Heterakis sp.* (%)	*Capillaria sp.* (%)
Papaya extract	19.70^{a}	27.30^{a}	22.70^{a}	16.70^{a}
Niclosamide-levamisole	9.10^{a}	77.30^{b}	15.20^{a}	10.60^{a}
Control	39.10^{b}	73.80^{b}	13.60^{a}	31.80^{b}

(Values in columns that do not share the same superscript letters are significantly different at the significance level of 0.05).

The number of oocysts or eggs per gram of faeces varied very little among the different experimental groups. The intensity of *Ascaridia* infestation was reduced by half ($p < 0.05$) in chicken flock units treated with dried papaya seed meal compared to the untreated control group.

Table 2: Infestation intensity in positive chicken flocks

Experimental Groups	*Ascaridia galli* (%)	*Eimeria sp.* (%)	*Heterakis sp.* (%)	*Capillaria sp.* (%)
Papaya extract	$493^{a} \pm 115$	$315^{a} \pm 55$	$505^{a} \pm 65$	$348^{a} \pm 73$
Niclosamide-levamisole	$806^{ab} \pm 329$	$323^{a} \pm 29$	$290^{b} \pm 62$	$330^{a} \pm 116$
Control	$1296^{b} \pm 173$	$341^{a} \pm 37$	$407^{b} \pm 93$	$343^{a} \pm 65$

(Values in columns that do not share the same superscript letters are significantly different at the significance level of 0.05).

Figure 1 shows the efficiency over time of dried papaya seed meal and the conventional deworming agent (Niclosamide-levamisol) treatment on *Ascaridia galli, Eimeria sp., Heterakis sp and Capillaria sp.* The efficacy of the conventional antiparasitic drug on *Ascaridia galli* and *Capillaria sp.* lasted the whole post-treatment experimental period. In contrast, *Carica papaya* extract treatment was effective on *Ascaridia galli* and *Eimeria sp.* infestation for 3 weeks post-treatment period. Neither the conventional deworming nor the meal of the *Carica papaya* dried seeds affected the flock *Heterakis sp.* infestation level.

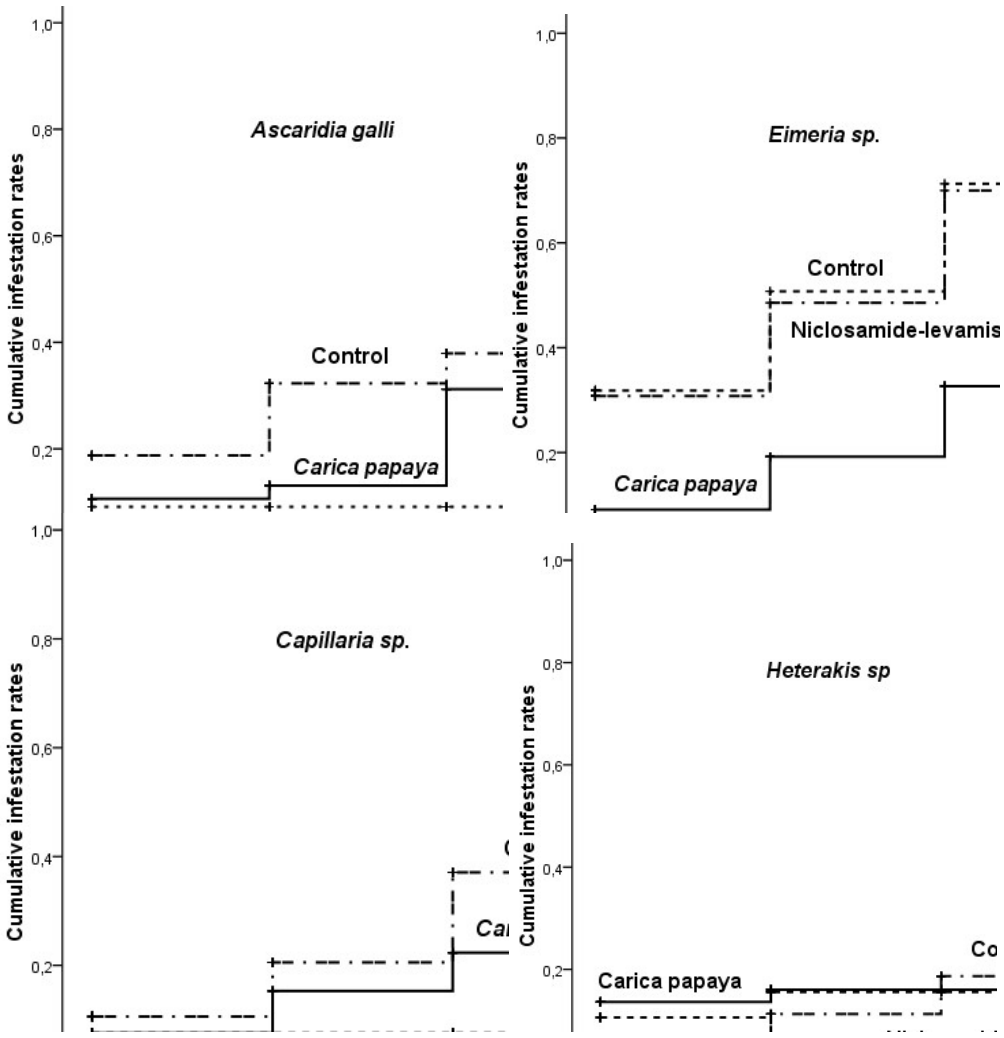

Figure 1: Cumulative infestation rates

5 DISCUSSION

The cumulative coccidial infestation rates were 74% and 27% for untreated and *Carica papaya* extract treated chicken flock units respectively. The meal of dried seeds of unripe *Carica papaya* fruit significantly reduced coccidial infection in chicken flocks that received this meal in drinking water with a reduction rate of 64% compared to the control untreated group. The low coccidial infestation rate (27%) observed in chicken flocks treated with *Carica papaya* extract during this study is similar to the prevalence of the disease recorded in semi-intensive litter-based exotic layer rearing system with the use in preventive mode of anticoccidians. This prevalence is 27% (Yunus et *al.,* 2008, Lunden et *al.,* 2010) and 36% according to Dakpogan and Salifou, (2013a). The *Carica papaya* fruit seed extract is thus effective in the same way as the conventional anticoccidial drugs used by poultry producers in small-scale commercial poultry production system. The effectiveness of *Carica papaya* extract on *Ascaridia galli* and *Capillaria sp.* infestation in the treated chicken flocks is more moderate, but statistically similar to that induced by the conventional antiparasitic drug the Niclosamide-levamisole complex. The cumulative infestation rates of *Ascaridia galli* and *Capillaria sp* were 9% and 11%, respectively, for the Niclosamide-levamisole complex and 17% and 19% for the dried papaya seed extract treatment. The findings are consistent with the results obtained by several authors using various parts of *Carica papaya* plant. The anthelmintic effect of the latex, leaves and seeds with elimination of human and animal intestinal worms was observed (Okenyi *et al.,* 2007, Sacramento *et al.,* 2010, Arvind *et al.,* 2013). The antiprotozoal effect of the seed extract was demonstrated by Ekanem *et al.* (2004) on fish parasite organism, *Ichthyophthirius multifiliis*. Dakpogan *et al.* (2018) observed the anticoccidial effect of papaya leaf extract on chicken coccidiosis caused by *Eimeria tenella*. The anthelmintic activity of papaya seeds can be attributed to carpaine, carpasemine (Kermanchai *et al.,* 2001) and proteolytic enzymes such as cysteine proteinase (Stepek *et al.,* 2005) and papain (Arvind *et al.,* 2013) from the fruit. Also, *Carica papaya* is known for its anti-inflammatory properties, certainly due to its vitamin A content, used against tumors, ulcers and can accelerate wounds healing (Beuth et *al.,* 2001). Dried papaya fruit seed meal and the conventional parasitic drug have no effect on *Heterakis sp*. The number of eggs per gram of faeces of parasites such as *Heterakis sp., Capillaria sp.* and the number of oocysts per gram of faeces of *Eimeria sp.* were not fundamentally affected by the herein applied treatments. However, *Carica papaya* extract significantly reduced the number of eggs per gram of faeces in *Ascaridia galli*. The Kaplan Meier curves obtained in this study made it possible to analyse the effectiveness of treatments over time. The experimental chicken flock parasite infestation level reduction effect induced by *Carica papaya* extract treatment on *Ascaridia galli and Eimeria sp* lasted almost 3 weeks. After the third week, the proportion of faecal samples positive for *Ascaridia galli* increased from 10 to 30%, a drastic increase of about 20%, and the proportion of samples positive for *Eimeria sp* increased from 30 to 55%, an increase of 25%. On the other hand, the effect of the conventional antiparasitic drug on *Ascaridia galli* and *Capillaria sp*. last more than four weeks and was constant throughout the experiment time period and beyond. The results of the herein experimental field study highlighted the effectiveness of dried seed meal of *Carica papaya* unripe mature fruit (green fruit) on *Eimeria sp.* and *Ascaridia galli* in traditional smallholder free-range local chicken production system. Difficulties related to producer's access to veterinary conventional drugs, the high cost of anticoccidial products (Dakpogan *et al.,* 2013b) on the one hand, and the problems related to the respect of drug exact posology and the presence of drug residues in poultry products on the other hand make the use of *Carica papaya* extract a great alternative to control parasitic diseases in family poultry

production system. The remedy is natural, accessible, easy to use and less costly. However, the most appropriate dose remains to be elucidated and the use of other organs of the plant for better efficacy need to be further investigated.

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