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Evaluation of the effect of *Phyllanthus amarus*, *Jatropha curcas* and *Piliostigma thonningii* on experimental chicken coccidiosis

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1 SUMMARY

The anticoccidial activity of *Phyllanthus amarus* (Hurricane weed), *Jatropha curcas* (purging nut) and *Piliostigma thonningii* (monkey bread) was tested on seventy five *Eimeria tenella* infected Isa-brown male day-old chicks in a completely randomized design as an alternative measure of controlling coccidiosis. Each chick was orally challenged with 15 000 *Eimeria tenella* sporulated oocysts. There were five groups infected chicks. The first, second and third groups received , the decoction of *Phyllanthus amarus, Jatropha curcas* and *Piliostigma thonningii, ad libitum* respectively for five days post-inoculation as drinking beverage. The fourth group was treated with Amprolium orally for also five days post-infection and the fifth group was the infected untreated control. Body weight gain, feed conversion ratio, lesion score, proportion of bloody droppings, survivability, morbidity and oocyst excretion were evaluated. The results showed an efficacy of *Phyllanthus amarus* in the reduction of oocyst excretion with a reduction rate of 87% compared with the infected untreated control group oocyst excretion. Moreover, macroscopic lesion intensity reduction and low presence of bloody diarrhoea were observed with the *Phyllanthus amarus* treated chicks. The oocyst excretion reduction rate was 74% with *Jatropha curcas* infected treated chicks. The growth performance results were similar among the infected treated chick groups. *Piliostigma thonningii* was less effective in reducing oocyst excretion compared with the other two medicinal plants. Further spectroscopic studies are needed to value the active anticoccidial ingredients in these plants.

2 INTRODUCTION

Coccidiosis is caused by intracellular protozoa that parasitize the host digestive tract. The family of Eimeriidae includes about 25 genera. The best known of them are the genera Eimeria, Isospora and Tyzzeria, with several species which are of great medical and veterinary importance. Avian coccidiosis is one of the major economically devastating parasitic diseases in poultry industry worldwide. Therefore, it is known as a limiting factor for poultry production (Bichet *et al.,* 2003). Chickens are infected by seven different species

of *Eimeria*: *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox and E. tenella*. The transmission between birds is done by ingestion of infectious oocysts (Del Cacho *et al.,* 2010). *Eimeria brunetti, E. necatrix* and *E. tenella* are associated with haemorrhagic coccidiosis and can be highly pathogenic, with high morbidity and some mortality (Long *et al.,* 1982). The remaining four species are usually less pathogenic, causing malabsorptive pathologies, although morbidity and mortality can occur depending on dose ingested, parasite strain-specific variation in virulence and host factors such as age, breed and immune status (Long *et al.,* 1976; Williams *et al.,* 2009). The different in-host stages of Eimeria spp. invade intestine cells (enterocytes), and replicate resulting in variable pathological changes (Tarek *et al.,* 2016). The changes range from local destruction of the mucosal barrier and underlying tissue, to systemic effects such as blood loss, and death (Yun *et al.,* 2000). Symptoms are associated with drop in egg production, impaired growth rate due to nutrients malabsorption in adults, necrotic enteritis due to *Clostridium perfringens*, and high mortality rates, especially, in young birds (Tewari and Maharana, 2011). Progress has been made in the prevention and control of coccidiosis by chemotherapy, particularly with the use of anticoccidial drugs and vaccination. The primary means of coccidiosis control is by prophylactic administration of in-feed anticoccidial drugs, although resistance is ubiquitous (Shirley *et al.,* 2007). The economical loss due to coccidiosis is estimated at US $ 3 billion globally (Dalloul and Lillehoj, 2006). Carington *et al.* (2007) in South Africa and Dakpogan *et al.* (2013) in Benin have estimated the financial cost of anticoccidial drugs used per laying hen during its life cycle at US $ 0.30. The intensive use of anticoccidial drug is increasingly worrisome because of anticoccidial drug-resistant coccidia strain emergence (Dakpogan *et al.,* 2018) and the presence of drug residues in poultry products prejudicial to consumer health (Cannavan *et al.,* 2000 ; Mortier *et al.,* 2005 ; Danaher *et al.,* 2008). Conventional vaccines were developed to control coccidiosis (Williams 2002; Chapman *et al.,* 2002; Li *et al.,* 2005). Vaccines include live vaccines or live attenuated vaccines which may cause moderate or more severe vaccine reactions (Vermeulen *et al.,* 2001). The replication of live vaccine in the intestinal cells can be a predisposing factor to many gastrointestinal bacterial pathogens such as *Clostridium perfringens and Escherichia coli*. Also, relative to cost, the requirement for multiple parasite lines and production capacity prove limiting (Shirley *et al.,* 2005). Notwithstanding all of these issues, live vaccines work well when applied in the field and there is to date no substantive evidence that their use over several decades has driven selection of parasite populations towards resistance and immune escape (Shirley *et al.,* 2005). However, the development and application of next generation subunit or vectored vaccines based on the expression of a single, or a small number, of *Eimeria* antigens could drive more targeted immune selection, leading to the rapid appearance and dissemination of vaccine resistance in the field (Blake *et al.,* 2017). Consequently, alternative methods of coccidiosis control have become increasingly attractive. The anticoccidial alternative methods include management and biosecurity (Peek, 2010), Phytotherapy (Youn and Noh, 2001 ; Naidoo *et al.,* 2008 ; Arczewska and Swiatkiewicz, 2010 ; Peek and Landman 2011 ; Kheirabadi *et al.,* 2014 ; Dragan *et al.,* 2014 ; Dakpogan *et al.,* 2018), Prebiotics (Elmusharaf *et al.,* 2006 ; McCann *et al.,* 2006) and probiotics (Peek, 2010 ; Abbas *et al.,* 2012) with promising reported results. The current study came up with the results of the anticoccidial effect of *Phyllanthus amarus*, *Piliostigma thonningii*, *and Jatropha curcas* fresh leaves extract on chick *Eimeria tenella* coccidiosis.

3 MATERIALS AND METHODS

3.1 Day-old chicks: Seventy five (75) day-old Isa-brown male chicks were housed in a deep litter-floured starting pen, under 22 hours lighting and held at initially 35 °C up to 22 day-old before being allocated to the experimental groups in the National Agricultural University poultry research station. The chicks had free access to feed and drinking water. Vaccination against Newcastle disease, Infectious bronchitis and Infectious bursal disease was the basic applied biosecurity measures.

3.2 *Eimeria tenella* oocysts and inoculation : *Eimeria tenella* oocysts preserved in 2% potassium dichromate solution were generously provided by the Department of Pathology and Pathogen Biology, Royal Veterinary College, North Mymms, Hertfordshire, UK and kept in a refrigerator (2-5 °C) until use. All the feces produced by each group of birds, during the 24 h preceding the experimental infection, were examined to confirm the absence of any oocyst. Each 23 day-old coccidia-free chick was challenged orally with a dose of 15 000 oocysts.

3.3 Herb extract and anticoccidial drug: The leaves of the three plants *Phyllanthus amarus, Jatropha curcas* and *Piliostigma thonningii* were collected at the flowering stage. They were washed and then dried in room temperature (30 °C) for 2 hours. After partial drying, the fresh leaves were weighed. One litre of boiled water was used for 100 g of fresh leaves. Tap water was boiled at 100 °C, removed from the fire 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 days post-infection corresponding to the period of oxidant insult induced by the coccidian parasite (Koinarski *et al.,* 2005). Amprolium was the conventional anticoccidial molecule used at the dose of 0.6 g per litre of water following the drug administration prescription.

Phyllanthus amarus

Jatropha curcas

Piliostigma thonningii

3.4 Experimental groups and data collection: Seventy-five (75) *Eimeria tenella* oocysts experimentally infected 23 day-old chicks were randomly assigned to five treatment groups on the basis of 15 subjects per treatment (3 per group with 5 replications) in a completely randomized design. There were *Phyllanthus amarus* treated chicks group, *Jatropha curcas* treated chicks group, *Piliostigma thonningii* treated chicks group, amprolium treated chicks

group and the infected untreated control chicks group. The effectiveness of herb extracts was assessed on the basis of bloody diarrhoea, survival rate, oocysts excretion, lesion score, body weight gain and feed conversion ratio. The proportion of blood in feces from the third to seventh day post inoculation was evaluated by considering the amount of bloody faeces per bird divided by the total number of faeces excreted per bird, during this time period. The survival rate was estimated from the number of surviving chicks divided by the number of initial chicks. Oocysts excretion (Soulsby, 1986) was recorded from 6 to 14 day post inoculation. The method consist in diluting one gram of faeces in 30 ml of saturated sodium chloride flotation fluid. The resulting fluid was homogenized by thorough stirring. The faecal suspension was filtred and a subsample immediately taken with a Pasteur pipette. The two fields of the McMaster counting chamber were filled with the faecal suspension. After 3 mn, the *Eimeri*a oocysts in both counting fields were counted under 10 photonic microscope magnification. The number of Oocysts Per Gram of faeces (OPG) was determined by multiplying the number of counted oocysts by 200. The lesion scores were assessed (Johnson and Reid, 1970) at the 6th day post-infection. The method consist in attributing the score 0 when there is a total absence of macroscopic lesions in the caecum and the score 1 when there are some few petechiae scattered on the caecal wall. The score 2 is given when the caecal wall is thickened and the score 3 when a large amounts of blood and tissue debris occupy the caecum. The score 4 is attributed when there is liquid blood or coagulated clot in a distended caecum. Chick body weights and feed consumption in each group were recorded at the starting of the experiment and at the end of the first and the second weeks after experimental infection.

3.5 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.

4 RESULTS

Body weight gain and feed conversion ratio of the infected chicks groups treated with amprolium, *Phyllanthus amarus*, *Jatropha curcas,* and *Piliostigma thonningii* were significantly higher than those of the untreated infected control chick group at the end of the first week post-infection ($p < 0.05$). However, these feed utilization efficiency parameters were the same at the end of the second week among all the groups ($p > 0.05$).

Table 1: Body weight gain and feed conversion ratio (M ± SE) of infected chicks

Experimental groups	Body weight gains (g)		Feed conversion ratio	
	Day 0 – day 6	Day 6 – day 14	Day 0 – day 6	Day 6 – day 14
Placebo	$6.77^{a} \pm 0.34$	$6.97^{a} \pm 0.85$	$4.04^{a} \pm 0.24$	$3.6^{a} \pm 0.27$
Amprolium	$10^{b} \pm 0.46$	$6.03^{a} \pm 1.09$	$2.82^{b} \pm 0.12$	$3.08^{b} \pm 0.06$
Phyllanthus amarus	$9.31^{b} \pm 0.40$	$5.60^{a} \pm 0.72$	$2.91^{b} \pm 0.27$	$3.37^{ab} \pm 0.05$
Piliostigma thonningii	$10.20^{b} \pm 0.53$	$6.37^{a} \pm 0.44$	$2.95^{b} \pm 0.17$	$3.65^{a} \pm 0.06$
Jatropha curcas	$10.58^{b} \pm 1.30$	$6.40^{a} \pm 0.71$	$2.46^{b} \pm 0.43$	$3.40^{ab} \pm 0.10$

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

The lesions scores and the proportion of bloody feces of the infected chick groups treated with amprolium and the medicinal plant extract such as *Phyllanthus amarus*, *Jatropha curcas,* and *Piliostigma thonningii* were significantly lower ($p < 0.05$) than those observed in the infected untreated control chick groups. The lowest values were recorded in the *Phyllanthus amarus* extract treated chicks. The overall morbidity rate was 100%, however, no death was ever recorded among all the groups during the experimentation time period.

Table 2: Lesion scores, bloody droppings and mortality

Experimental groups	Lesions scores (M ± SE)	Proportion of bloody feces (M ± SE)	Survivability (%)	Morbidity (%)
Placebo	$3.40^{a} \pm 0.2$	$13.81^{a} \pm 0.60$	100	100
Amprolium	$0.80^{b} \pm 0.3$	$1.33^{b} \pm 1.33$	100	100
Phyllanthus amarus	$0.60^{b} \pm 0.4$	$1.11^{b} \pm 1.11$	100	100
Piliostigma thonningii	$0.60^{b} \pm 0.4$	$1.33^{b} \pm 1.33$	100	100
Jatropha curcas	$1.20^{b} \pm 0.5$	$2.16^{b} \pm 1.32$	100	100

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

The oocyst excretions varied significantly among the experimental chick groups during the first 7 days post-infection. Chicks infected and treated with *Phyllanthus amarus* extract excreted significantly fewer oocysts (45.860) than all the other chick groups including the conventional anticoccidial drug treated chick group (180.556). The oocysts excretion reduction rate of *Phyllanthus amarus* extract treated chick group was 87% compared with that of the infected untreated control group. These reduction rates were 74 and 65% for *Jatropha curcas* and *Piliostigma thonningii* respectively.

Table 3: Number of Oocysts Per Gram (OPG) (Mean x 10^2)

Patent period	Placebo	Amprolium	*Phyllanthus amarus*	*Piliostigma thonningii*	*Jatropha curcas*
Day 1	468^{a}	630.40^{b}	213.20^{c}	504^{bc}	259.60^{bc}
Day 2	1129.20^{a}	518.40^{b}	138.40^{c}	189.20^{bc}	241.20^{bc}
Day 3	925.60^{a}	268.40^{b}	76.40^{b}	124^{b}	189^{b}
Day 4	630.80^{a}	106.80^{b}	19.40^{b}	71.20^{b}	129.20^{b}
Day 5	83.66^{a}	69.36^{ac}	9.20^{b}	39.20^{bc}	45.60^{ab}
Day 6	33.36^{a}	12.20^{ab}	2^{b}	17.20^{ab}	10^{b}
Day 7	173.20^{a}	200^{a}	0^{b}	320^{a}	50^{b}
Total OPG	**3443.82^{a}**	**1805.56^{c}**	**458.6^{b}**	**1264.8^{c}**	**924.6^{b}**

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

4 DISCUSSION

The best growth performance and parasitological results were observed in *Eimeria tenella* infected chicks, treated with herb extracts with higher body weight gain, lower feed conversion ratio, light post mortem ceacal lesions, lower proportion of bloody droppings and significant fewer oocysts excretion, comparable to the conventional anticoccidian

effect. The beneficial effect of medicinal plants in the control of avian coccidiosis was observed by several authors (Gotep *et al.,* 2016; Dakpogan *et al.,* 2018). The herein results agreed with many other findings about the effectiveness of herb extracts utilization against chicken coccidiosis. The most appealing include the anticoccidial activity of: *Sofora flavescens* (Youn and Noh, 2001), *Tulbaghia violacea* (Naidoo *et al.,* 2008), *Andrographis paniculata* (Arczewska and Swiatkiewicz, 2010), *Artemisia spp.* (Oh *et al.,* 1995 ; Allen *et al.,* 1997 ; Tamasaukas *et al.,* 1997 ; Arab *et al.,* 2006 ; Kheirabadi *et al.,* 2014; Dragan *et al.,* 2014 ; JinYing *et al.,* 2018), *Carica papaya* and *Vernonia amygdalina* (Dakpogan *et al.,* 2018). *Phyllantus amarus* has proven to be the most effective anticoccidian among the three medicinal plants used in this study in terms of number of oocysts per gram in feces reduction with a reduction rate of 87%, when compared with the untreated infected control. *Phyllanthus amarus* is a plant of the family of Euphorbiaceae, perennial and widely distributed in tropical and subtropical countries (Mazumder *et al.,* 2006, Tahseen and Mishra, 2013). Ethnobotanically, several different organs of the plant are used against diabetes, inflammation, gastrointestinal disorders, urinary disorders, ulcers, cancer and microbial infections (Rajeshkumar *et al.,* 2002; Khatoon *et al.,* 2004; Saranraj and Sivasakthivelan, 2012; Ushie *et al.,* 2013). The antioxidant activity (Lim and Murtijaya 2007) and the antiplasmodic effect (Ajala *et al.,* 2011) of *Phyllanthus amarus* were also reported. The anti-parasitic activity of *Phyllanthus amarus* was tested and known by many researchers. Tolulope *et al.* (2011), in Nigeria observed the efficacy of *Phyllanthus amarus* on the resistant form of *Plasmodium yoelii* in albino rats as a preventive and curative treatment measures. The antioxidant activity of *Phyllanthus amarus* was observed in malarious albino rats with serum increase of antioxidants such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase and decrease of malonic aldehyde. The serum increase of malonic aldehyde is a manifestation of the oxidative stress observed in the pathogenesis of parasitic diseases (Ojezele *et al.,* 2017). The pathogenesis of coccidiosis is associated with oxidative stress caused by increased production of Reactive Oxygen Species due to parasite activities as well as the immune response of the host organism causing depletion of antioxidants enzyme levels, such as glutathione, a potent redox and the increase of lipid peroxidation of enterocytes and cells surrounding the intestines (Gotep *et al.,* 2016). The significant reduction in lesion score and oocysts excretion induced by *Phyllantus amarus* could be attributed to its chemical compounds content such as phyllantine and hypophyllantine (Kushwaha *et al.,* 2013), normalizers of Reactive Oxygen Species (ROS), which are oxidants of unsaturated fatty acids with a destructive effect on cellular organelles especially mitochondria. The anticoccidial activity of *Jatropha curcas* observed in this study is similar to that of *Phyllanthus amarus* with a better feed conversion ratio and a reduction rate of oocysts per gram of feces of 74% when compared to the infected untreated control group. Both plants belong to the same family of Euphorbiaceae. *Jatropha curcas* contains several chemical compounds such as alkaloids, saponins, steroids, diterpernoids known for their anti-parasitic effects (Catherine *et al.,* 1997; Staubmann *et al.,* 1999a; Aiyelaagbe *et al.,* 2007; Igbinosa *et al.,* 2009). The leaves of *Jatropha curcas* are used to treat wounds, parasitic diseases including malaria, microbial infections, dysentery (Matsuse *et al.,* 1999; Staubmann *et al.,* 1999b; Parveen *et al.,* 2007; Igbinosa *et al.,* 2009).

Piliostigma thonningii was less effective than the two previous plants in terms of oocyst excretion reduction with a reduction rate of 65% compared to the control group oocysts excretion. The antibacterial activity of *Piliostgma thonningii* (Akinpelu and Obuotor, 2000) and its prophylactic and curative effect on lipid peroxidation involved in cardiovascular disorders (Ighodaro and Omole, 2012) were reported.

Indeed, the herein results demonstrated the anticoccidial activity of Benin three medicinal

plants *Phyllanthus amarus, Jatropha curcas* and in a lesser extent *Piliostigma thonningii*. These plants are used in traditional medicine to prevent and cure malaria. However, further investigations are needed to determine the comparative efficacy in preventive and curative treatment of the plant extracts, the appropriate extraction technique, the best active dose on coccidia and the best organ with potential anticoccidial activity.

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