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Effect of Adlai (*Coix lacryma-jobi* L.) roots on small ruminants naturally infected with gastrointestinal helminth parasites and Coccidia

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Abstract

Parasites pose a significant threat to the health of small ruminants. Natural products such as Adlai roots have anthelmintic potentials. The study aimed to determine the Anthelmintic potential to the small ruminants. A total of 12 goats and sheep regardless of sex and age were used in the study and randomly distributed into four (4) Treatments with Three (3) replications following Randomized Complete Block Design. There were four different levels of Treatments. Treatment 1 as the control, Treatment 2 (100ml of Adlai roots decoction), Treatment 3 (150ml of Adlai roots decoction), and Treatment 4 (200ml of Adlai roots decoction). Freshly excreted feces were collected and placed in plastic cups and immediately examined through Modified Mc Master Test to evaluate the Anthelmintic activity of the designated treatments. The evaluation criteria were based on the frequency counts of egg parasites and oocysts per 2 grams of feces under different treatments. Results were analyzed using the analysis of variance (ANOVA) of Randomized Complete Block Design (RCBD). Tukey's test was used to compare treatment means showing significant difference. Statistical results were non-significant among Treatment means in all of the parameters except for the strongylid counts for the month of April which showed significant difference among treatment mean at ($P < 0.05$) where sheep treated with Treatment 4 (200ml of Adlai roots decoction). Adlai roots decoction has anti-parasitic activity.

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Introduction

Small ruminants form an integral and important component of the pattern of animal production in Asia. The importance of small ruminants is primarily associated with their small size, which is significant and to the advantage of mankind for three important reasons: economic, managerial, and biological (Devendra and Burns1983). Goat and sheep production in the Philippines is currently a sunrise industry or 99% backyard (PSA 2016). It stands at a meager 3.71M head in 2016. This small population base is traceable to two basic factors: high slaughter rate and low productivity. Low productivity stems from lack of knowledge on improved management practices and a lack of access to quality breeders. Growth is slow and weight averages only 15kg at market age (PSA 2016).

Eleven regions recorded decreases in production, ranging from 0.4 percent to 6.2 percent. The highest decline in production was reported in Central Luzon, from 2,727 metric tons live weight in 2018 to 2,557 metric tons, live weight in 2019 respectively. The decline was due to poor nutrition, lack of health management, and low productivity (PSA, 2019). Sheep and goats share many health problems however, there are some important differences between the species (Pezzanite, et. al. 2009). Serious problems with internal parasites indicate that changes in management are required. Practices that increase the resistance of the animals and exposure to infectious stages of the parasites can avoid the need for regular deworming (Macey 2002).

Adlai (*Coix- Lacryma- jobi L.)* commonly known as Job's Tears, is a broad-leaved, grain, leaving tropical plant of the family Poaceae considered as nutritious healthy food in Asian countries. Its roots and grain are also used as traditional medicine and reported different biological activities including anti-inflammatory, anti-tumor, anti-allergic, anti-mutagenic, anti-microbial effect and anthelmintic (Siddique KI. et.al. 2017). The intent of this paper is to evaluate the effects of Adlai roots as anthelmintic through decoction process in small ruminants.

Materials and methods

Time and Place of the Study

The study was conducted last October 2018-May 2019 atcmU Goat and Sheep Project.

Facilities and Equipment

CMU Goat and Sheep Project was used. Materials used were empty sacks, weighing scale, basin, casserole, chopping board, chopping knife, strainer, pitcher, syringe, plastic cups, ice water cellophane, burner stove, LPG, and cellophane.

Experimental Animals

Twelve (12) naturally infected weaned goat and sheep kid weighing 10 to 20kg about 2 to 5 months of age and regardless of the sex at the CMU Goat and Sheep project was used in the study. In the selection of experimental animals, there were considerations involved such as the initial weight of each kid, age, body score and fecal egg count of each animal should not be less than 250 eggs per gram (EPG) of feces. The Fecal analysis was done at the Animal Disease Diagnostic Laboratory, College of Veterinary Medicine. For the feeding system, the animals were fed with their usual diet and was subjected to confinement together with the herd.

Goat and Sheep subjected for treatment were marked with a paint spray and with their corresponding ear notch for identification purposes. This was done to avoid confusion during the administration of Adlai roots decoction and in the collection of fecal sample in each animal.

The kids were drenched with different levels of Adlai roots decoction facilitated with a bottle three times a week (Monday, Wednesday, and Friday). Week after the administration of treatment, fecal samples were taken every end of the week for three months. Each sample taken was subjected to fecal analysis. Weekly fecal egg count results of post- treatment were recorded and compared with pre-treatment fecal egg count results using RCBD design to compare means and evaluate the efficacy of the Adlai roots decoction.

Experimental Treatment

The following was the experimental treatments:

Treatment 1 = 0 ml of adlai roots decoction

Treatment 2 = 100 ml of adlai roots decoction

Treatment 3 = 150 ml of adlai roots decoction

Treatment 4 = 200 ml of adlai roots decoction

Preparation of Treatment

The Adlai roots were collected at the Agricultural Experiment Station (AES), Musuan, Bukidnon. After collection, the 1kl. Of Adlai roots were washed with running tap water and then placed into the casserole and boiled in 1liter of water within 40 minutes. After that, the Adlai roots decoction was kept warm and transferred into a clean container using the measuring cups. The Adlai roots decoction was measured before given to animals.

Data Collected

The following data collected for statistical analysis were:

1. Total eggs per gram (EPG) of feces counts and oocysts per gram in each kids (Modified McMaster Egg Counting Technique)
2. Present predominant species of gastrointestinal helminth parasites through the rectal fecal sample (Modified McMaster Egg Counting Technique). Modified McMaster Egg Counting Technique

1. Weigh 4 g of feces and mixed thoroughly with 60 ml of any floatation solution into a plastic cup (1:15).
2. Pour fecal suspension through a wire mesh screen or a fine container.
3. Mix the filtrate thoroughly with the aid of spatula and strainer 10 times by transferring alternately from 1 cup to the other in order to ensure that there was a uniform suspension of fecal materials.
4. Transfer aliquot of fecal suspension to each chamber, loading egg counting chamber with 0.15 ml.
5. Count all the eggs with in ruled area (1cm2) of each chamber using the 10x objective. The number of eggs per 2g feces was calculated by multiplying the counted eggs with both chambers by 50.

Statistical Analysis

The data that were collected and tabulated, analyzed using a student RCBD and Tukey's Test were used for any significant Treatment mean.

Results and discussion

There are two predominant types of gastrointestinal Parasites infectingcmU Goat and Sheep as shown in Fig.s 1, 2 and 3. These are *strongylids*, *coccidia* and *moniezia*.

Coccidia

Coccidia are protozoan unicellular organisms of the phylum Apicomplexa that parasitize most major lineages of vertebrates as well as invertebrates (Fayer, 1980). Due to taxonomical complications and the vast amount of data being compiled within the coccidia field, a specialist website has been erected in an attempt to record, store and resolve taxonomical redundancy associated with these organisms. According to the "Coccidia of the World" compiled by Don Duszynski, Steve Upton and colleges, there are currently over above 2000 named species of coccidia distributed over 8-13 families. There is no doubt this is a true underestimation of the real diversity of coccidia, and many thousands remain undescribed. Coccidian parasites are responsible for several of the most severe diseases are known in animals and man (Levine, 1973). For example, in domestic animals, *Eimeria tenella* is responsible for considerable decrease in growth and development of domestic poultry flocks by damage caused in the intestinal lining during infection, and costs the industry that exceed US$1.5 billion annually (Sharman *et al*., 2010).

Coccidia are complex unicellular parasites of vertebrates and invertebrates. They parasitize their host intracellularly. They are classified according to the following characteristics: Possession of an environmentally resistant oocyst wall (Lee *et al*., 2000). Sporozoites develop within a secondary cyst within the oocyst known as a sporocyst, with the exception of *Cryptosporidium* where they develop directly within the oocyst (Lee *et al*., 2000, Robinson *et al*., 2010).

Strongylids

The most common parasite *strongylid* nematode of goats in the Philippines is *Haemonchus contortus*.

Haemonchus contortus, known as the *barber's pole worm* is relatively common in ruminant abomasums. The common name of this parasite is related to the macroscopically visible entwining of the blood-filled intestine and white uterus in the female worm. Hyper infested pastures containing numerous third-stage larvae are the source of infection. Lambs are particularly, at risk. Larvae on grasses are ingested by the host and enter the abomasum, where they may lie dormant within the gastric glands. After development to adults, they exit to the abomasal surface and attach via a buccal tooth. Eggs pass in the feces, completing the life cycle. *Haemonchus* are blood feeders and can cause severe anemia, hypoproteinemia, and resultant edema. This edema is characteristically present in the intermandibular space, resulting in a physical resemblance to a bottle ("bottle jaw"). As with any process resulting in anemia and hypoproteinemia, there are pale mucous membranes, stunting, and diarrhea. Diagnosis is by fecal egg counts and at necropsy by semi quantification of abomasal parasite load along with the attendant lesions of anemia and hypoproteinemia. At necropsy the carcass is pale and has generalized edema and fluid in all body cavities secondary to hypoproteinemia. Abomasal contents are fluid and discolored from free blood. Foci of mucosal hemorrhage are present at sites of worm attachment (Gelberg, 2017).

Other Parasite Present

Moniezia expansa

Moniezia expansa as shown in Fig. 3. belongs to Kingdom Animalia, Phylum Plathelminthes, Class Cestoda, Order, Cyclophylidea, Family, Anoplocephalidae, Genus, Moniezia, Species M. expansa, *Moniezia expansa* worms occur worldwide in the small intestine of ruminants, reach a length of 10m and their posterior proglottids are very broad (~2.5cm). these proglottids are characteristics by the presence of a double set of sexual organs being situated at the 2 lateral sides of the proglottids. Intermediate hosts are oribatid mites, which are fed together with grass. The prepatent period took about 30-52 days; patency lasted to 3-8 months. Anemia, diarrhea, loss of weight are the most common signs of disease. Diagnosis was done by finding the characteristic proglottids around the anus or by the determination of eggs in the faeces (Mehlhorn, H. 2008).

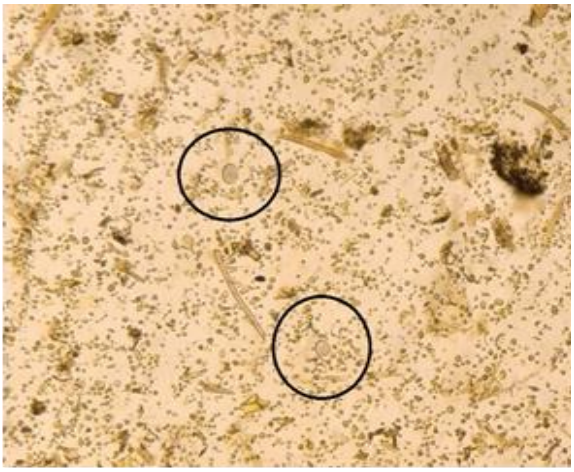

Fig. 1. *Coccidia* oocysts present in experimental Goat.
Source: ADDL

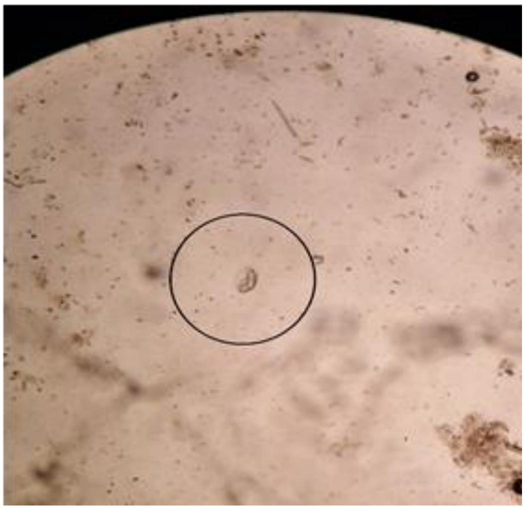

Fig. 2. *Strongylid* egg and *Coccidia* oocysts present in experimental Sheep.
Source: ADDL

Fig. 3. *Moniezia expansa*
Source: ADDL

Initial Coccial count on Pre-Treatment in Experimental Goats

Table 2 shows the coccidial counts of an experimental goat on Pre-treatment. Treatment 2 with 70,300.00 got the highest number of coccidial oocysts followed by Treatment 1, 3, and 4 respectively. According to (Pezzanite, L. et.al, 2017). Goats are generally more susceptible to internal parasites. The group most susceptible to parasites are young animals. It follows that the experimental goats are infected with coccidia. Coccidia is usually found in animals in confinement or intensive system, as a result of poor sanitation, overcrowding and stress. (Pezzanite, L. et.al 2017). These values however, are non-significantly different from each other which implies that the coccidial count is homogenous.

Table 1. The initial count of Coccidia on Pre-Treatment for Experimental Goats.

Treatment	Replication 1	2	3	Mean ^{ns}
1	20600.86	7399.44	6049.73	11350.01
2	201699.80	2199.61	7000.00	70300.00
3	0200.00	2749.95	7349.63	6766.52
4	2599.98	2449.26	4900.00	3316.41

CV= 95.67%

ns= not significant

Coccidial count after Post Treatment (October-December) Tables 3, 4 and 5 show the effect of the treatment on experimental goats on 1st, 2nd, and 3rd months readings respectively. In this study, the result shows a decrease in the coccidial counts a week after the treatment particularly Treatments 3 and 4 (150ml of Adlai roots decoction) and (200ml of Adlai roots decoction) there is also a decrease seen in Treatments 1 and 2. In the month of November, it was observed that Treatment 1 (control) got the highest number of coccidial counts while Treatments 2, 3 and 4 were observed to have a decreasing number of coccidial counts.

Similarly, in the 3rd month (December), there was a notable decreasing number of coccidial count from Treatments 1, 2, 3, and 4. Even though this does not reach statistical fecal significance, there are variations for the mean egg count.

It was found out in the phytochemical screening, that *Coix lacryma- jobi L.* in seeds, leaves, and roots indicated the presence of saponin, phenols and tannin, and alkaloids (Sajan, D. et.al, 2017). The study of (Sajan, et.al, 2017) reveals that leaf, seeds, and roots extract of *Coix lacryma- jobi L.* tested for anthelmintic actively showed a better effect. Anthelmintic effect of plants is normally ascribed to secondary metabolites such as proanthocyanidins, alkaloids, or polyphenols, known as condensed tannins. The Presence of such secondary metabolites might be responsible for such a period of effect. (Sajan, D. et.al, 2017).

Table 2. Coccidial count of Experimental Goats (October).

Treatment	Replication 1	2	3	Mean ^{ns}
1	15322.22	6283.33	10183.33	10596.29
2	14683.33	4233.33	2833.33	49249.98
3	4100.00	2383.33	3433.33	3305.55
4	9150.00	450.00	1433.33	3647.77

CV= 230.70%

ns= not significant

Table 3. Coccidial count of Experimental Goats (November).

Treatment	Replication 1	2	3	Mean ^{ns}
1	24637.50	1375.00	25587.50	17199.83
2	12262.00	4550.00	6850.00	7887.3
3	2187.50	4812.50	6687.50	4562.50
4	7637.50	387.50	4825.00	4283.33

CV= 75.83%

ns= not significant

Table 4. Coccidial count of Experimental Goats (December).

Treatment	Replication 1	2	3	Mean ns
1	18350.00	1337.50	7740.00	9142.50
2	1270.00	10930.00	7740.00	6646.66
3	3620.00	590.00	3530.00	2580.00
4	850.00	1310.00	4370.00	2176.66

CV= 110.72%

ns= not significant

The initial Coccidia counts in the experimental Sheep Pre Treatment

The feces of the experimental sheep were examined before the treatment was administered to ascertain that the sheep are naturally infected with gastrointestinal parasites. Table 6 shows initial coccidia number of oocysts /2g feces. Statistical Analysis showed non-significant. Occurrence of internal parasites were also observed because the experimental animals did not undergo deworming, hence the sheep used in this study had fulfilled the criteria regarding parasitic egg or oocysts excretion before the administration of the treatment. Gastrointestinal parasitism is associated with economic losses, lower productivity, reduced animal performance (Badran et.al., 2012) as well as mortality and morbidity (Negasi *et al.*, 2012).

Table 5. The initial count of Coccidia on Pre-Treatment in Experiment Sheep.

Treatment	Replication 1	2	3	Mean ns
1	50.00	800.00	600.00	483.33
2	5700.00	1300.00	1200.00	2733.33
3	2550.00	650.00	600.00	1266.66
4	1200.00	1150.00	6200.00	2850.00

CV= 367.16%

ns= not significant

Coccidial count (no. of oocysts/2g feces) Post-treatment in experiment Sheep

Tables 7, 8 and 9 show the effect of treatment on the experimental sheep on (February, March, and April) monthly readings respectively.

Results showed no significant differences among treatment means. Even though it does not reach significance level, there were observable differences on the oocysts per gram of the different treatments, as treatments administered with an increasing level of Adlai roots decoction. It showed a decreasing number of coccidial oocysts compared to the Treatment 1 as (control), it was most likely due to the bioactive compounds contained in Adlai roots of *Coix lacryma-jobi L.* These bioactive compounds have a broad spectrum of anthelmintic acting by inhibiting microtubules polymerization after binding to the colchicine. It carves a decrease in microtubules in intestinal cells and uptake of glucose by adult and larval forms of parasites (Sajan, et.al 2017).

Table 6. Coccidia count of Experimental Sheep (February).

Treatment	Replication 1	2	3	Mean ns
1	487.50	533.33	862.50	627.77
2	850.00	11075.00	183.33	4036.11
3	2987.50	9162.50	1637.50	4595.83
4	1062.50	1612.50	500.00	1058.33

CV= 114.54%

ns= not significant

Table 7. Coccidia count of Experimental Sheep (March).

Treatment	Replication 1	2	3	Mean ns
1	775.00	283.33	275.00	444.44
2	350.00	8812.50	87.50	3083.33
3	775.00	1712.50	862.50	1116.66
4	662.50	1875.00	262.50	933.33

CV= 168.84%

ns= not significant

Table 8. Coccidia count of Experimental Sheep (April).

Treatment	Replication 1	2	3	Mean ns
1	4925.00	337.50	300.00	1854.16
2	362.50	3475.00	187.50	1341.66
3	575.00	500.00	925.00	666.66
4	562.00	775.00	275.00	604.00

CV= 128.01%

ns= not significant

Initial Strongylid and Pre-Treatment in Experiment Sheep

The initial strongylid count is presented in Table 10 were the average egg count per treatment is 500, 3550, 1200, 5716.66 for Treatments 1, 2, 3 and 4 respectively. Statistical analysis showed nonsignificantly among the treatment mean. Sheep can be a host to variety of internal parasites and parasitic worms lay eggs, more called oocysts which are passed out through the feces (PCAARRD, 2003).

Table 9. The initial count of Strongylid on Pre-Treatment in Experiment Sheep.

Treatment	Replication 1	2	3	Mean ns
1	750.00	750.00	0.00	500.00
2	7900.00	2250.00	500.00	3550.00
3	500.00	1500.00	1600.00	1200.00
4	0.00	350.00	16800.00	5716.66

CV= 116.63%

ns= not significant

Strongylid counts at Post-treatment in the experimental Sheep.

Tables 11, 12 and 13 present the results of strongylid fecal egg count for February, March, and April. After administration of the treatment, it was observed that Treatment 4 was an effective anthelmintic for strongylid since there was a noticeable decrease in the number of fecal egg count of strongylid from Treatments 2, 3 and 4 based on the numerical values indicated on the Tables 10, 11, and 12.

It was observed that the higher levels of Adlai roots decoction administered to the experimental sheep the lower the number of fecal egg count. Although it did not reach the statistically significant level in February and March, however in the month of April statistical analysis showed a significant result at ($P<0.05$) (Muller-Harvey et.al 1992) stress that saponins, alkaloids, polyphenols, and tannin are active compounds of the plant which have been considered to have Anthelmintic effects. The study of (Sajan, D. *et al,* 2017) indicated that *Coix lacryma-jobi* L. tested for Anthelmintic activity showed that there is paralysis and death of susceptible gastrointestinal parasites occur slowly due to insufficient energy for the production of Adenosine Triphosphate and their clearance from the gastrointestinal tract.

Table 10. Strongylid count of Experimental Sheep (February).

Treatment	Replication 1	2	3	Mean ns
1	175.00	262.50	237.50	225.00
2	0.00	2975.00	62.50	1012.50
3	325.00	7225.00	150.00	2566.66
4	512.50	3375.00	537.50	1475.00

CV= 124.87%

ns= not significant

Table 11. Strongylid count of Experimental Sheep (March).

Treatment	Replication 1	2	3	Mean ns
1	1487.50	325.00	425.00	745.83
2	125.00	8375.00	287.50	2929.16
3	775.00	4800.00	2062.50	2545.83
4	225.00	662.50	150.00	345.83

CV= 150.96%

ns= not significant

Table 12. Strongylid count of Experimental Sheep (April).

Treatment	Replication 1	2	3	Mean*
1	200.00	833.33	200.00	411.11
2	75.00	4000.00	1100.00	1725.00
3	125.00	1125.00	0.00	416.66
4	0.00	1850.00	116.66	655.55

CV= 93.84%

ns= not significant

*= significant

Comparison between Pre and Post-treatment of Parasites in Goat.

Fig.s 4, 5 and 6 show the comparison between Pre-Post-treatment of parasite eggs in goat and sheep. The Fig.s indicated that there was a trend observed with the results. The higher the amount of Adlai roots decoctions, the lower the number of fecal egg count. Consistently Treatment 4 (200ml of Adlai roots decoction) got the lowest number of fecal egg count and oocysts both goat and sheep while Treatment 1 (Control) got the highest number of fecal egg and oocysts count.

This implies the administration of Adlai roots decoction reduced the number of parasitic eggs. Adlai roots, stem and leaves (*Coix lacryma-jobi* L.) were tested for phytochemical screening, and it was found out that there was the presence of secondary metabolites (Chabbra & Gupta, K. 2015).

Similarly, Aswar *et.al.* (2008) stressed that secondary metabolites of plant derive extracts (saponins, alkaloids, tannins and flavonoids and cumarines) were responsible for anthelmintics activity. These anthelmintic may have a wide therapeutic index and are capable of killing or inhibiting egg production of gastrointestinal parasites.

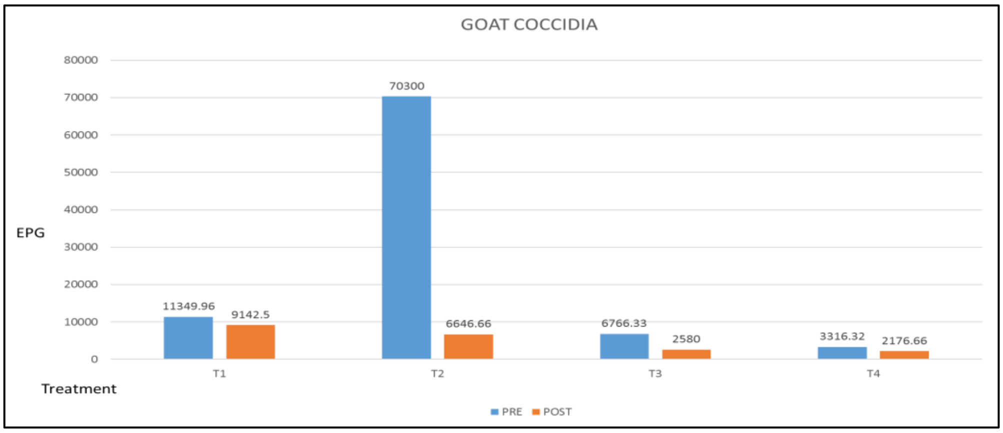

Fig. 4. Goat coccidial count pre and post treatment comparison.

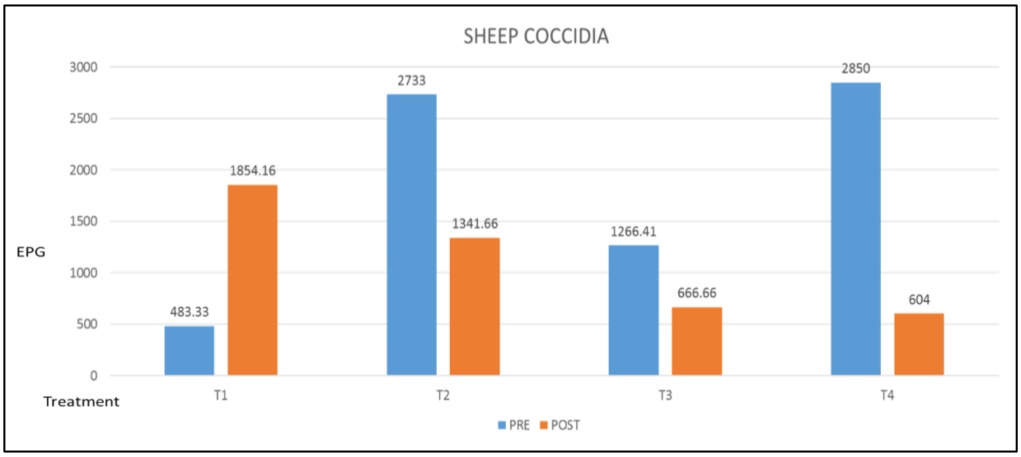

Fig. 5. Sheep coccidial count pre and post treatment comparison.

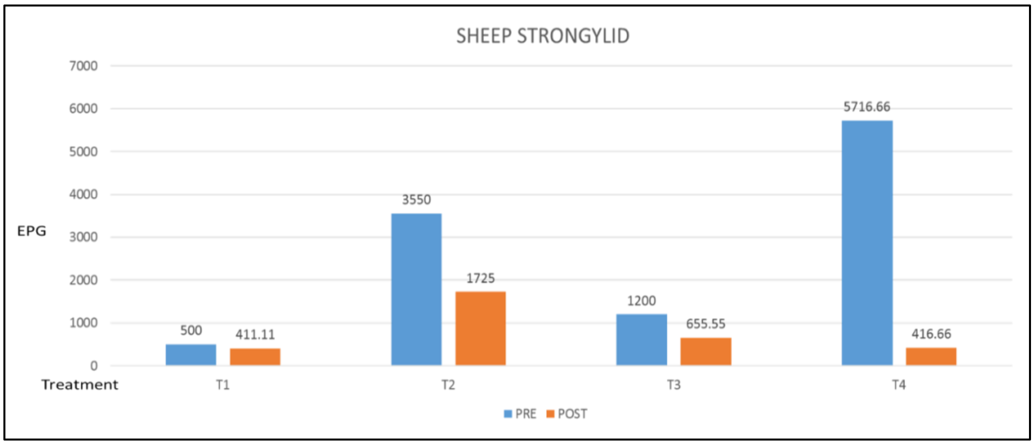

Fig. 6. Sheep strongyloides count pre and post treatment comparison coccidia oocysts s and strongylid fecal egg count reduction percentage in goat and sheep.

Table 14, shows the coccidia oocysts reduction percentage in Goat at post Treatment. No significant effect on coccidial oocysts reduction percentage. Although Treatment 2 got the highest fecal egg count reduction percentage with 86.50%, 40.33%, 21.95% and 13.72% T4, T3, and T1 respectively. Tables 15 and 16 present the coccidia oocysts and strongylid fecal egg count reduction percentage in sheep at post treatment.

Statistical Analysis showed nonsignificantly. Treatment 3 (150ml Adlai roots decoction) got the highest reduction percentage with 85.49%. Even though it did not reach statistical significance, there were observed differences in the mean percentage of oocysts and fecal egg count of the different treatments.

Again this result could be due to the bioactive compounds in the Adlai roots that are extracted through decoction process are having anthelmintic properties (Future, A. 2017).

Table 13. Coccidia oocysts reduction percentage in Goat.

Treatment	October	December	% Percentage
T1	10596.29	9142.50	13.72
T2	49249.98	6646.66	86.50
T3	3305.55	2580.00	21.95
T4	3647.77	2176.66	40.33

Table 14. Coccidia oocysts reduction percentage in Sheep.

Treatment	February	April	% Percentage
T1	627.77	1854.16	195.36
T2	4036.11	1341.66	66.76
T3	4595.83	666.66	85.49
T4	1058.33	604.00	42.93

Table 15. Strongylid fecal egg count reduction percentage in Sheep.

Treatment	February	April	% Percentage
T1	225.00	411.11	(82.72)
T2	1012.50	1725.00	70.37
T3	2566.66	416.66	83.77
T4	1475.00	655.55	55.56

Conclusion

Based on the results the following conclusions are derived:

1. CMU goat and sheep were found to have a gastrointestinal parasite called Genus Coccidia and Strongyloididae.
2. Number of eggs per gram of feces was constantly reduced every month after administration of Adlai roots decoction.
3. Coccidial oocysts and the number of eggs of strongylids in both goats and sheep reduced its number, thus Adlai roots decoction was an effective anti-parasitic.
4. The higher the amount of levels of Adlai roots decoction administered to the experimental animals showed better results consistently. Thus, indicating the use of Adlai roots decoction as Anthelmintic in small ruminants.
5. *Moniezia expansia* is another type of parasites found in the experimental sheep.

Recommendation(S)

1. Isolation of bioactive components of *Coix lacryma-jobi L.* (Adlai Roots) decoction to determine what specific components from these compounds is having a lethal effect against eggs of parasites.
2. Increase the levels of concentration of Adlai Roots Decoction to improve the effectivity of the Adlai Roots decoction as Anthelmintic or Dewormer.
3. To evaluate the potential anthelmintic effect of Adlai Roots decoction using other experiments (i.e in vitro) in different species classified similarly as gastrointestinal helminths; and
4. Also further studies on Adlai Roots decoction toxicity and duration of storage would be helpful.

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