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Anticoccidial activity of *Khaya senegalensis*, *Senna siamea* and *Chamaecrista rotundifolia* in chicken (*Gallus gallus*)

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ABSTRACT

Anticoccidial drug residues in poultry products, prejudicial to consumer health and resistance problem of some *Eimeria* strain to conventional drugs have led to the search of alternative ways of controlling coccidiosis. The aim of this study was to evaluate the anticoccidial activity of the extract of *Khaya senegalensis, Chamaecrista rotundifolia,* and *Senna siamea* in a completely randomized design. The study was carried out on 23 day-old male chicks (*Gallus gallus*) experimentally infected with 15 000 oocysts of *Eimeria tenella* and treated with the extract of these plants. There were five (5) experimental groups. Three (3) infected groups treated with leaf extract of *Senna siamea*, *Chamaecrista rotundifolia* and tree balk extract of *Khaya senegalensis.* The tow (2) remaining groups were the infected treated with amprolium a conventional anticoccidial drug and the infected untreated control. Body weight gain, feed efficiency, lesion score, proportion of bloody droppings, and excretion of coccidia oocysts were assessed during 14 days post infection. The results showed the effectiveness of the extract of *Senna siamea* leaves (cassia) and *Khaya senegalensis* bark, through the improvement of body weight gain and feed conversion ratio and the reduction of lesion scores and oocysts excretion. The oocysts excretion reduction rate was 80% for *Senna siamea* and *Khaya senegalensis* compared to the infected untreated control group. *Senna siamea* and *Khaya senegalensis* extract demonstrated an obvious anticoccidial effect in the current study. However, further investigations are needed to determine the appropriate doses, the best mode of the herbs utilization, preventive or curative, the best extraction method of active ingredient and the parts of the plants with better anticoccidial effect.

Keywords: *Khaya senegalensis, Chamaecrista rotundifolia, Senna siamea,* coccidiosis, *Eimeria tenailla,* chicken.

INTRODUCTION

Poultry production plays a fundamental role in food security because of its short production cycle and remains an animal protein source easily accessible. Chicken is one of the most efficient source of animal-derived protein (Smil, 2002). Thus, pathogens that compromise the efficiency of chicken

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production can pose a serious threat to global food supplies and human poverty (Perry et al., 2002; Godfray et al., 2010). Through a combination of parasite ubiquity, fecundity and pathogenicity, coccidiosis is among the 10 most economically significant endemic livestock diseases (Perry et al., 2002, Bennett and Ijpelaar, 2005).

Coccidiosis is a parasitic disease that affects animals such as poultry, mammals, rodents and lagomorphs, etc. and caused by a protozoan of the phylum Apicomplex, family Eimeridae and genus *Eimeria* (Dakpogan et al., 2018). It is a digestive parasitosis due to the presence and replication of coccidian sporozoïtes in the digestive epithelium of birds and is characterized by a massive destruction of epithelial cells with clinical signs such as severe haemorrhagic diarrhea that can lead to death with 50 % or even 90% mortality rate especially in young chickens (Vermeulen et al., 2001). Seven species of pathological importance are known to affect chicken: *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria necatrix, Eimeria tenella, Eimeria praecox* and *Eimeria mitis* (Mc Dougald, 2003). The replication of sporozoites in the intestinal tract leads to lesions of epithelial tissue, with interruption of nutrition, digestive process and nutrient absoption (Mc Dougald, 2003). The consequences of these lesions are: laying reduction, growth impairment and high mortality among young animals (Tewari and Maharana, 2011).

Coccidiosis prevention and control measures are based on the use of anticoccidial products in the feed and the routine anticoccidial preventive treatment and vaccination. However, *Eimeria* resistant strain emergence (Bichet et al., 2003, Chapman, 2007, Abbas, 2008), the presence of drug residues in poultry products (Cannavan et al., 2000, Mortier et al., 2005; Danaher et al., 2008) prejudicial to consumer health and the sub-clinical form of the disease caused by the replication of coccidia vaccine strains in enterocytes with necrotic enteritis (Shirley et al., 2007 Bostvironnois and Zadjian 2011), pose serious threats to the chicken sector. In addition, the economic incidence of the disease is estimated at 2.3 billion Euros worldwide, with 70% of the losses attributable to the subclinical form of the disease, which depresses body weight gain and food consumption (Sorensen et al., 2006). The use of medicinal plants (Dakpogan et al., 2018), probiotics and prebiotics (Abbas et al., 2012; McCann et al., 2006) and biosecurity (Peek, 2010) as alternative coccidiosis control methods were reported with more or less satisfactory results.

The current study came up with the anticoccidial effect of *Khaya senegalensis, Senna siamea* and *Chamaecrista rotundifolia* extract in chicken (*Gallus gallus*).

MATERIALS AND METHODS

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 Benin 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.

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 *Eimeria tenella* oocysts.

Herb extract and anticoccidial drug

The leaves of *Senna siamea, Chamaecrista rotundifolia,* at the flowering stage and fresh tree bark of *Khaya senegalensis* were collected. They were washed and then dried in room temperature (30 °C) for 2 hours. After partial drying, they were weighed. One liter of boiled water was used for 100 g of fresh leaves and tree bark. Tape water was boiled at 100 °C and added to the leaves and tree bark. 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 liter of water following the drug administration prescription.

Experimental groups and data collection

Seventy-five (75) 23 day-old chicks experimentally infected with *Eimeria tenella* oocysts were randomly allocated 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 *Khaya senegalensis* treated chicks group, *Senna siamea* treated chicks group, *Chamaecrista rotundifolia* treated chicks group, amprolium treated chicks group and the untreated control chicks group. The effectiveness of herb extracts was accessed on the basis of bloody diarrhea, 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. 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 lesion scores were assessed (Johnson and Reid, 1970) at the 6^{th} day post-infection. 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.

Statistical analysis

The descriptive and inferential analyses applied to oocysts excretion, body weight gain, feed conversion ratio and lesion scores 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

The average daily body weight gain (table 1) of infected chicks treated with amprolium and medicinal plant extracts was significantly higher ($p < 0.05$) than that of the infected untreated control group in the first week post inoculation period. The daily body weight gain was reduced in the second week post inoculation period with statistically the same value ($p > 0.05$) among all the groups. The lowest body weight gains in the second week post inoculation period were observed in *Senna siamea* and amprolium infected treated chicks groups. Feed conversion ratio did not vary among the experimental groups in the first week. However, in the second week, the lowest feed conversion ratio ($p < 0.05$) was observed in *Khaya senegalensis* and *Senna siamea* treated chicks (Table 1).

Lesion scores and proportion of bloody dropping of infected chicks treated with conventional anticoccidial drug and herbal extracts were significantly lower ($p < 0.05$) than those of the infected untreated control group (table 2). A complete absence of bloody droppings was observed in infected chicks treated with *Khaya* *senegalensis* and *Chamaecrista rotundifolia* extract. All the infected birds expressed the disease, however, no mortality was ever recorded during the study (Table 2).

Oocyst excretion results are shown in Table 3. *Eimeria tenella* oocyst excretion of the infected treated chicks groups was significantly lower ($p < 0.05$) than that of the infected untreated control chick group in the first 3 days patent period. This difference remained statistically constant until the end of the experiment. The lowest oocyst excretions were recorded in the amprolium treated chick group (1020×10^2) followed by *Senna siamea* (1176×10^2) and *Khaya senegalensis* (1203×10^2) treated chicks groups.

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

Experimental groups	Body weight gains (g)		Feed conversion ratio	
	D 0 – D 6	D 6 – D 14	D 0 – D 6	D 6 – D 14
Control	$8.85^{a} \pm 0.30$	$3.72^{a} \pm 0.12$	$3.47^{a} \pm 0.77$	$3.81^{a} \pm 0.21$
Amprolium	$10.47^{b} \pm 0.18$	$2.88^{bc} \pm 0.16$	$3.67^{a} \pm 0.06$	$3.72^{ac} \pm 0.16$
Khaya senegalensis	$11.95^{cd} \pm 0.32$	$3.27^{ab} \pm 0.16$	$3.33^{a} \pm 0.21$	$3.08^{c} \pm 0.26$
Senna siamea	$11.29^{bc} \pm 0.42$	$2.67^{c} \pm 0.13$	$3.60^{a} \pm 0.31$	$3.03^{bc} \pm 0.18$
Chamaecrista rotundifolia	$12.38^{d} \pm 0.51$	$3.47^{a} \pm 0.22$	$3.56^{a} \pm 0.12$	$3.40^{ac} \pm 0.38$

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

Table 2: Lesion scores, bloody droppings and mortality of the infected chicks.

Experimental groups	Lesion score (M ± SE)	Proportion of bloody feces (M ± SE)	Survivability (%)	Morbidity (%)
Control	3.80a ± 0.2	$25.08^{a} \pm 7.19$	100	100
Amprolium	$1.80^{b} \pm 0.8$	$11.49^{b} \pm 4.99$	100	100
Khaya senegalensis	$1.60^{b} \pm 0.74$	$0.00^{b} \pm 0.00$	100	100
Senna siamea	$0.60^{b} \pm 0.24$	$1.81^{b} \pm 1.81$	100	100
Chamaecrista rotundifolia	$1.60^{b} \pm 0.81$	$0.00^{b} \pm 0.00$	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).

Table 3: Excretion of oocysts (OPG) in experimental chicks groups (Mean x 10^2).

Patent period	Control	Amprolium	*Khaya senegalensis*	*Senna siamea*	*Chamaecrista rotundifolia*
Day 1	1768.20^a	102.40^b	45.60^b	280.13^b	348.60^b
Day 2	1853^a	40.60^b	288^b	1.40^b	0.20^b
Day 3	706.80^a	106^b	96.80^b	95^b	264^b
Day 4	317.20^a	118.40^a	139.80^a	363.60^a	330.80^a
Day 5	296^a	$157a^b$	81.20^b	84.80^b	101.20^a
Day 6	239.20^a	222.40^a	451.40^a	160.40^a	270.40^a
Day 7	686.80^a	272.40^b	98.80^b	171.60^b	298^b
Total OPG	5875.58	1020.65	1203.31	1176.26	1615.50

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

DISCUSSION

In the first week post inoculation period, treatment with *Senna siamea*, *Khaya senegalensis* and *Chamaecrista rotundifolia* extract improved the infected chick body weight gain, feed conversion ratio comparable to the results induced by the conventional anticoccidial drug. Also, lower lesion scores, proportion of bloody droppings, and a reduction in the *Eimeria tenella* oocysts excretion were observed in infected treated chicks groups. The oocyst reduction rate was 80% both for *Senna siamea* and *Khaya senegalensis* extract treated chicks groups, compared to the control group. This rate is 73% for *Chamaecrista rotundifolia*. The findings are consistent with the results of Christaki et al. (2004) who obtained a reduction in lesion scores, bloody diarrhea and oocysts per gram in feces (OPG) with *Agrimonia eupatoria*, *Echinacea angustifolia*, *Ribes nigrum* and *Cinchonas uccirubra* extract treatment against chicken coccidiosis. Also, Dakpogan et al. (2018), reported a reduction in oocyst excretion rate of 56% in *Eimeria tenella* experimentally infected chicks, treated with *Carica papaya* extract. Other authors have reported the anticoccidial activity of several medicinal plants (Youn and Noh 2001, Sujikara 2000, Arczewska and Swiatkiewicz 2010, Emilio et al., 2013, Loredana et al. 2015). *Senna siamea* is a plant of the family Fabaceae commonly used in Benin to cure malaria and different forms of fevers (Adomou et al., 2012). The bark of *Senna siamea* contains compounds such as emodin and upoleole recognized as active on multi-resistant strains of *Plasmodium falciparum* (K1) (Ajaiyeoba et al., 2008). Alkaloids called Cassiarin A and B were isolated from the leaves and demonstrated an antiplasmodic effect (Hiroshi et al., 2007). Jignasu et al. (2013), identified flavenoid and phenolic compounds in the aerial parts of *Senna siamea* that have antioxidant and anti-inflammatory effects. Indeed, the efficacy of *Senna siamea* in lesion score and oocysts excretion reduction observed in the herein study might be ascribed to the beneficial action of antioxidants contained in the leaves, upon the oxidative stress induced by parasite activity and the immune response of the host organism, which can be destructive to cell's organelles. Also, tree bark of *Khaya senegalensis* acts like the leaf extract of *Senna siamea* upon coccidiosis pathogenesis, because it also contains antioxidants capable of reducing oxidative stress. Oxidative stress is a Reactive Oxygen Species (ROS)-induced oxidative reaction of unsaturated fatty acids, observed during the development of the disease, which cause chains reactions with harmful effect on cells, according to Gotep et al. (2016). The oxidative reactions entail in one hand, the depletion of antioxidant enzymes level such as glutathione a strong oxidoreducer and in the other hand, the increased of lipid peroxidation of enterocytes and cells surrounding the intestines. The plants extracts have an absorptive effect on cell's free radicals and a regulatory property toward the immune system demonstrated in rats (Dhkil et al., 2012). *Chamaecrista rotundifolia* did not remain inactive on *Eimeria tenella* oocysts excretion. However, its rate of reduction was lower than that of the other two plants and lower than that of the conventional anticoccidial product used. However, *Chamaecrista rotundifolia* induced better feed utilization and lesion score reduction.

Conclusion

The efficacy of *Senna siamea* leaf extract, *Khaya senegalensis* tree bark and *Chamaecrista rotundifolia* on *Eimeria tenella* coccidiosis was obvious in this study and was demonstrated through the improvement of the disease evaluation parameters. However, further investigations are needed to determine the appropriate doses, the best mode of medicinal plants utilization, preventive or curative, the best extraction method of active ingredient and the parts of the plants with better anticoccidial effect.

COMPETING INTERESTS

The authors declare that they have no competing interests regarding this study.

AUTHORS' CONTRIBUTIONS

A part from the sporulated oocysts, all the other materials used in the herein works were provided by all the authors. The study was designed by all of them and its carrying out, performed by the first, the second and the third authors. The proofreading was done by all the authors.

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