

Research Article



Assessment of Anti-Coccidial Efficacy of Ethanolic Extract of *Aloe vera* Leaf in Kabir Chicken in Cameroon

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Abstract | Coccidiosis caused by *Eimeria* species is a serious disease in the poultry industry. Anticoccidial efficacy, growth and haematological parameters of ethanolic extract of *Aloe vera* were tested against coccidiosis in Kabir chicken. Fresh *A. vera* leaves were harvested, dried in gentle heat, ground and the sieved powder was used to prepare an ethanolic extract. Chicken were infected with approximately 3200 *Eimeria* oocysts until they began shedding oocysts in their faeces. The extract was administered at different doses of 0.16 g/chicken/day (T1), 0.32 g/chicken/day (T2) and 0.84 g/chicken/day (T3) directly into the mouth of the chicken. Chicken in the T4 group were given sulfaquinoxalina-LH, those in T5 were infected but not treated while T6 was the neutral group. Carcass and haematological parameters were determined. There were significant differences ($P < 0.05$) in the feed intake between experimental groups. Chicken of T3 had the highest body mass gain (11.7%) while those of T5 had the least (3.1%). The highest feed conversion ratio (14.7%) was recorded in chickens of T3 while the overall feed conversion ratio was recorded in T4 (18.6%). The weights of different organs were similar ($P > 0.05$) in all groups. The highest oocyst count reduction rate was observed in T4 (98.7%) and the value reduced in a dose dependent manner in the groups receiving *A. vera* extract. Red blood cell count (RBC) was significantly highest ($P = 0.043$) in T4 ($2.8 \pm 0.3 \times 10^{12}$ /uL) and lowest in T5 ($0.8 \pm 0.3 \times 10^{12}$ /uL). White blood cell count (WBC) was also significantly highest ($P = 0.031$) in T4 ($98.8 \pm 0.3 \times 10^9$ /uL) and lowest in T5 ($18.3 \pm 11.8 \times 10^9$ /uL). Haemoglobin levels were not significantly different between treatment groups. This extract could be incorporated into the feed or drink of chicken for the prophylactic treatment of coccidiosis before outbreaks.

Keywords | *Aloe vera*, Efficacy, Coccidia, Kabir chicken

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INTRODUCTION

In developing countries, poultry production is largely based on traditional extensive poultry production systems (Bawankar et al., 2014). Village chicken represent an appropriate system to provide high-quality protein to the fast growing human population. They also provide income to resource-poor smallholder farmers, especially women. However, high mortality rates, mainly due to diseases, including coccidiosis, constitute one of the greatest constraints on chicken development. Coccidiosis causes considerable economic loss in the poultry

industry, especially in broiler chicken as it is associated with reduced growth rate and impaired feed conversion thus leading to poor performance of chicken and mortality (Usman et al., 2011). Chicken are susceptible to at least nine species of coccidia. The commonest species are *Eimeria tenella*, which causes caecal coccidiosis and *E. acervulina* as well as *E. maxima* which cause chronic intestinal coccidiosis. Currently, coccidiosis control programmes largely rely on chemotherapy and immunoprophylaxis. However, the development of drug resistance in strains in the field and the withdrawal period for these drugs prior to slaughter necessitate the exploration of alternative

methods of treatment such as the use of medicinal plant extracts for controlling the disease. Usman et al. (2011) tested fifteen herbal extracts for anticoccidial activity in chicks and had varying degrees of results. Plant extracts such as *Carica papaya*, *Moringa indica*, *Mentha arvensis* and *Aloe vera* have been used traditionally to treat various gastrointestinal ailments in man and animals in different parts of the world (Hashemabadi and Kaviani, 2008).

Aloe vera is a cactus-like plant. It is stemless with triangular, fleshy leaves ranging in colour from grey-green to bright green and the margins of the leaves have small white 'teeth'. The leaves are composed of three layers: an inner gel, a yellow sap and the outer thick layer of 15-20 cells known as rind (Surjushe et al., 2008; Hashemabadit and Kaviani, 2010).

Reports on the medicinal properties of *A. vera* include the external healing of different kinds of wounds, excellent soothing of minor burns, scrapes, ulcers, and alleviation of arthritis, constipation and piles (Hashemabadit and Kaviani, 2010). The leaf and juice of *A. vera* have been used in animals internally or externally. It is believed to possess pharmacological antibacterial, antivenin, and immunological properties. Some resource-poor smallholder farmers usually use *Aloe* species to reduce chicken mortalities. Common examples of *Aloe* used in Southern Africa include *A. vera excelsa*, *Aloe christiana*, and *Aloe spicata* which are easy to use and are available all year round (Trivedi et al., 2008). However, the potential for using *A. vera* in livestock health management is not widely documented (Bawankar et al., 2014). There is therefore a need to investigate and document the therapeutic importance of the aloes, which may in turn result in the preservation and protection of the natural plant resource-base. Smallholder farmers are able to contribute in the conservation efforts if they use the herb for ethno-veterinary and medicinal purposes. Although ethno-veterinary medicines are widely used, the optimum dosage and the dose response characteristics are not known (Gueye and Van't-Hooft, 2000; Hashemabadit and Kaviani, 2010).

Commonly, haematological parameter measurements provides valuable information in human and animal medicine. Unfortunately, due to lack of information of such parameters, blood profile is not used widely in avian medicine (Mukherjee and Wahile, 2006; Kayode et al., 2009). Although there is limited information in this field, evaluation of blood profiles of broiler strains has been assessed by some studies. Significant increases in haematological parameters with age have been reported in broiler chicks such as WBC counts including lymphocytes, monocytes, heterophils, eosinophils and basophils (Arczewska-Wlose and Swiatkiewz, 2012; Ajay et al., 2013) and RBC, haemoglobin (Hb) and packed cell volume (PCV) as reported by Aliasghar et al. (2012). Such studies need to be validated in different localities in order to appreciate the medicinal val-

ue of the plant extract as this could reduce the challenges faced by chicken and chicken farmers as a result of coccidiosis. Against this background, this study was undertaken to evaluate the anticoccidial efficacy of the ethanolic extract of *A. vera* in Kabir chicken by assessing the oocyst load, growth and haematological parameters.

MATERIALS AND METHODS

STUDY AREA AND POPULATION DESCRIPTIONS

The study was carried out in the Africa Brazil Marketplace project poultry farm located in Lyongo village, Buea, South West Region, Cameroon. The locality is located at the foot of Mount Cameroon and is very rich in vegetation. The experimental animals consisted of Cameroonian Kabir chicken aged 9 weeks comprising 8 fowls per experimental group and of both sexes. They were of mixed breed, displaying a variety of feathering and plumage colours.

POULTRY FARM MANAGEMENT

After leaving the hatchery the experimental chicks were grown under uniform brooder conditions from a day old to experimental ages. The birds were housed in a disinfected deep litter system with wood shavings as bedding material. Each treatment occupied an area of 2.25 m² where feed and water were provided *ad libitum*. Incandescent bulbs were used to provide light and heat day and night during the brooding period.

STUDY DESIGN

Kabir chicken aged 9 weeks old and of both sexes with naked necks were divided into 6 pens (Groups) containing 8 chicken each. Groups 1-5 (T1-T5) were infected with approximately 3200 *Eimeria* oocysts at the 8th week. The faeces of the chicken was monitored on a daily basis for the presence of oocysts. On the 4th day post-infection, oocysts were found in the stool of the birds and treatment commenced and went on for six days after which blood was extracted and the chicken sacrificed for carcass analyses. Groups 1-3 (T1-T3) were given the ethanolic plant extract of *A. vera* in different doses of 0.16 g, 0.32 g and 0.84 g/chicken/day respectively. Group 4 (T4) was given the commercial anticoccidial drug (Sulfaquinoxalina-LH); 1 tea spoonful of powder in 2.5 mL of water in their drinkers for four days according to the manufacturer's instruction. Group 5 (T5) was the control group which was infected with the *Eimeria* parasites but not treated at all and group 6 (T6), the neutral group was not infected at all, but given only water. All the chicken in each group were of equal numbers (8/group), sexes and plumage, same feed intake and composition, under the same environmental conditions with *ad libitum* access to feed and water. A reservoir chicken for coccidian oocysts was used and oocyst output per gram of faeces was quantified using the McMaster technique and found to supply mean output of 3200 opg

of faeces. This reservoir chicken was treated for other parasitic, bacterial and viral infections except that of coccidia. A gram of filtered faeces was obtained from the reservoir chicken, dissolved and administered orally to each chicken in T1-T5 except T6. By implication, the chicken received approximately 3200 *Eimeria* oocysts. The McMaster technique was used to monitor the oocyst output in the stool of the infected chicken (Christensen et al., 1987).

PREPARATION AND ADMINISTRATION OF PLANT EXTRACTS

Fresh *A. vera* leaves were harvested, dried in gentle heat, ground and sieved. 95% of ethanol was diluted to 70% ethanol using Gay-Lussac's dilution method to form alcoholic water which was used for the preparation of the ethanolic extract (<http://www.spc.ac-aix-marseille.fr/labospc/spip.php?article329> accessed May, 2015). *A. vera* leaf powder (500 g) was put into 1000 ml of 70% ethanol and covered to avoid evaporation. This was macerated and stirred after every 6 hours, for 72 hours after which, the mixture was filtered through filter papers and the liquid was evaporated to dryness using an evaporator at 40°C. The powder was given in 0.16 g, 0.32 g and 0.84 g for T1, T2 and T3 respectively per chicken per day. T4 contained sulfaquinoxalina-LH and was administered 1 teaspoon full daily in 2.5 L of water. It was taken for 4 days. The weights of these birds were taken after every two days using a sensitive balance while oocysts per gram of faeces was quantified by the McMaster counting technique (Christensen et al., 1987).

HAEMATOLOGICAL AND CARCASS PARAMETER ANALYSES

At the end of the experiment, chicken from each treatment were sacrificed, blood samples were collected from their aortic veins into EDTA tubes for haematological analysis. The chicken were de-feathered, and opened for the evaluation of carcass and internal organ parameters to evaluate the effects of these parasites and different *A. vera* extract doses on their weights. The internal organs were carefully removed, observed and weighed using an electronic balance (Ohaus CS200, USA). The organs concerned were live weight (LWT), carcass weight (CWT), heart (HT), liver (LV), lungs (LGS), spleen (SPL), fabricius bursa (FB), gizzard (GZ), proventriculus (PV), thymus (TH), caecal weight (CEWT), caecal length (CEL), kidney (KN), empty caecal weight (ECEWT) and bile (BL). The blood was transported in ice and carried to the Laboratory of Animal Health (LASAN) of the Department of Animal Production, of the Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon for analysis. Haematological parameters (WBC, RBC and Hb) were analysed using the haematology analyser machine for animals (Beckman Coulter, Coulter AC. T different Haematology Analyser, BR-13692A).

STATISTICAL ANALYSIS

The data collected was entered and analysed using SPSS

Package version 20. The data was subjected to ANOVA at 95% confidence level ($P < 0.05$) to compare means, and when there were significant differences between means, these were separated using the Duncan multiple range test. The mean oocyst counts that were calculated before and after the administration of the different extracts were used to evaluate percentage in reduction or reduction rate, using the formula below:

$$\text{Reduction rate} = (\text{Initial oocyst counts} - \text{final oocyst counts} \times 100) / (\text{Initial oocysts counts})$$

Also, some production parameters such as the average feed intake (FI) which is the total amount of feed consumed by the chicken from the day of the administration of the plant extracts till the last day, average daily weight gain (AWG) between treatments were calculated. These parameters were calculated as follows:

$$\text{Average daily weight gain (AWG)} = \text{Total weight gain per chicken per group} / \text{Total number of days}$$

$$\text{Feed consumption rate (FCR)} = \text{FI} / \text{Total AWG}$$

Where, FI = feed intake and AWG is average weight gain (Unigwe et al., 2014).

Table 1: The feed intake, cumulative average weight gain, growth rate and feed conversion ratio of the Kabir chickens treated with ethanolic *A. vera* leaf extract

Treatment (Ts)	Growth parameters			
	AFI	AWG	%AGR	%FCR
T1 (0.16)	1195.4±58.9	720.9±18	4.0 ^{ab}	7.1 ^b
T2 (0.32)	1173.2±75.5	799.3±29.7	5.9 ^a	7.8 ^b
T3 (0.84)	1300.0±128.3	817.5±44.2	11.7 ^b	14.8 ^a
T4(Sulfa--)	1204.6±109.3	835.6±34.0	6.6 ^a	18.6 ^a
T5 (Pos)	1170±73.5	731.7±31.0	3.1 ^a	7.5 ^b
T6 (Neg)	1274.4±176.3	799.4±25.61	6.7 ^a	11.6

Values in a column followed by the same superscripts are not significantly different ($P > 0.05$); SEM = standard error of means; FI = feed intake; AWG = average weight gain; AGR = average growth rate; FCR = feed conversion rate.

RESULTS

GROWTH PARAMETERS

The Growth parameters of *Eimeria*-infected Kabir chicken treated with ethanolic *A. vera* leaf extract are presented in Table 1. Results obtained showed that there were significant differences in the feed intake between the different groups receiving the ethanolic *A. vera* leaf extract ($P < 0.05$). A significant difference ($P = 0.014$) was observed in the average weight gain between groups and these are shown by superscripts from Turkey multiple comparison test in Table 1. The highest average growth rate was ob

Table 2: Average weight of some internal organs of *Eimeria*-infected Kabir chicken treated with ethanolic extract of *Aloe vera*

Organs examined	Experimental groups and Weights±SEM (g) of organs					
	T1 (0.16g)	T2 (0.32 g)	T3 (0.84 g)	T4-Sulfaquinoxalina-LH	T5-control	T6-neutral
Liver	643±59.6	711.8±29	805.2±104.3	763.8±91.2	685.5±107	758.7±79.2
Carcass	426.7±49.6	475.8±17.5	544.8±76.3	526.7±81.3	457.2±73.1	564.2±65.1
Heart	3.6±0.3	3.6±0.3	5.6±0.8	4.6±0.4	4.2±0.8	5.6±0.7
Liver	20.5±1.7	21.8±2.2	25.3±0.6	19.8±2.5	21.4±1.4	22.5±2.1
Lungs	3.4±0.6	4.4±0.1	4.4±0.8	4.5±0.6	3.7±0.7	5.3±1.2
Spleen	1.1±0.1	1.3±0.1	1.6±0.4	1.6±0.4	1.3±0.2	1.3±0.2
Fabricus	0.5±0.1	0.7±0.1	0.8±0	0.7±0.1	0.7±0.1	1.6±0.3
Gizzard	20±1	23.7±0.5	22±2.4	26±3.5	23.6±3.6	20.1±2.6
Proventriculus	3.8±0.5	4.7±0.1	4.9±0.6	6.2±1.4	3.6±0.7	3.4±0.3
Thymus	0.8±0.2	1.9±0.5	2±0.1	1.4±0.1	2.5±0.5	0.6±0.3
Caecal	6.1±1.6	4.9±0.7	7.3±1.4	7.7±1.2	6.6±1.6	9.1±1.2
Caecal length (cm)	15.3±1.2	11.5±0.8	13.1±1.7	12.9±0.1	15.3±2	14.1±0.5
Kidney	4.6±0.1	5.7±0.4	5.6±0.6	5.4±0.2	6.1±1	5.9±0.6
Empty caeca	3.8±0.1	3.2±0.2	3.6±0.4	4±0.2	3±0.5	4.9±1.1
Bile	0.8±0.1	0.8±0.4	0.7±0.5	0.6±0.1	1±0.5	0.5±0.2

No statistical significance at $p < 0.05$ with the different organs in all the groups

served in chicken of T3 (11.7%) while the lowest was observed in fowls of T5 (3.1%). Moreover, the highest feed conversion ratio was observed in T4 (18.6%) while the least was observed in fowls of T1 (7.1%).

CARCASS CHARACTERISTICS

The Carcass characteristics of *Eimeria*-infected Kabir chicken treated with ethanolic *A. vera* leaf extract are presented in Table 2. The results obtained revealed no significant difference in the weights of organs between groups at $P > 0.05$.

Table 3: Reduction rate of *Eimeria* oocyst counts in Kabir chicken treated with ethanolic extracts of *A. vera* leaves

Treatment (Ts)	Oocyst counts (OPG)		%Reduction	Status
	Initial	Final		
T1(0.16)	3462.5±1952.2	685.7±158	80.2 ^a	M-L
T2 (0.32)	32600±1588.6	700±286.4	97.8 ^a	H-L
T3 (0.84)	9887.5±3049.8	206.5±158.1	97.9 ^a	M-L
T4 (Sulf)	11987.5±5114.3	150±50	98.7 ^a	H-L
T5 (Pos)	9750±1145.3	19112.5±2532.5	-49.0 ^b	M-H

a, b on the same column, the values assigned the same letter are not significantly different ($P > 0.05$); **H-L** = high to low, **M-H** = Medium to high OPG

FAECAL OOCYST COUNTS

The final faecal oocyst counts of *Eimeria*-infected Kabir chicken treated with ethanolic *A. vera* leaf extract in different groups showed significant reduction in oocyst count between treatment groups (Table 3). The oocyst counts in the untreated group (T5) increased continuously from the

initial count on Day 4 as opposed to the treated group. The highest oocyst count reduction rate was observed in T4 (98.7%) which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was observed in T3 (97.9%), and reduced in a dose-dependent manner to 97.8% in T2 and then 80.2% in T1, the differences in oocyst reduction between the treated groups (T1-T4) were not significant at $p > 0.05$ except with the untreated group at $P < 0.05$.

HAEMATOLOGICAL PARAMETERS FOR *EIMERIA*-INFECTED KABIR CHICKEN TREATED WITH ETHANOLIC EXTRACTS OF *A. VERA* LEAVES

The haematological parameters for *Eimeria*-infected Kabir chicken treated with ethanolic extracts of *A. vera* leaves are presented in Table 4. There was a statistically significant difference in RBC and WBC counts at $P \leq 0.043$ and $P \leq 0.031$ respectively between experimental groups treated with ethanolic *A. vera* leaf extracts.

DISCUSSION

Coccidiosis constitutes a major health problem to the poultry industry and has primarily been controlled by the use of standard medication under field conditions in spite of limitations like drug resistance and other concerns in relation to food chain contamination. As a substitute, the use of plants and their products as immunomodulators and therapeutics have traditionally been used. According to Farnsworth's report in 1999, more than 64% of the world's population use botanical drugs to combat health

problems. In this regard, therapeutic properties of *A. vera* have been studied in different animal models and human beings. These include anti-inflammatory immunomodulatory, wound healing, promotion of radiation damage repair, antibacterial, antiviral and antifungal (Arczewska-Włosek and Świątkiewicz, 2012).

Table 4: Effects of treatment with ethanolic *A. vera* leaf extracts on the RBC, WBC and Hb of *Eimeria*-infected Kabir chicken

Treatment	RBC ($\times 10^{12}/L$)	WBC ($\times 10^9/L$)	Hb (g/dL)
T1	2.7 \pm 0.2 ^a	85.9 \pm 6.9 ^a	8.8 \pm 0.9
T2	2.3 \pm 0.1 ^a	96.2 \pm 2.5 ^a	8.4 \pm 0.8
T3	2.6 \pm 0.1 ^a	96.1 \pm 1.5 ^a	8.6 \pm 0.3
T4	2.8 \pm 0.3 ^a	98.8 \pm 0.3 ^a	9 \pm 1.1
T5	0.8 \pm 0.3 ^b	18.3 \pm 11.8 ^b	7.0 \pm 0.1
T6	1.7 \pm 0.4 ^a	56.7 \pm 17.1 ^a	8.2 \pm 0.6

a, b on the same column, the values assigned the same letter are not significantly different ($P > 0.05$)

The results of this study showed that the feed intake of the chicken between groups was significantly affected by the plant extracts. It can therefore be assumed that the beneficial effect (high percentage weight gain of 11.7% in T3) of the *A. vera* extract obtained in infected chicken was as a consequence of its stimulating influence on the chickens' appetite, but not necessarily as a result of its anticoccidial properties (Arczewska-Włosek and Świątkiewicz, 2012). In line with this finding, Guo et al. (2004), Alffi (2007) and Durrani et al. (2007) reported significant differences in feed consumption rate of broilers fed with *A. vera* leaf and other herbal extracts.

Chicken of T3 that received the highest dose of the ethanolic *A. vera* leaf extract on the other hand, had the highest average percentage weight gain (11.7%) while T5 had the lowest with 3.1%. This could be attributed to the fact that the *A. vera* leaf extract has a feed stimulatory enzyme which assists in feed consumption rate and intake. As a result of significant food intake with *A. vera* this also led to a statistically significant average weight gain ($P = 0.014$) of the Kabir chicken. On the contrary, Sarad et al. (2008) in Tanzania reported no significant difference in food and water intake with fowls treated with *A. vera* extract. Chandrakesan et al. (2009) reported that coccidial challenge significantly affected body mass gain and feed conversion ratio of chicks. However, Kurkure et al. (2006) on the contrary reported that coccidial challenge had no effect on body mass gain and feed conversion on fowls treated with *A. vera* extract.

When carcass parameters were evaluated, the plant extracts did not have any significant effect on the major body organs although higher values were recorded in treated groups in a dose-dependent manner. Findings of the pres-

ent study are in agreement with the reports of Sungirai et al. (2013) who all reported no significant differences in major body organs with broilers infected with oocysts and treated with *Aloe* extract.

Significantly ($P < 0.05$) higher body weight gain, dressed weight and lower feed conversion ratio were observed for chicken in the test group when compared with control groups. Similar findings have been reported by Wheeler et al. (1994), Guo et al. (2004), Chand et al. (2005), Jiang et al. (2005), Mehmet et al. (2005), Durrani et al. (2007) and Sarad et al. (2008). The higher body weight gain of the chicken given the ethanolic extract of *A. vera* could be due to better performance of the chicken and the diversified antimicrobial activities of *Aloe* gel. This was also demonstrated by Swaim et al. (1992) in chicken. *A. vera* juice is known for healing wounds caused by trophozoites at the level of the caeca which in this present work showed no significant difference in terms of empty caecal weights (ECEWT).

There was a significant reduction in faecal oocyst count with an increase in *A. vera* concentration. The reduction in oocyst count was probably as a result of the anti-coccidial activities of the *A. vera* extract. Sungirai et al. (2013) reported similar findings in Zimbabwe with treated and untreated groups of broiler chicken with *Aloe* extract. Gadzirayi et al. (2010) reported similar results after adding *A. vera* powder to the drinkers of experimental chicken. The same author also found out that *A. excelsa* was as good as a synthetic coccidiostat in controlling coccidiosis. It has been reported that *Aloe* juice contains organic chemicals known as 1, 8 dihydroxyanthraquinone and its derivatives include *Aloe* emodin, aloetic acid and isobarbaloin (Hamman, 2008; Yim et al., 2011). These chemical constituents in *Aloe* extract act as laxative agents by interacting with the gastrointestinal mucosa and inducing bowel motility. This leads to the quick discharge of coccidial oocysts that are lodged in faecal matter thereby reducing the oocyst count. Gadzirayi et al. (2010) reported similar findings with *Aloe excelsa* extract.

Results obtained also showed that ethanolic *A. vera* leaf extract had significant rise on the RBC ($P = 0.048$) and WBC ($P = 0.024$) counts of the chicken between treatments. In line with this study, Sarad et al. (2008) reported significantly higher WBC level in a similar study with experimental broilers treated with *Aloe* extract as compared to others. Sham et al. (2003), Gautam et al. (2004), Balwinder et al. (2005), Valle et al. (2005) and Durrani et al. (2007) also reported increased levels of immunities in chicken given various herbal extracts in drinking water. This is probably due to the fact that *A. vera* has immune stimulatory properties and it is this property that it uses to raise antibodies against coccidial infections. Since antibodies are raised to fight these parasites, it is therefore

suggested that this is the reason for the high WBC in the *Aloe* treated group. Blood loss due to coccidial infection was also minimized and this is probably the reason for the higher RBC levels with respect to other groups.

CONCLUSIONS

Aloe vera extract proved to be of significant importance in fighting coccidiosis and showed a significant reduction of oocyst counts of 97.9% and significant increases in WBC and RBC levels. Thus, the extract could be used as organic alternative to synthetic chemicals to combat coccidiosis and improve chicken health especially in poor communities.

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