

Efficacy of different concentrations of *Aloe chabaudii* leaf gel as a substitute of a sulphonamide on the control Avian Coccidiosis

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Abstract

The objectives of this study were to investigate the effectiveness of *Aloe chabaudii* Sconland juice as a potential substitute of sulphonamide drugs and to measure its effects when administered at different concentrations in the control of avian coccidiosis. Thirty Cobb 500 broiler chickens were used for the experiment. The broilers were randomised into six groups of five birds each and the groups were assigned to six treatments in a Complete Randomised Design (CRD). Four of the groups were given any one of the following treatments, different concentrations of Aloe (weight/volume: 10%, 20% and 30%) and a sulphonamide. There was a control group which was not given a treatment and one cohort group which was neither infected nor treated (Control). At two weeks of age, 25 of the birds were infected with coccidia via drinking water using infected chicken faecal matter, while the remaining five birds were left uninfected. Infection proceeded for 1 week and after the infection period, faecal oocysts were counted from each individual bird using the McMaster technique. Treatments started one day post infection and continued for one week where after 20 gram samples of faecal matter were taken from each bird per treatment and oocysts were counted. After oocyst counting, all birds were slaughtered and lesion scoring was done on the intestines using the Johnson and Reid technique. Data was analysed for oocysts count in different treatments, faecal egg count reduction, relative risk of coccidiosis and the effectiveness of aloe concentrations as a substitute for a commercial sulphonamide. The results showed that the concentrations of aloe used in the experiment were not effective as compared to the sulphonamide in controlling coccidiosis as the faecal egg reduction was below 90% for all concentrations. The relative risk of coccidiosis infection in the farm was found to be 100% meaning coccidiosis is a disease of economic importance at the farm. However there was a reduction in the faecal oocyst count with increase in aloe juice concentration to control coccidiosis, though this could be not be compared to the sulphonamide used (ESB₃) which was more effective ($p < 0.05$). This study has shown that there is potential for use of *Aloe chabaudii* leaf gel as a chemotherapeutic though much research is needed to determine absolute concentrations which will make it comparable to commercially available drugs in terms of efficacy.

Keywords: *Aloechabaudii*, coccidiosis, sulphonamide, efficacy.

Introduction

Avian coccidiosis is among the most costly and widespread infectious diseases in the poultry industry (Chapman, 2003). In Zimbabwe, the

disease is of socio-economic importance among newly resettled and rural poultry farmers (Gadzirayi *et al.*, 2004). The land reform programme which started in the year 2000 led to an increase in the number of small scale broiler farmers countrywide. Many of these farmers practice extensive to semi-extensive poultry production. This comprises mainly of a wide range of free range and broiler chicken farming, where disease incidence of poultry coccidiosis has been on the rise (Gadzirayi *et al.*, 2004).

The majority of resettled farmers still use primitive production structures such as poor poultry housing and this coupled together with use of untreated water from unprotected water sources such as dams, open wells and rivers increases the chances of prevalence of diseases such as avian coccidiosis. Due to the liquidity challenges currently facing the country, resource poor farmers are unable to purchase commercial drugs, coupled with the closure of veterinary shops in rural areas and growth points. On the other hand some resettled farmers are located far from these shops and have to travel long distances to urban areas to buy drugs.

A remedy can be found in the use of ethno veterinary medicines. Ethno veterinary medicines have been

known to be used in the control of different livestock ailments, and aloe is one good example. The ethno veterinary uses of aloe, dates far back into history as in the Greek, Egyptian and Roman eras. Its therapeutic advantages have survived for over 4000 yrs with the earliest records being dated back to 2100 BC (Bassetti and Stefano, 2005). Aloe comes in a wholesome number of varieties and different sub species. These include *A. Excelsa*, *A. Spicata* and *A. Vera* and these have a good reputation for their use in research concerning ethno veterinary science (Durrani *et al.*, 2008). *Aloe chabaudi* is a traditional medicine which also has been used to control avian coccidiosis, over years in most rural parts of eastern Zimbabwe and Mozambique (Mwale *et al.*, 2005). Majority of communal farmers, who use it, claim the herb is an efficient remedy to such ailments. However, unlike its former mentioned sub species, there is not much evidence in terms of quantifiable data to substantiate the claims. It is still being used as a cheaper and more readily available alternative for the control of coccidiosis by a majority of small scale producers in the unavailability of chemotherapeutic agents.

It is therefore important to have knowledge on the most appropriate dosages of the aloe leaf juice that will

elicit an effective therapeutic response from the chickens. This will go a long way in addressing losses due to the financial liquidity challenges faced by communal farmers in accessing veterinary drugs, and also address issues concerning disease resistance due to continued use of a single drug. This research was therefore carried out to determine the results of using different concentrations of *Aloechabaudiischon land* juice in the control of coccidiosis in small scale broiler production, as a substitute of a popular chemo therapeutic sulphonamide.

Materials and Methods

Study area description

This research was carried out at Family Aids Caring Trust, located 1 km from Rusape town centre in Makoni district of the Manicaland province in Zimbabwe. This area falls under agro-ecological region IIb with sandy loam soils. The annual rainfall ranges between a 1000mm- 1200mm with an average maximum temperature of 22°C. The terrain is of gently rugged slopes falling in the catchment area of the Rusape river and dam basin located at an altitude of 4,610 feet above sea level (1,410m).

Methodology

Experimental birds, design and management

Thirty Cobb 500 broilers raised from day old were used in this experiment. The birds were grown under uniform brooder conditions up to one week of age with an average mass of 150-180g live mass. At this age, 25 birds were infected with an average 20 000 coccidian oocysts via drinking water using infected faecal matter of positively diagnosed birds obtained from a local veterinary station. Infection continued for a week to complete the incubation period of the mature oocysts which is 4-6 days as according to (Conway and McKenzie, 2007). After the last day of infection, the 25 birds infected were chosen and randomised to 5 groups in a Complete Randomised Design, where each group contained 5 birds. A sixth group which containing uninfected birds was added. The birds were housed in a disinfected deep litter system with wood shavings being the bedding material. Each treatment occupied an area of 1m² where feed was provided *ad libitum* and birds were allowed 2 litres of water per day. The feed which was used was in the form of starter crumbs

(21% CP) for the first two weeks, and broiler grower pellets (16% CP) for the last two weeks of the experiment. The birds were already vaccinated against Newcastle disease by the supplier and vaccination for this disease was not repeated, however, birds were vaccinated against Infectious Bursal Disease at two weeks of age.

***Aloe chabaudii* identification and chemical analysis**

A search was made for *aloechabaudii* to be used in the study. An aloe species was positively identified and confirmed as *aloe chabaudii* *conland* by experts of botanical sciences from the University of Zimbabwe. Ten *aloe chabaudii* leaves were then collected from different plants within the area neighbouring that of study and were stored overnight in a refrigerator at a temperature of 4° C to prevent oxidation of heat degradable component of the sap. The samples were then sent the following morning to the University of Zimbabwe Bio chemistry department for chemical composition analysis. Results showed that 100g aloe leaf extract had 10% mass per unit volume (10% m/v) concentration, this having 98.5% water and 0.5% dissolved solutes. On

average, a full grown leaf weighed 200g equivalent to 50g of aloe extract.

Treatments

This experiment had six groups, with five birds per group and each bird per group was an experimental unit, therefore each treatment was replicated five times. 200g of fresh aloe leaves were cut into small cubical pieces and were soaked in 2 litres of chicken drinking water (approximately 10% m/v extract), and left over night for the leaf juice to diffuse into solution and was treated in the first group. Simultaneously the second and the third group were treated with 400g (approx 20% m/v extract) and 600g (approx 30% m/v extract) of aloe leaves respectively. 10% m/v aloe extract, was taken as the first treatment for the lowest aloe extract concentration level, 20% m/v being the second and 30% m/v being the highest aloe extract concentration level in this case. The fourth group represented the cohort study so as to prove the importance of avian coccidiosis as a disease of economic importance in this experiment. This group contained 5 birds that were not infected and were not given any treatment neither in their feed nor drinking water. The 5th group was the control which had infected birds but was not assigned for any

treatment dosages. The 6th group had infected birds which were receiving a sulphonamide drug treatment which was sulphaclozine (ESB₃). Dosages started from the third week and continued for one week with all other variables being kept constant and the group to treatment orientation was as follows:

Data collection

Oocyst counts

After a week of giving the assigned treatments, individual birds per group had their faecal samples collected by means of plastic collecting bags. About 10g from each sample collected was taken to the central veterinary laboratories for oocysts counting using the McMasters chamber oocysts counting technique. Briefly in this technique, 10 g of faecal matter were soaked in 100 ml of tap water for 24 hours at 4°C. The mixture was then shaken vigorously and the litter was filtered through a single thickness of muslin (q.s. filtrate to 100 ml). A 10 ml centrifuge tube was filled with filtrate and centrifuged for five minutes at a speed that concentrates the solids. The debris was removed and the pellet was re-suspended in a few milliliters of saturated salt solution (sodium chloride). Samples were taken from

the solution using a Pasteur pipette and were poured on a Mc Masters fill chamber. The number of oocysts was counted under a microscope and the actual number of oocysts was calculated using the following formula:

$$\text{Number of per gram of litter} = \frac{n}{0.10} \times 100 \times 0.1$$

Where: n = number of oocysts counted,

0.10 = volume of the McMaster counting chamber,

100 = 100 ml of water that the litter is soaked in, and

0.1 = correction for 10 g of litter originally taken.

Lesion scoring

Individual birds per treatment were sent for gastro intestinal lesion analysis once at the end of the treatment period, after faecal oocyst count samples were taken and analysed. Each bird was dissected and their gastro intestinal tracts removed and opened to analyse them for lesions. Observations were recorded as data in the form of scores as according to the lesion scoring

technique by (Johnson & Reid, 1970). The data collected was transformed before analysis.

Relative Risk

The relative risk for the incidence of coccidiosis by measuring association between exposed (control) and non exposed (cohort) broilers was done using the following formula (Table 1)

Table 1: Two x two table to calculate relative risk

	Infected	Non infected	totals
Exposed (control)	a	b	a + b
Non exposed(cohort)	c	d	c + d

Risk for exposed = $\frac{a}{a+b}$ and risk for
 exposed = $\frac{c}{c+d}$

Were a = number of infected birds in the exposed group

b = number of non infected birds in the exposed group

c = number of infected birds in the non-exposed group

d = number of non infected birds in the non-exposed group

Therefore, Relative Risk of Disease = Risk in exposed group/ Risk in unexposed group × 100

Statistical analysis

Differences in initial level of infection and after treatment oocysts counts were compared using Feecal Egg Reduction Technique (FERT) using the following formula:

$$\frac{\text{initial count} - \text{final count}}{\text{initial count}} \times 100$$

One way analysis of variance was used to analyse the transformed data for lesion scores and that of oocyst counts using the Genstat Discovery Edition. Mean values were separated using the least significance differences (LSD) and differences were considered significant at $p < 0.05$

Results

Feecal Egg Reduction Technique for oocyst counts

Table 2: Feecal egg reduction (FERT/10g fecal matter)

Treatment	Aloe 10	Aloe 20	Aloe30	cohort	control	Esb3
Mean final oocyst count	352	290	233	800	3487	30
Mean Initial oocysts count	2000	2000	2000	2000	2000	2000
FECR %	82.4	85.5	88.4	60	74.35	98.5

ESB₃ treatment was only one which had over 90% FERT percentage, thus it was the only one effective in disease control (Table 2).

Oocyst count

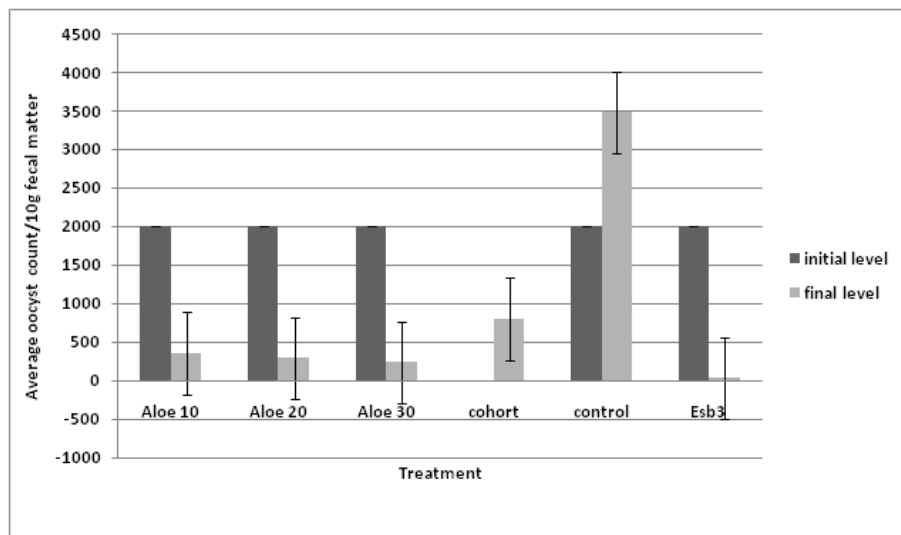


Figure 1: Changes in average pre and post treatment oocyst counts for all treatments

CV(%) =1.6, S.E = 14,11

From Figure 1, there was a significant reduction in the faecal oocyst count ($p < 0.05$) with increase in aloe juice concentration to control coccidiosis, with aloe 30% having the highest percentage reduction in oocysts. The mean final oocyst count value for ESB₃ (30 oocysts/10g of faecal matter) was significantly lower ($p < 0.05$) than that of the most effective aloe concentration (aloe 30%) which had a value of 233 oocysts/ 10g of faecal matter. However, aloe treatments managed to reduce oocysts numbers to a large extent comparing with their

initial levels of infection oocysts count (from an average of 2000 oocysts/ 10g faecal matter. In the control there, was an increase in oocyst count value to a higher level of 3487 oocysts/10g faecal matter, from that of the average initial level of infection which was 2000 oocysts/ 10g faecal matter. The cohort analysis group showed a significant level of infection by coccidian having a final group average oocyst count mean value of 800 oocyst/ 10g faecal matter, having started the experiment without infection.

Lesion scores

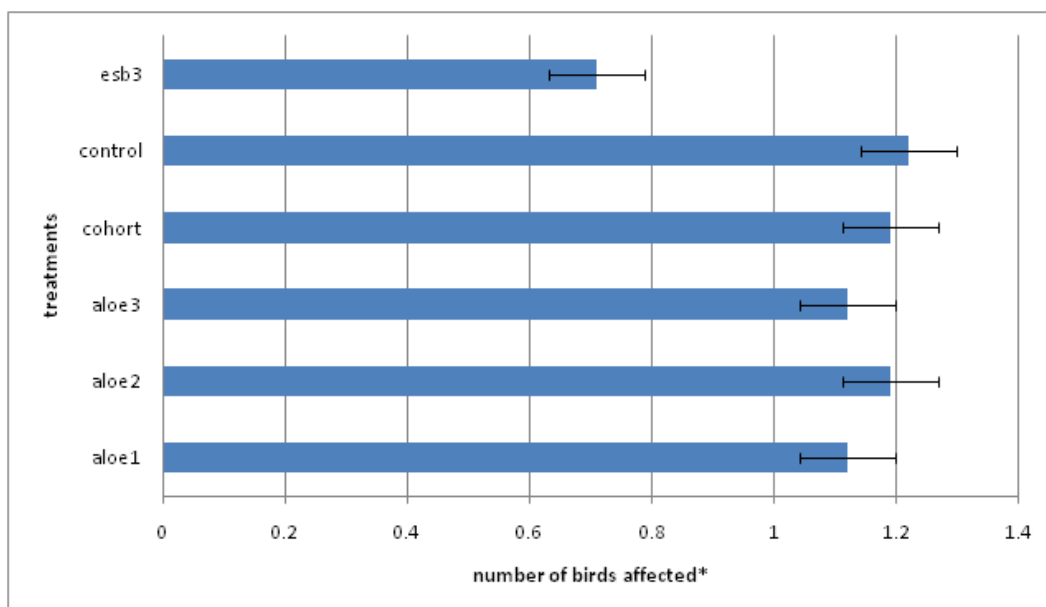


Figure 2: Distribution of number of birds found to have lesions per treatment

Figure 2 shows that there were no significant differences ($p \geq 0, 05$) in the numbers of birds affected with lesions for all aloe treatments, together with the control and cohort. The control showed the greatest occurrence of lesions. However, it is also shown that the ESB₃ treatment was significantly different from all the other treatments ($p < 0.05$) and had the least range in terms of number of birds.

Relative risk

$$\text{Risk for exposed} = \frac{5}{5+0} = 1$$

$$\text{Risk for none exposed} = 1$$

The relative risk of infection with coccidiosis = Risk in exposed group / Risk in non-exposed group $\times 100 = 100\%$

Discussion

Aloe managed to reduce oocyst levels to minimum levels but the results were not comparable to that of ESB₃. Results obtained by (Gadzirayi *et al.*, 2004) showed that treatment with 0,8g of *Aloe Excelsa* ground powder in drinking water significantly reduced oocyst numbers.

In his he showed that differences in oocyst counts proved *A. Excelsa* juice to be as effective as ESB₃ in controlling coccidiosis. However, their results also showed that the aloe drug although effective, had a lower curative value as compared to that shown by the ESB₃ drug. This is similar to this case, where *A. chabaudii* gel was not as efficient in its control as compared to ESB₃. The most possible reason for this could be that, the concentration levels of aloe used in this experiment were not enough to conclude that *A. Chabaudii* leaf gel could not reach an oocyst reduction level like that observed for ESB₃. This implies that, since the reduction in oocysts with increase in aloe juice concentration was not exhausted to the threshold level, further increasing the aloe concentration could possibly have increased oocyst reduction in the broilers.

Another reason why aloe was proved not to be as effective as ESB₃, though it reduced oocyst numbers, is due to the differences in the mode of action towards coccidian oocysts control between the two agents. ESB₃ is a coccidiocidal drug meaning it actually kills disease agents by inhibiting protein synthesis within their systems and thus kill the microbes. This is

different from aloe juice's mode of action which is more coccidiostatical, meaning, it arrests the intra and extra cellular growth of the disease agent though development may resume after drug withdrawal. This is supported by work done by (Yim *et al.*, 2011) which looked at the protective effects of aloe vera based diets in *eimeria maxima*, in which aloe treatment proved successful. The study discussed that aloe was able to control coccidiosis due to the chemistry of its gel. The chemistry of aloe juice comprises of a class of organic chemicals known as 1,8 dihydroxyanthraquinone derivatives including aloe emodin, aloetic acid and isobarbaloin (Hamman, 2008). These chemical constituencies in the aloe juice act as laxative agents by interacting with the gastrointestinal mucosa and inducing bowel motility and allowing for quick discharging of coccidia lodged in fecal matter.

Though effective, ESB₃ was not able to reduce the number of oocysts to zero to show complete disease control. This could be explained by the issue of emergence of drug resistant *eimeria* strains due to the continued use of ESB₃ to control coccidiosis as it is the most available anticoccidial. As explained by (Shane,

2008), there has been a concern in the rise of ionophore and sulphonamide resistant strains of coccidia in many broiler production systems of the world and much research is being done to investigate such problems.

(Durrani *et al.*, 2008) also found out that there were significant decreases in oocyst counts by increasing aloe concentration in a related study, same way with what was observed in this study. However lesion score results from this study showed differences from what was obtained by (Yim *et al.*, 2011) as there were no differences in the range of the number of birds that had lesion scores for all treatment only with ESB₃ showing significant lesion score control. This can be due to differences in the pathogenicity that occur between *eimeria* strains. Since this experiment was based on a mixed *eimeria* strain exposure, it could be that there was an uneven distribution between the most virulent and less virulent strains of *eimeria* during the time of infection as the eight species known to infect poultry are not known to be equally important. For instance, *E. Mitis* is very pathogenic, but doesn't necessarily result in lesions as compared to *E. acervulina* and *E. Maxima* (De Gussem, 2007). In another experiment involving using *A. Secundiflora* gel as anticoccidial, (Waihenya *et al.*, 2002) observed that aloe treated birds had a glyco protein aloe layer, lining the intestinal lumen

of the gastro intestinal tract. This layer prevented the adherence and invasion of the intestinal lining by mature oocysts such that the birds had less clinical lesions.

Lesion score results showed no significant differences in terms of the number of birds infected between *A. Chabaudi* treated groups, which is different from what was shown with the oocyst count results. (De Gussem, 2007) explains this by stating that there is weak correlation between lesion scores and oocysts per gram (OPG) meaning that increase in lesions is not directly linked to amount of oocyst per gram faecal matter and vice versa. According to (Ott, 1970), *eimeria* have a high morbidity, resilience and incidence may occur due to transferring by animals, insects, dust, contaminated feed, water and equipment. This probably explains the incidence of disease in the prospective cohort analysis of this study proving the importance of coccidiosis as a disease in poultry production. The uninfected birds which received no treatment were housed in the same run as with all the other treatments thus mature oocyst could have possibly been transferred to these birds.

Conclusion

Coccidiosis has been shown to be a disease of economic importance under poor habitat, health and

nutrition. This thus implies that there is need to control cases upon incidence, and proper management should be effected to prevent disease prevalence. *Aloe chabaudii* has been shown to be a potential chemotherapeutic for coccidiosis as seen by the reduction in faecal oocysts. However it is not as effective as ESB₃ in controlling coccidiosis, therefore, it can be used as a coccidiostat to control the disease in the absence of more effective drugs. Future studies are recommended to increase the concentration (m/v) of *Aloe chabaudii* levels beyond those used in this study. There is also need to increase the number of birds per group and repeat data collection more than once to increase amount of data and accuracy of results. Lastly, future studies should determine the cost effectiveness of using aloe in controlling coccidiosis as compared to use of sulphonamides.

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