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Investigation of the effect of three medicinal plants on chicken *Eimeria tenella* coccidiosis

Hervé Brice Dakpogan[1*], Razaki Adiho Ossè[1], Sabbas Attindehou[1], Luc Hippolyte Dossa[2], Frédéric Houndonougbo[2], Armand Bienvenu Gbangboche[1] , Guy Apollinaire Mensah[3], Sahidou Salifou[4]

[1]*School of Animal Production System Management, P.O.BOX 43 Ketou, National Agricultural University, 01 P.O.BOX 55 Porto-Novo, Benin*

[2]*School of Animal Production Sciences and Techniques, Faculty of Agronomic Sciences 01 P.O.BOX 526, University of Abomey-Calavi, Benin*

[3]*National Agricultural Research Institute of Benin (INRAB), 01 P.O. BOX 884, Cotonou, Benin.*

[4]*National Veterinary Parasitology Laboratory, Polytechnic School of Abomey-Calavi P.O.BOX 2009, University of Abomey-calavi, Benin*

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Abstract

Resistance of some coccidia strains to conventional anticoccidial products and the presence of anticoccidial drug residues in poultry products, detrimental to consumer health have led to the search for alternative means of controlling coccidiosis. The aim of the current study was to evaluate the effect of leaf extract of *Morinda lucida*, *Combretum micranthum* and *Paullinia pinnata* on *Eimeria tenella* experimental coccidiosis in a completely randomized design. There were five experimental groups: *Morinda lucida*, *Combretum micranthum, Paullinia pinnata,* amprolium treated chicks group and finally the infected untreated control chicks group. Body weight gain, feed conversion ratio, lesion score, proportion of bloody droppings, survivability, morbidity and oocyst excretion were evaluated. The results demonstrated the efficacy of *Morinda lucida* on *Eimeria tenella* coccidiosis comparable to that of amprolium, the conventional anticoccidial product, in terms of improved feed conversion ratio, total absence of bloody droppings, and reduction of oocyst excretion with a reduction rate of 81% compared to the untreated infected control group. *Morinda lucida* is followed in its effectiveness by *Combretum micranthum* in a lesser extent. *Morinda lucida* has proven effective in chicken coccidiosis controlling. However, investigations are needed to determine the appropriate dose, the appropriate extraction method, the parts of the plant with better anticoccidial activity, and the adequate mode of utilization, curative or preventive.

* **Corresponding Author:** Hervé Brice Dakpogan ✉ dakpogan2002@yahoo.fr

Introduction

Chicken (*Gallus gallus*) is one of the most efficient protein sources (Smil, 2002) and pathogens that could compromise its efficient productivity would pose serious threats to food security and human survival. Through the combination of ubiquity, fecundity and pathogenicity of its causative agent, coccidiosis is among the ten most endemic and economically disastrous diseases that affect livestock in both developed (Perry *et al*., 2002; Bennett and Ijpelaar, 2005; Al-Gawad *et al*., 2012) and developing countries.

The genus *Eimeria*, is a protozoan parasite, of the phylum Apicomplexa and the family Eimeridae, that causes coccidiosis in all animals, including chickens (McDougald, 2003). Seven species, *Eimeria acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox and E. tenella*, are known to infect domestic chicken (Williams *et al*., 2009). It is a widespread disease, present everywhere poultry farming is practiced (McDougald, 2003). Coccidia are present in the external environment as spores surrounded by a fairly resistant shell called oocyst (Williams, 1999). They become infectious two days after excretion and are directly ingested by healthy chickens. The life cycle of chicken coccidia is direct without an intermediate host. Sporozoites replication in the intestinal tract causes damage to the epithelial tissue, with interruption of nutrition, digestive processes, and nutrient absorption (McDougald, 2003). This, results in decreased weight gain, secondary infections and significant mortality (Tewari and Maharana, 2011).

To control coccidiosis, several anticoccidial products were successfully developed and used for several years (Long, 1982). Unfortunately, the effectiveness of anticoccidians in the control of coccidiosis becomes problematic because of the side effect of certain anticoccidians on birds, their misuse by poultry producers, their natural ineffectiveness and finally the resistance of some coccidian strains against anticoccidians (Long, 1982; Shirley *et al*., 2007; Chapman *et al*., 2010). Sørensen *et al*. (2006) estimated the total annual cost of clinical coccidiosis losses, its sub-clinical form and its control to more than € 2.3 billion worldwide. Similarly, the presence of anticoccidial residues in poultry products is prejudicial to consumer health (Cannavan *et al*., 2000; Mortier *et al*., 2005; Danaher *et al*., 2008). Improved use of the immune competence of birds for the control of coccidiosis has led to the development of recombinant vaccines based on vaccine serotypes and parasite-specific molecules (McDougald, 2003). However, live vaccines, regardless of their efficacy, must replicate in epithelial host cells to confer active immunity to the body. This replication phase leads to a subclinical morbid state with major effects of decreased performance and predisposition of the intestinal tract to opportunistic gastrointestinal bacterial infections such as necrotic enteritis (Mathis and Broussarde, 2006; Peek, 2010). Apart from the use of anticoccidial drugs and vaccines to control coccidiosis, several studies have addressed the use of medicinal plants against the disease with promising reports (Oh *et al*., 1995; Youn and Noh, 2001; Elmusharaf *et al*., 2006; McCann *et al*., 2006; Peek and Landman, 2006; Abbas *et al*., 2012; Kheirabadi *et al*., 2014; Drägan *et al*., 2014).

The aim of this study was to evaluate the anticoccidial activity of *Combretum micranthum, Morinda lucida* and *Paullinia pinnata* on chicken *Eimeria tenella* coccidiosis.

Materials and Methods

Day-old chicks

Seventy-five (75) day-old Isa-brown male chicks were reared on a deep litter-floured starting pen. The rearing initial temperature was 35 °C and 22 hours lighting up to 22 day-old before being allocated to the experimental groups in the Poultry Research Station of the Benin National Agricultural University. Chicks had free access to feed and drinking water and were vaccinated against Newcastle disease, Infectious bronchitis and Infectious bursal disease.

Experimental infection

Oocysts of *Eimeria tenella* preserved in 2% potassium

dichromate solution were generously provided by the infectiology laboratory of INRA, Tours, France and kept in a refrigerator (2-5 °C) until use. The coccidian-free status of experimental infection-candidate chick was confirmed by faecal examination 24 hours preceding the inoculation. Each 23 day-old coccidia-free chick was challenged orally with 15 000 infectious oocysts.

Herb extract and anticoccidial drug

Leaves of *Combretum micranthum*, *Morinda lucida and Paullinia pinnata,* collected at the flowering stage were washed and dried in room temperature (30 °C) for 2 hours. After partial drying, the fresh leaves were weighed. One liter of boiled water was used for 100 g of fresh leaves. Water was boiled at 100 °C, and added to the leaves. After 30 minutes time period, the infusion was filtered, cooled at room temperature (30 °C) and served to the bird. This operation was repeated every morning during the five days treatment period. The infected chicks received the infusion *ad libitum* for five day post-infection (Koinarski *et al.,* 2005). Amprolium was the conventional anticoccidial molecule used at the dose of 0.6 g per liter of water, following the drug administration prescription.

Experimental groups and data collection

Seventy-five (75) *Eimeria tenella* experimentally infected 23 day-old chicks were randomly allocated to five treatment groups on the basis of 15 chicks per treatment (3 per group with 5 replications) in a completely randomized design. There were *Combretum micranthum*, treated chick group, *Morinda lucida* treated chick group and *Paullinia pinnata,* treated chick group, amprolium treated chick group and the untreated control chick group. The effectiveness of herb extracts was assessed on the basis of bloody diarrhea, survival rate, oocyst excretion, lesion score, and body weight gain and feed conversion ratio. The proportion of blood in feces from the third to seventh day post inoculation was evaluated. The survival rate was estimated from the number of surviving chicks divided by the number of initial chicks. Oocyst excretion (Soulsby, 1986) was recorded from 1 to 14 day patent period. The lesion scores were assessed (Johnson and Reid, 1970) at the 6th day post-infection. Feed consumption was recorded daily and chick body weight was recorded at the starting of the experiment and at the end of the first and second weeks post infection.

Statistical analysis

The descriptive and inferential analyses applied to oocyst excretion, body weight gain, feed conversion ratio and lesion score were made using the General Linear Model (GLM) procedure of SAS (vo. 9.2). Frequency procedure with Fisher test was used for survivability and morbidity estimation and comparison.

Results

Body weight gain and feed conversion ratio

In the first week post inoculation period, the average daily body weight gains observed in infected chick groups treated with *Combretum micranthum leaf extract*, amprolium the conventional anticoccidial drug and the untreated control chick groups were significantly higher ($p < 0.05$) than those of infected chick groups treated with *Morinda lucida* and *Paullinia pinnata* leaf extract (Table 1).

Table 1. Body weight gain and feed conversion ratio (Mean ± Standard Error).

Experimental groups	Body weight gains (gram)		Feed conversion ratio	
	Day 0 – day 6	Day 6 – day 14	Day 0 – day 6	Day 6 – day 14
Morinda lucida	$9.51^{a} \pm 0.80$	$7.40^{a} \pm 0.51$	$2.93^{a} \pm 0.22$	$2.62^{a} \pm 0.08$
Combretum micranthum	$12.48^{b} \pm 0.46$	$5.71^{ab} \pm 0.51$	$2.44^{bc} \pm 0.04$	$3.64^{bc} \pm 0.27$
Paullinia pinnata	$10.14^{a} \pm 0.79$	$5.77^{ab} \pm 0.69$	$2.81^{ab} \pm 0.20$	$3.26^{ab} \pm 0.23$
Amprolium	$12.99^{b} \pm 0.73$	$6.12^{ab} \pm 0.74$	$2.33^{c} \pm 0.11$	$3.55^{bc} \pm 0.36$
Control	$13.10^{b} \pm 0.49$	$5.24^{b} \pm 0.53$	$2.43^{bc} \pm 0.07$	$4.06^{c} \pm 0.21$

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

This trend was reversed in the second week; with the average daily body weight gain of the infected untreated control chick group lower ($p < 0.05$) than that of the infected chicks treated with *Morinda lucida* leaf extract. The highest values of feed conversion ratio were recorded in chicks infected and treated with medicinal plant leaf extract in the first week post inoculation period (Table 1). On the other hand, in the second week, the best feed conversion ratio was observed in the infected chicks treated with *Morinda lucida* leaf extract.

Table 2. Lesion scores, bloody dropping, morbidity and mortality.

Experimental groups	Lesion score (M ± SE)	Proportion of bloody feces (M ± SE)	Survivability (%)	Morbidity (%)
Morinda lucida	$3.00^{a} \pm 0.40$	$0.00^{a} \pm 1.97$	100	100
Combretum micranthum	$2.20^{a} \pm 0.90$	$4.34^{ac} \pm 2.74$	100	100
Paullinia pinnata	$2.80^{a} \pm 0.80$	$2.66^{ac} \pm 2.66$	100	100
Amprolium	$0.00^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	100	100
Control	$2.40^{a} \pm 0.90$	$9.81^{bc} \pm 4.45$	100	100

M: Mean, **SE:** Standard Error, %: percentage, (Values in columns that do not share the same superscript letters are significantly different at the significance level of 0.05).

Lesion scores, bloody diarrhea and mortality

Results of lesion scores, proportion of bloody dropping, mortality and morbidity are showed in Table 2. Lesion score was significantly lower in infected chicks treated with the conventional anticoccidial drug ($p < 0.05$). It is followed by the chick group infected and treated with *Combretum micranthum* leaf extract. A total absence of bloody droppings was observed in infected chicks treated with *Morinda lucida* leaf extract and amprolium ($p < 0.05$). Indeed, the entire infected chicks were diseased with coccidiosis specific diarrhea. However, no mortality was ever recorded among the chicks during the experiment.

Oocyst excretion

Oocyst excretion observed in chicks treated with the medicinal plant leaf extracts was lower ($p < 0.05$) and comparable of that of the conventional anticoccidian treated group (Table 3). The 7th day patent period was marked by a complete absence of oocysts in the feces of all the experimental groups except, the infected chick group treated with *Combretum micranthum* leaf extract and the infected untreated control chick group. Oocysts excretion variability was more marked in the first two-day patent period.

Discussion

Three medicinal plant leaf extract were used in this study against *Eimeria tenella* experimental coccidiosis: *Morinda lucida, Combretum micranthum* and *Paullinia pinnata*. Among them only *Morinda lucida* leaf extract treatment results were comparable to the results of amprolium, the conventional anticoccidial product, with lower feed conversion ratio, total absence of blood in the feces and reduced oocyst excretion. *Morinda lucida* leaf extract treatment against coccidiosis is followed in its effectiveness by *Combretum micranthum*. Several authors have reported the anticoccidial activity of various medicinal plants: *Sophora flavescens* (Youn and Noh, 2001), *Andrographis paniculata* (Sujikara, 2000), *Allium sativum, Salvia officinalis, Echinacea purpurea, Thymus vulgaris* and *Origanum vulgare* (Arczewska-Wlosek and Swiatkiewicz, 2010), *Fomes fomentarius* (Shazia, 2013), *Artemisia annua* (Emilio *et al.,* 2010; Loredana *et al.,* 2015), *Khaya senegalensis* (Gotep *et al.,* 2016), *Carica papaya* (Dakpogan *et al.,* 2018). *Morinda lucida* is a plant of the family Rubiaceae found in the tropical forest areas of West Africa. The current results agreed with the efficacy of *Morinda lucida* acetone-extract observed by Ola-Fadunsin and Ademola (2014) on broiler

chicks naturally infected with field strains of *Eimeria* species in Nigeria with improved growth performance and coccidian oocyst production inhibition. *Morinda lucida*-induced growth performance in terms of improved daily body weight gain and feed conversion ratio observed in the herein study might be ascribed to the less damage on intestinal epithelium and the significant coccidia oocyst production inhibition. *Morinda lucida* is one of the four best herbs used for medicinal purposes against fevers, various organs of the plant such as roots, bark, leaves are used in decoction or infusion against yellow fever, malaria, trypanosomiasis, feverish conditions during childbirth (Ademola *et al.,* 2013).

Table 3. Excretion of oocysts (OPG) in experimental chicks groups (Mean × 10²).

Patent period	*Morinda lucida*	*Combretum micranthum*	*Paullinia pinnata*	Amprolium	Control
Day 1	42.20^{ab}	54.70^{ab}	40^{ab}	1.40^{a}	104.30^{b}
Day 2	308.80^{ab}	683.80^{ab}	945.96^{ab}	26.10^{a}	1700.90^{b}
Day 3	242.00^{a}	308^{a}	525.44^{a}	350.80^{a}	820.80^{a}
Day 4	191.10^{a}	300^{a}	603.40^{a}	179.20^{a}	1543.20^{b}
Day 5	59.90^{a}	7.96^{a}	175.22^{a}	9.40^{a}	138.40^{a}
Day 6	2.80^{a}	2.80^{a}	3.40^{a}	1.20^{a}	48.80^{a}
Day 7	0.00^{a}	3.640^{b}	0.00^{a}	0.00^{a}	5.60^{b}
Total	846.80	1393.66	2293.42	568.10	4362.00

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

The major constituents of *Morinda lucida* are various types of alkaloids and anthraquinones (Adesogan, 1973). Ten (10) anthraquinone compounds were isolated from the stem of the plant and characterized (Lawal *et al.,* 2012). Ogunlana *et al.* (2008) reported antioxidant properties of *Morinda lucida* phenolic compounds. Allen *et al.* (1998) observed that antioxidant-active plants have anticoccidial activity.

The anticoccidial efficacy of *Morinda lucida* observed in this study can be ascribed to the antioxidant properties of the various chemical constituents of the plant. These antioxidants are involved in inhibition of oxidative stress caused by coccidia and the immune system response of the host organism.

The efficacy of *Morinda lucida* leaf extract in reducing coccidian oocyst excretion of experimentally infected chicks is followed by that of *Combretum micranthum* commonly referred to as Kinkeliba. According to Eloff *et al.* (2008), species of Combretaceae family possess chemical compounds that are active on microbes such as viruses, bacteria and fungi. Antibacterial (Banfi *et al.,* 2014) and antiviral activities (Mouzouvi *et al.,* 2014) of Combretum *micranthum* were reported. The second plant *Paullinia pinnata* reduced to almost less than half level the oocyst excretion of infected untreated control chick group.

It is a plant of the family Sapindaceae whose constituents are tannins, steroids (Tokoudagba *et al.,* 2018) with antioxidant properties (Jimoh *et al.,* 2007) used against human parasitic diseases, especially the root hydroalcoholic extract of the plant (Spiegler *et al.,* 2016).

Conclusion

Indeed, *Morinda lucida* and *Combretum micranthum* revealed an anticoccidial activity comparable to that of amprolium, the conventional anticoccidial product, used in this study.

However, future investigations are needed to determine the effectiveness of different parts of the plant, the appropriate effective dose, the development of the active ingredient by spectroscopic study and the best mode of the plant extract utilization preventive or curative.

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