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REGULAR ARTICLES

Anticoccidial effects of *Khaya senegalensis* aqueous stem bark extract on broiler chickens experimentally infected with Eimeria species

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Abstract

Graded concentrations (200, 400 and 800 mg/kg) of the aqueous stem bark extract of *Khaya senegalensis* was evaluated for its therapeutic efficacy against experimentally induced coccidiosis in broiler chicken. The phytochemical analysis shows the presence of tannins, saponins, cardiac glycosides and steroids. There was significant reduction in oocyst count across the groups in a graded dose manner with 800 mg/kg being the most efficacious dose. There was also weight gain across the treatment groups with immunomodulatory and erythropoetic activities observed. Also, a significant ($p < 0.05$) graded dose-dependent reduction in the oocyst count in the treatment groups. A significant ($p < 0.05$) increase in mean weight gain was also recorded across the experimental groups except the negative control. The haematology also showed a dose-dependent increase in red blood cells, haemoglobin and packed cell volume of the treatment groups. The extract had no significant difference ($p > 0.05$) on the white blood cells, but a slight decrease in the white blood cells and heterophil counts was observed at 400 mg/kg. Furthermore, the aspartate amino transaminase level showed a significant difference ($p < 0.05$). Fluctuating levels of other serum biochemical parameters such as total protein, albumin and potassium were observed. No significant difference ($p > 0.05$) in the sodium concentration was observed. In addition, oxidative stress biomarkers such as catalase significantly increased ($p < 0.05$) in all the experimental groups in addition to the concomitant increase in reduced gluthathione (GSH) and superoxide dismutase (SOD) levels. Conclusively, the aqueous extract of *K. senegalensis* was effective in the management of coccidiosis thus supporting its folkloric use.

Keywords *Eimeria* · *Khaya senagalensis* · Anticoccidial · Antioxidant · Broiler chicken

Introduction

Poultry farming is one of the fastest growing sectors of animal production in the world (Mottet and Tempio 2017). In Nigeria, many people depend on small-scale poultry production systems for food, currency, work and business, but diseases such as coccidiosis are a major limiting factor (Perry et al.2002).

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Coccidiosis is one of the most important diseases of poultry worldwide that is characterized by enteritis. It is caused by genus *Eimeria* in chicken, which undergo a direct life cycle with transmission between hosts by way of a resistant oocyst (Habibi et al. 2016). The disease has significant economic impact on the poultry industry causing high mortality, poor growth, decreased productivity and high medical cost (Williams 1999). Currently, this disease has been mainly controlled by the use of anticoccidial drugs in feed or water (Mcdonald and Shirley 2009) and also the use of vaccines (Yang et al. 2015). Although most of the diseases of infectious origin affecting the poultry industry have been controlled successfully using protective vaccines, coccidiosis caused by several *Eimera* spp. is still considered as the most challenging (Tewari and Maharana 2011).

The use of plant extracts as medicants may alleviate these difficulties, as they are not only natural products but may comprise new therapeutic molecules to which resistance has not yet developed (Naidoo et al. 2008). The use of herbal remedies in poultry diets has been proposed because of their natural stimulation of the immune system, enhanced growth performance and/or anticoccidial effects (Habibi et al. 2016). In the last decade, plant extracts have been widely investigated that are used for controlling avian coccidiosis and improving poultry performance worldwide (Mathis et al. 1995; Allen and Fetterer 2002; Abbas et al. 2012; Ola-Fadunsin and Ademola 2013; Gotep et al. 2016).

Khaya senegalensis commonly known as African mahogany is a popular medicinal plant in Nigeria and African traditional medicine (Danquah et al. 2013). It belongs to the family *Meliaceae*. In Nigeria, the tree has many local names: 'Madaci' in Hausa, 'Dalehi-Kahi' in Fulfulde, 'Aganwon' in Yoruba and 'Ono' in Igbo languages (Makut et al. 2008; Offiah et al. 2011). The Hausa and Fulani tribes in northern Nigeria use *K. senegalensis* as a remedy for several human and animal ailments (Makut et al. 2008). The stem bark of *K. senegalensis* is also used traditionally in the treatment of malaria, intestinal worms, diarrhoea, dysentery and venereal diseases (Adebayo and Kretti 2011).

Therefore, this study aimed to evaluate the phytochemicals and therapeutic efficacy of the aqueous stem bark extracts of *Khaya Senegalensis* using oocyst count, weight gain, haematological parameters, some serum biochemical parameters and oxidative stress markers in chickens experimentally infected with *Eimeria* oocyst.

Materials and methods

Plant collection and preparation

The stem bark of *Khaya senegalensis* (KS) was harvested from trees within the vicinity of the National Veterinary Research Institute (NVRI), Vom-Nigeria. The plant was identified and authenticated by a botanist at the Federal College of Forestry, Jos, Nigeria, and assigned voucher number, FHJ199. The collected plant material was washed, air-dried and ground into powder under aseptic conditions. Approximately 800 g of the grounded plant was macerated with distilled water for 72 h. After extraction, the mixture was sieved and filtered. The filtrate concentrated by drying in the oven at 40 °C. The dried extracts were stored at 4 °C until needed.

Phytochemical screening

The active components of the aqueous stem bark of *Khaya senegalensis* extract were screened for the presence of some phytochemicals using standard methods (Clarkson et al. 1983). The procedure was carried out at the toxicology laboratory, National Veterinary Research Institute (NVRI) Vom-Nigeria.

Source of oocyst

Mixed *Eimeria* oocyst suspension (*Eimeria tenella*, *E. necatrix* and *E. brunetti*) was obtained from the Parasitology Division of the National Veterinary Research Institute (NVRI). The oocysts were harvested from the caecum of a known coccidian-infected chicken using standard procedure as described in our previous article (Gotep et al. 2016).

Experimental animals

Ethical clearance for this experiment was obtained from Animal Ethical Committee, NVRI, with reference number AEC/03/22/15. The animals were properly handled in accordance with the principles and guide for the care and use of laboratory animals (National Research Council 1992). Apparently healthy day old broiler chicks were obtained from a hatchery in Jos, Nigeria, and brooded under standard conditions for 2 weeks before commencement of the study. The chicks were fed standard pelletized broiler starter feed (Vital feed® Grand Cereals, Nigeria Plc., Jos, Nigeria) and water given ad libitum. Birds were housed in individual cages with proper lighting. The birds were vaccinated against infectious bursal disease (IBD), Newcastle disease using NVRI Vom Vaccines.

Experimental design

Twenty-five broiler chicks, 2 weeks old, weighing between 262 and 416 g were used for this study. The chicks were divided into five groups: five chickens per group namely, 200 mg/kg body weight (bw) of KS, 400 mg/kg bw of KS, 800 mg/kg bw of KS, negative control (infected, untreated) and positive control (infected, treated with a standard drug; Amprolium 250 WSP, Kepro®

B.V., Holland). Each bird was housed individually in wooden cages. Each bird was infected with 2185 (0.1 ml) sporulated oocysts as a single oral gavage according to Biu et al. (2006). Faecal samples were collected daily to monitor the oocyst count. The birds were monitored daily for development of clinical signs of the disease. At the peak of oocyst count which was at day 7 post-infection, treatment commenced. The aqueous extract was reconstituted and administered to the birds per-os. The treatment was carried out for 5 days and the oocyst count was monitored daily.

Monitoring of experimental birds

Evaluation of faeces for the oocyst per gram (OPG) counts was performed using modified McMaster's technique (Kaufmann 1996) on a daily basis. The weight of individual chicks was measured daily. The experiment was terminated 24 h after the administration of the last dose of the extract. About 2 ml and 5 ml of blood samples was collected from the wing vein into sample bottles with and without an anticoagulant for haematological and biochemical evaluation, respectively, at the end of the experiment. Finally, the birds were sacrificed, and the intestine were harvested and stored in normal saline for oxidative stress assay.

Haematological analysis

Both erythrocytic (red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) concentration) and leucocytic (white blood cell (WBC) count) indices were determined using standard methods as described by Jain (1986). Haematological parameters such as mean corposcular volume (MCV), mean corposcular haemoglobin (MCH) and mean corposcular haemoglobin concentration (MCHC), together with absolute count of heterophils and lymphocytes, were calculated using standard formula (Campbell 1995).

Serum biochemical analysis

We evaluated some serum biochemical parameters such as total proteins, albumin, sodium and potassium and aspartate amino transaminase (AST) which are marker indication of injury/insult to the liver and kidney. These parameters were estimated using Randox diagnostic kit according to manufacturer's instructions.

Tissue preparation and homogenization

The harvested small intestinal segment was rinsed with phosphate-buffered saline (PBS) and blotted on filter paper before taking their weight. They were subsequently chopped and homogenized using buffer (0.1 M phosphate buffer, pH 7.4) as described previously (Gotep et al. 2016).

Table 1 Phytochemical constituents of aqueous stem bark extract of *Khaya senegalensis*

Phytochemicals	*Khaya senegalensis*
Tannins	+
Saponin	+
Alkaloids	−
Flavonoid	−
Cardiac glycosides	+
Resin	−
Anthraquinones	−
Steroids	+

Oxidative stress assays

Biomarkers indicative of oxidative stress such as superoxide dismutase (SOD), catalase, malondialdehyde (MDA) and reduced glutathione (GSH) levels including total protein were performed using standard protocols (del Carmen Contini et al. 2012).

Statistical analysis

Data obtained from the study were summarized as means ± standard deviation. Student's *t* test analysis was used to determine the differences between the means determined at 5% level of significance using the Graph pad prism version 5.

Results

Extraction and phytochemical screening

The yield of the plant extract was 7.02%. The phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides and steroids (Table 1).

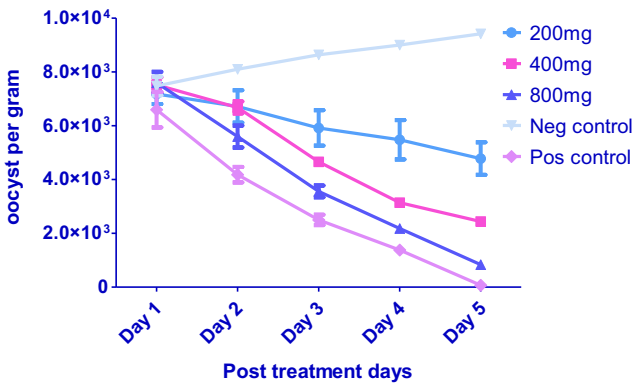

Fig. 1 Therapeutic effect of aqueous stem bark extract of *Khaya senegalensis* on oocyst count of chickens infected with *Eimeria* oocyst. Neg. control (infected, untreated). Pos. control (infected, treated with a standard drug: Amprolium 250 WSP)

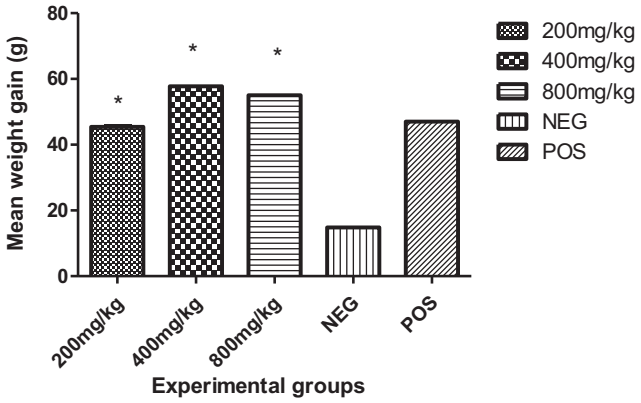

Fig. 2 Therapeutic effect of aqueous stem bark extract of *Khaya senegalensis* on mean weight gain of chickens infected with Eimeria oocyst. Neg. control (infected, untreated). Pos. control (infected, treated with a standard drug: Amprolium 250 WSP). Asterisk (*) indicates significant difference ($p < 0.05$) against negative control

Effect of the extract on oocyst count and weight gain

There was a significant ($p < 0.05$) graded dose-dependent reduction in the oocyst count from day 1 post-infection (Pi) to day 5 Pi in all the treated groups with the exception of the negative control. The dosage of 800 mg/kg was the most effective therapeutic dose almost comparable to the standard drug (Fig. 1). A significant ($p < 0.05$) increase in mean weight gain was also recorded across the experimental groups compared with the negative control (Fig. 2).

Effect of the extract on some erythrocytic indices and haematological parameters

There was a dose-dependent numerical increase, though not statistically significant ($p > 0.05$) in RBCs, HB and PCV of the treatment groups compared to the negative control (Table 2). Haematological parameters such as MCV showed numerical increase at 800 mg/kg compared to 200 mg/kg whereas MCHC showed a numerical decrease level at 800 mg/kg when compared with 200 mg/kg (all not statistically significant). MCH had a graded dose response with the highest response at 800 mg/kg just slightly lower than the response observed with the standard drug (positive control) (Table 2).

Effect of the extract on white blood cell count

The extract had no significant difference ($p > 0.05$) on the total white blood cell count for all the treatment groups with the controls. The number of heterophils decreases with increasing concentration of the extract. Eosinophils and basophils were not detected (Table 3).

Effect of the extract on some biochemical parameters

The AST level showed a significant difference ($p < 0.05$) with the different treatment groups compared to the negative control. Fluctuating levels of other serum biochemical parameters such as total protein, albumin and potassium were also observed with significant increased level in total protein and decreased level in albumen for all the treated groups compared to negative control group. However, potassium concentration showed a significant decreased level at higher concentration of the extract and positive drug control compared to negative untreated control group. No significant difference ($p > 0.05$) was observed in the sodium concentration (Table 4).

Oxidative stress biomarkers

The catalase activity significantly increased ($p < 0.05$) in all the experimental groups when compared to the negative control (Table 5). Reduced gluthathione (GSH) levels also increased at graded dose dependent across the experimental group when compared to the negative control; however, this increase was not statistically significant ($p > 0.05$) (Table 5). The levels of malondialdehyde (MDA) decreased in dose-dependent manner across the experimental group at a

Table 2 Effect of aqueous stem bark extract of *Khaya senegalensis* on some erythrocytic and haematological parameters of chickens infected with *Eimeria* oocysts

Groups	RBCs (%)	HB (%)	PCV (%)	MCV (%)	MCHC (%)	MCH (%)
200 mg/kg	2.49 ± 0.07^{c}	12.93 ± 0.48^{a}	29.67 ± 0.33^{c}	84.87 ± 1.0^{c}	44.18 ± 1.24^{b}	52.25 ± 1.03^{d}
400 mg/kg	2.42 ± 0.13^{c}	13.15 ± 0.23^{a}	30.00 ± 1.00^{c}	81.60 ± 3.5^{c}	47.20 ± 0.98^{b}	57.00 ± 2.85^{d}
800 mg/kg	2.74 ± 0.07^{c}	13.30 ± 0.61^{a}	31.00 ± 1.15^{c}	87.13 ± 3.5^{c}	42.83 ± 1.28^{b}	58.33 ± 0.33^{d}
Neg. control	2.06 ± 0.03^{c}	11.27 ± 0.13^{a}	27.67 ± 2.33^{c}	63.10 ± 0.6^{c}	40.47 ± 1.84^{b}	51.00 ± 0.00^{d}
Pos. control	2.91 ± 0.45^{b}	12.50 ± 0.32^{a}	31.00 ± 1.15^{c}	103.0 ± 0.6^{a}	42.37 ± 1.79^{b}	62.00 ± 0.00^{d}

[a,b,c] Column values with different letter superscript differ significantly ($p < 0.05$) with the negative control

Neg. control (infected, untreated)

Pos. control (infected, treated with a standard drug: Amprolium 250 WSP)

RBC red blood cell count, *HB* haemoglobin concentration, *PCV* packed cell volume, *MCV* mean corpuscular volume, *MCHC* mean corpuscular haemoglobin concentration, *MCH* mean corpuscular haemoglobin

Table 3 Effect of aqueous extracts of *Khaya senegalensis* on white blood cells of chickens infected with *Eimeria* oocysts

Groups	TWBC	Heterophils	Lympho	Mono	Eosino	Baso
200 mg/kg	10.60 ± 0.30 [a]	30.00 ± 0.00[b]	52.50 ± 2.06 [a]	17.20 ± 0.12[a]	ND	ND
400 mg/kg	9.26 ± 0.36 [a]	22.00 ± 1.15[b]	80.00 ± 1.15 [a]	ND	ND	ND
800 mg/kg	10.24 ± 0.35 [a]	21.67 ± 0.88[b]	65.19 ± 1.91 [a]	13.19 ± 1.12[a]	ND	ND
Neg. control	12.93 ± 0.52[a]	30.33 ± 2.40[b]	69.67 ± 2.40[a]	ND	ND	ND
Pos. control	10.40 ± 0.30 [a]	23.00 ± 4.43[b]	87.50 ± 3.50 [a]	ND	ND	ND

[a, b] Column values with same superscripts do not differ significantly ($p > 0.05$) with the negative control

ND not detected, *Lympho* lymphocytes, *Mono* monocytes, *Eosino* eosinophils, *Baso* basophils

Neg. control (infected, untreated)

Pos. control (infected, treated with a standard drug: Amprolium 250 WSP)

significant level with the 800-mg/kg dose when compared to the negative control (Table 5). Finally, a non-statistically significant ($p > 0.05$) dose-dependent increase in the superoxide dismutase (SOD) activity was observed (Table 5).

Discussion

Plants are used medicinally in different countries and regions of the world. These plants are rich sources of therapeutic agents (Kubmarawa et al. 2007). The indiscriminate use of anti-coccidial drugs, inadequate accessibility of farmers to veterinarians and vaccine failure have made poultry farmers opt for medicinal plants in the management of avian coccidiosis. The presence of phytochemicals confers this priceless quality in medicinal plants for new drug development.

The presence of tannins, saponins, cardiac glycosides and steroids in the aqueous stem bark extract of *K. senegalensis* has also been reported by Kubmarawa et al. (2009) and Elisha et al. (2013). Tannins have been associated with anti-inflammation by increasing the supply of digestible proteins which uncouples oxidative phosphorylation, causing a decrease in gastrointestinal metabolism (Tiwari et al. 2011). Saponins have also been associated with anti-diarrhoeal and anti-protozoan activities by impairing the digestion of protein and the uptake of minerals and vitamins in the gut of the host (Androulakis et al. 2006). Both steriods and glycosides also have anti-diarrhoeal activity because steroids enhance intestinal absorption of Na^+ and water while glycosides inhibit the release of autocoids and prostaglandins (Kumari et al. 2014).

The significant increase in the mean weight gain across all the extract and standard drug-treated groups is suggestive of the enhanced feed conversion ratio (FCR) and inhibition of inflammation to the intestinal mucosa. This is in consonance with the observation by Nwosu et al. (2012). Also, the dose-dependent reduction in oocyst count observed across all the experimental groups shows the efficacy of the aqueous stem bark extract of *K. senegalensis.* This corroborates the observation of other researchers (Fajimi and Taiwo 2005; Maikai et al. 2007). These observations could be attributed to the various phyto-compounds present in the extract and their wide range of biological activities.

The increase in the RBC, Hb concentration and PCV across the treatment groups shows the anti-anaemic property of the extracts. This also suggests that the extracts have an erythropoietic inducing ability. This is in line with the findings of Sanni et al. (2005) who reported the anti-anaemic effect of *K. senegalensis* aqueous extracts at doses of 250 and 500 mg/kg body weight on phenylhydrazine-induced anaemia in rats. Decreased levels of PCV, Hb and RBC counts in the

Table 4 Clinico-chemical parameter of liver and kidney functions of chickens experimentally infected with *Eimeria* oocyst and treated with aqueous stem bark extract of *Khaya senegalensis*

Groups	TP (g/L)	ALB (mg/dL)	AST (U/L)	Na (mEq/L)	K (mEq/L)
200 mg/kg	28.76 ± 0.55[c]	8.70 ± 2.04[a]	29.02 ± 3.04[b]	147.70 ± 0.97[a]	4.18 ± 0.12[c]
400 mg/kg	31.34 ± 4.61[c]	8.23 ± 0.69[b]	26.12 ± 1.75[b]	143.50 ± 3.30[a]	4.07 ± 0.78[c]
800 mg/kg	27.70 ± 0.95[c]	10.13 ± 2.25[b]	25.66 ± 2.68[b]	147.50 ± 1.38[a]	2.48 ± 1.24[d]
Neg. control	24.37 ± 2.42[b]	13.01 ± 1.57[c]	40.51 ± 4.57[a]	140.20 ± 0.36[a]	5.82 ± 0.49[c]
Pos. control	36.01 ± 1.39[c]	17.79 ± 2.08[d]	26.78 ± 2.80[b]	147.90 ± 1.71[a]	1.61 ± 0.38[a]

[a,b,c,d] Column values with different superscripts differ significantly ($p < 0.05$) with the negative control

Neg. control (infected, untreated)

Pos. control (infected, treated with a standard drug: Amprolium 250 WSP)

TP total protein, *ALB* albumin, *AST* aspartate transaminase, *Na* sodium, *K* potassium)

Table 5 Effect of aqueous stem bark extract of *Khaya senegalensis* on intestinal oxidative stress biomarkers of chickens infected with *Eimeria* oocyst

Dosage (mg/kg)	Catalase (μM/min/mg protein)	GSH (mg/ml)	MDA (μM/mg protein)	SOD (U/mg protein)
200	12.48 ± 3.99^{a}	2.11 ± 0.36^{a}	$3.0 \times 10^{-6} \pm 7.6 \times 10^{-7a}$	1.45 ± 0.21^{a}
400	17.21 ± 2.55^{b}	2.91 ± 1.08^{a}	$2.5 \times 10^{-6} \pm 5.6 \times 10^{-7a}$	1.50 ± 0.32^{a}
800	16.24 ± 4.85^{c}	2.78 ± 0.35^{a}	$1.1 \times 10^{-6} \pm 2.9 \times 10^{-7b}$	1.85 ± 0.25^{a}
Neg. control	4.59 ± 0.68^{d}	2.52 ± 0.25^{a}	$3.4 \times 10^{-6} \pm 2.2 \times 10^{-7a}$	1.0 ± 0.26^{a}
Pos. control	16.04 ± 5.30^{e}	3.54 ± 1.14^{a}	$2.7 \times 10^{-6} \pm 1.5 \times 10^{-7a}$	1.15 ± 0.35^{a}

^{a,b,c,d} Row values with different superscripts differ significantly ($p < 0.05$) with the negative control

Neg. control (infected, untreated)

Pos. control (infected, treated with a standard drug: Amprolium 250 WSP)

GSH gluthathione, *MDA* malondialdehyde, *SOD* superoxide dismutase

infected untreated group (negative control) might be correlated with loss of blood (haemorrhage) in the intestine and caeca. During tissue injury, histamine may be released thereby causing increased permeability of the capillaries and venules leading to the exudation of large amount of fluid (Akhtar et al. 2015).

The decrease in the heterophil count with a concomitant increase in the lymphocyte count as compared to the negative control suggests the cellular immuno-modulatory effect of the extract since it has a direct activity on the parasitic load (Raja et al. 2011). Oyagbemi and Adejinmi (2012) also reported similar observations of decreased heterophils following the supplementation of the diets of broiler chickens with *Azadirachta indica* and *Vernonia amygdalina*. It is still unclear why we were not able to detect monocytes in some of the groups. Increased monocyte count was obtained in chickens infected with mixed species of *E. tenella* and *E. brunetti* (Adamu et al. 2013).

The increased level of catalase, reduced glutathione and superoxide dismutase is suggestive of the antioxidant-inducing properties of the plant in the chickens. Due to the implication of oxidative stress and inflammation in coccidiosis, the innate immune system of these chickens protects them by producing reactive oxygen species (ROS) in a process termed 'oxidative burst' in an attempt to destroy the *Eimeria* pathogens (Lillehoj and Trout 1996). These reactive species produced are not pathogen-specific, eventually damaging host tissues when protective antioxidants are inadequate. Also, lipid peroxidation was evident from the increased malondialdehyde (MDA) level in the negative control indicative of oxidative damage to the membrane lipids of the intestinal lining. In the treatment groups, the level of MDA decreased. However, amelioration seen across the treatment groups which compared favorably to positive control (infected and treated with amprolium) reveals the free radical scavenging capacity and inhibition of lipid peroxidation ability of the extract. Therefore, the antioxidant capacity of the aqueous stem bark extract of *K. senegalensis* extracts suggests a good maintenance of the chickens' defense systems against coccidiosis.

In conclusion, the study indicates that *Khaya senegalensis* exhibits a significant anticoccidial activity by reduction in oocyst count, improved weight gain, increased erythropoietic activities and significant improvement in the antioxidant status of the host.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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