

Efficacy of Different Concentrations of *Aloe chabaudii* Leaf Gel as a Substitute for a Sulfonamide for the Control of Avian Coccidiosis

Marvelous Sungirai, Moses Mucheni, Lawrence Masaka

Department of Livestock and Wildlife Management, Midlands State University, Gweru, Zimbabwe
Email: sungiraim@msu.ac.zw

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ABSTRACT

The objective of this study was to investigate the effectiveness of *Aloe chabaudii schonland* juice as a potential substitute of sulphonamide drugs and to measure its effects when administered at different concentrations in the control of avian coccidiosis. 30 Cobb and 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. At two weeks of age, 25 of the birds were infected with coccidia via drinking water using infected chicken fecal matter, while the remaining five birds were left uninfected. Infection proceeded for 1 week and after the infection period, fecal oocysts were counted from each individual bird using the McMaster technique. Treatments started one day after the infection and continued for one week after 20 gram samples of fecal 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, fecal 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 fecal egg reduction was below 90% for all concentrations. The relative risk of coccidiosis infection in the farm was found to be 100%, meaning that coccidiosis is a disease of economic importance at the farm. However, there was a reduction in the fecal oocyst count with increase in *Aloe* juice concentration to control coccidiosis, though this could not be compared to sulphonamide (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: *Aloe chabaudii*; Coccidiosis; Sulphonamide; Efficacy

1. Introduction

Avian coccidiosis is among the most costly and widespread infectious diseases in the poultry industry [1]. In Zimbabwe, the disease is of socio-economic importance among newly resettled and rural poultry farmers [2]. The land reform programme which started in the year of 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 is comprised mainly of a wide range of free range and broiler chicken farming, where disease incidence of poul-

try coccidiosis has been on the rise [2].

The majority of resettled farmers still use primitive production structures such as poor poultry housing, and this coupled together with the use of untreated water from unprotected water sources such as dams, open wells and rivers increases the chance of incidences of diseases such as avian coccidiosis. Due to the liquidity challenges the country currently faces, resource-poor farmers are unable to purchase commercial drugs, and this coupled together with the closure of veterinary shops in communal and resettlement areas leads to challenges in the con-

trol of livestock diseases. In addition, some resettled farmers are located far from these shops and have to travel long distances to urban areas to purchase 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 a good example. The ethno veterinary use 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 [3]. *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 [4]. *Aloe chabaudii schonland* is a traditional medicine which has also been used to control avian coccidiosis, over years in most rural parts of eastern Zimbabwe and Mozambique [5]. Majority of communal farmers, who use it, claim that 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 chemothe-rapeutic 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 addressing 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 *Aloe chabaudii schonland* juice in the control of coccidiosis in small scale broiler production, as a substitute for a popular chemo therapeutic sulphonamide.

2. Materials and Methods

2.1. Study Area Description

This research was carried out at Family Aids Caring Trust, located 1km 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 1000 mm - 1200 mm 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 4610 feet above sea level (1410 m).

2.2. Methodology

2.2.1. Experimental Birds, Design and Management

30 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 - 180 g live mass. At this age, 25 birds were infected with an average 20,000 coccidian oocysts via drinking water using infected fecal 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 [6]. 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 1 m² where feed was provided *ad libitum* and bird 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.

2.2.2. *Aloe chabaudii* Identification and Chemical Analysis

A search was made for *Aloe chabaudii* to be used in the study. An *Aloe* species was positively identified and confirmed as *Aloe chabaudii schonland* 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 200 g equivalent to 50 g of *Aloe* extract.

2.2.3. 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. 200 g 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 400 g (approx 20% m/v extract) and 600 g (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 sulfaclozine (ESB₃). Dosages started from the third week and continued for one week with all other variables being kept constant.

2.2.4. Data collection

1) Oocyst counts

After a week of giving the assigned treatments, individual birds per group had their fecal samples collected by means of plastic collecting bags. About 10 g from each sample collected was taken to the central veterinary laboratories for oocysts counting using the McMaster’s chamber oocysts counting technique. Briefly in this technique, 10 g of fecal 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 McMaster’s fill chamber. The number of oocysts was counted under a microscope and the actual number of oocysts was calculated using the following formula:

Number of oocytes per gram of litter

$$(1.1) \left(\frac{n}{0.1} \right) \times 100 \times 0.1$$

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.

2) Lesion scoring

Individual birds per treatment were sent for gastro intestinal lesion analysis once at the end of the treatment period, after fecal 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 [7]. The data collected was transformed before analysis.

3) 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**).

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

4) Statistical analysis

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

$$(1.4) \left(\frac{\text{initialcount} - \text{finalcount}}{\text{initialcount}} \right) \times 100$$

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

3. Results

3.1. Fecal Egg Reduction Technique for Oocyst Counts

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

Table 1. 2 × 2 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 = (1.2) $\frac{a}{a+b}$ and risk for non-exposed group = (1.3)

$\frac{c}{c+d}$, where 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.

Table 2. Fecal egg reduction (FERT/10 g fecal matter).

| treatment | <i>Aloe</i> 10 | <i>Aloe</i> 20 | <i>Aloe</i> 30 | 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 |

3.2. Oocyst Count

From **Figure 1**, there was a significant reduction in the fecal 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 fecal 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 fecal 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 fecal matter. In the control there, was an increase in oocyst count value to a higher level of 3487 oocysts/10g fecal matter, from that of the average initial level of in-

fection which was 2000 oocysts/10g fecal 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 fecal matter, having started the experiment without infection.

3.3. Lesion Scores

Figure 2 shows that there were no significant differences ($p > 0.05$) 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.

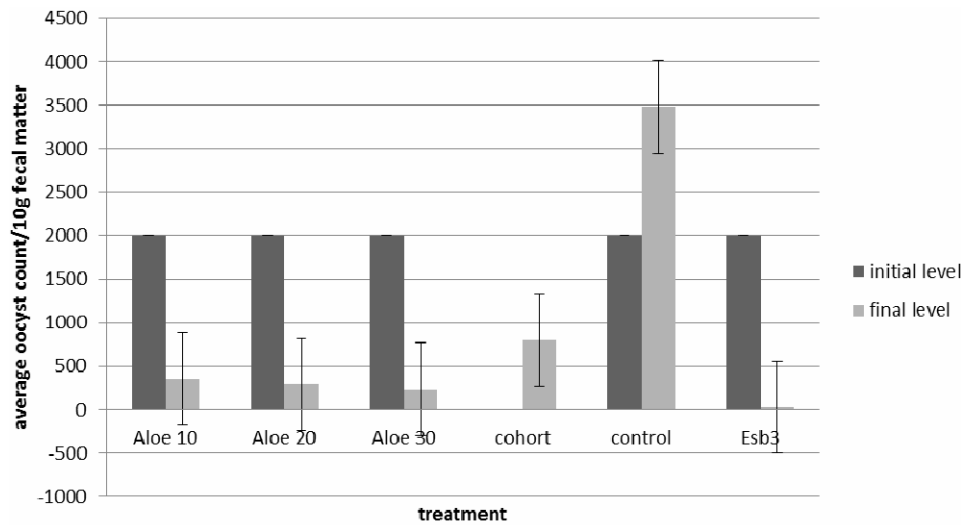


Figure 1. Changes in average pre and post treatment oocyst counts for all treatments 1: Changes in average pre and post treatment oocyst counts for all treatments. CV (%) = 1.6, S.E = 14.11.

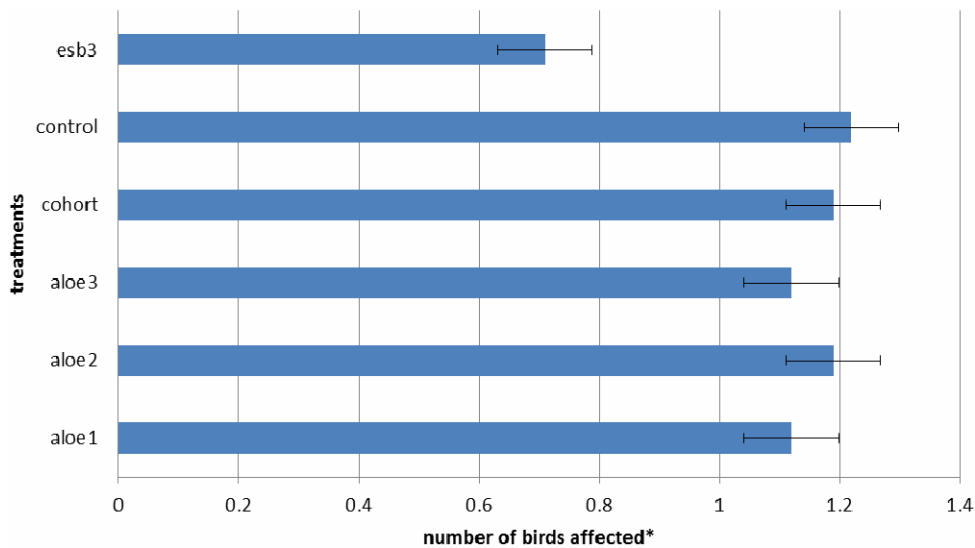


Figure 2. Distribution of number of birds found to have lesions per treatment.

3.4. Relative Risk

The relative risk of infection with coccidiosis was calculated and found to be 100%.

$$(1.5) \left(\frac{\text{riskin exposedgroup}}{\text{riskinnon exposedgroup}} \right) \times 100$$

4. Discussion

Aloe managed to reduce oocyst levels to minimum levels but the results were not comparable to that of ESB₃. Results obtained by [2] showed that treatment with 0.8 g 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 [8] 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 [9]. 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 [10], 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.

[4] 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 [8] 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* [11]. In another experiment involving using *A. secundiflora* gel as anticoccidial, [12] 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. chabaudii* gel treated groups, which is different from what was shown with the oocyst count results. [11] 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 fecal matter and vice versa. According to [13], *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.

5. Conclusion

Coccidiosis has been shown to be a disease of economic importance under poor habitat, health and nutrition. This thus implies that there is a 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 fecal 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* to levels beyond those used in this study. There is also a 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 the use of sulphonamides.

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