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Research Article

Effects of Slender Carpetweed (*Mollugo oppositifolia*) Extract on Intestinal Worms of Native Chicken *(Gallus gallus domesticus)*

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ABSTRACT

Poultry is the fastest growing component and one of the most efficient and effective means of food supply to the society in the form of meat and egg. Parasitic infections lead to low productivity and increased mortality in poultry. A total of forty-five (45) apparently healthy Philippine native chickens, regardless of sex and weight, ranging from 4-6 months of age, were randomly distributed in five (5) treatments. Each treatment was replicated thrice with three (3) birds per replication following the Completely Randomized Design (CRD). Simple Test Tube Flotation was used to establish which parasite groups are present in the fecal samples. The McMaster technique was also used as a counting chamber which enables a known volume (2 x 0.15 ml) of fecal suspension to be examined microscopically. The number of eggs per gram was calculated by counting the number of eggs within the grid of each chamber which gave the data on eggs per gram (EPG) of feces. The results revealed in T4 (1ml) slender carpet weed extract and T5 (1.5ml) have anthelmintic potentials when used in native chickens. Thus, slender carpet weed extract is an effective deworming agent. Slender carpet weed extract is effective in lowering the egg per gram of intestinal parasites found in native chickens. The optimum volume to ensure effectivity is 1.5 ml which revealed the strongest effect in reducing intestinal worms.

Keywords: *Agri-Education, Experimentation, Native chicken, Slender carpetweed*

Introduction

Native chicken farming is typically raised in the backyard of rural household and this has been the practice since native chicken was introduced as household poultry. Most farmers raise native chicken as additional income resources for meat and egg production. Native chicken meat in the market is highly in demand for its distinct taste and among health-conscious Filipinos. Native chicken meat is highly preferred since it is naturally raised, with the absence of drug residues, and savory. When

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cooked than those purely confined broiler chickens. Their eggs are distinctly palatable because of the intense yellow pigmentation. Native chicken farming is increasingly gaining attention among many farmers because of its potential as an income generator and accelerator is becoming apparent. Raising native chickens in the backyard is relatively a simple process since it does not demand high cost of maintenance as long as housing is available. Feed sources can be available from the vegetation in the backyard such as varieties of edible leaves, worms, and acher crops.

Slender carpet (*Mollugo oppositifolia***)** is a slender or ascending, smooth, branched, annual herb, with branches 10-40 cm in length. The leaves are opposite or whorled, spatulate, oblanceolate to oblong-obovate, 1-3 cm long, and up to 1cm wide weed. Slender carpet weed, locally known as Papait or Malagoso, is a common weed that grows at low and medium altitudes throughout the country which has been found to have plenty of therapeutic value. According to Juliana *et al.* (2015), slender carpet weed is claimed to be rich in iron and could be a good source of calcium. It is potential source natural antioxidants, antidiabetic, anticancer, and antimicrobial property.

Phytochemical screening of slender carpetweed states that the plant is rich in alkaloids saponins and terpenoids in excess together with tannins, glycosides, steroids, and flavonoids. In this study, the slender carpet weed was tested for its anthelmintic efficacy in native chickens.

Objectives of the Study

Generally, this study was conducted to determine the anthelmintic effects of slender carpet weed plant extract on intestinal worms of native chickens.

Specifically, the study aimed to:

1. Determine the optimum volume of slender carpet weed plant extract which inhibits the growth of intestinal worms in native chickens;
2. Determine which among the intestinal worms of native chicken is susceptible to slender carpet weed plant extract.

Methods

Research Design

Experimental Design and Treatments

A total of forty-five (45) apparently healthy Philippine native chickens, regardless of sex and weight, ranging from 4-6 months of age, were randomly distributed in five (5) treatments. Each treatment was replicated thrice with three (3) birds per replication following the Completely Randomized Design (CRD). The treatments, in varying volumes of slender carpet weed extract, were administered as dewormer specified as follows:

Treatment 1 – negative control (1 ml water)
Treatment 2 – positive control (levamisole 36 mg/kg)
Treatment 3 – 0.5 ml of slender carpetweed extract (SCE)
Treatment 4 – 1 ml of slender carpetweed extract (SCE)
Treatment 5 – 1.5 ml of slender carpetweed extract (SCE)

Preparation of Slender Carpetweed Leaf Extract

Two (2) kilos of fresh slender carpetweed plants were collected from Camiling, Tarlac. Collected plant samples were washed with clean water and air-dried for an hour before extraction. In extraction, plant samples were removed from the root of the plant, were cut into small pieces, and were crushed using the mortar and pestle. Samples were extracted and filtered using clean cheesecloth to separate the liquid extract from the solid residue. Plant extracts were placed in a clean and dark container.

Deworming Procedure

Prior to the administration of slender carpet weed leaf extract to chickens, feeds were suspended for 18 hours to completely utilize the deworming agents. Only fresh clean drinking water was given to the native chickens. Administration of slender carpet weed extract was administered after 18 hours of feed suspension following the treatments and was given orally to experimental birds through drenching using improvised syringe. Administration of plant extracts were done in the

morning at day 3, 5, 9, 11, 15, 17, 21, and 23, respectively, before fecal collection.

Collection of Fecal Samples

Fecal samples were collected from the experimental birds at 6:00 AM. Sterile plastic was attached to the vent of each animal at 6:00 PM before the day of collection commenced. Collections were done during day 0, 7, 13, 19 and 25, respectively, in the morning. Fecal samples in each collection were placed in labeled sterile plastic cups and were stored in -20°C freezer until further analysis. Fecal samples were subjected for laboratory examination and confirmation at the Regional Animal Disease Diagnostic Laboratory (RADDL) in San Fernando, Pampanga. During transport, fecal samples were placed in coolers filled with ice in order to prevent the hatching and development of eggs.

<u>On the Procedure for the Identification of Nematodes Egg Parasites</u>

Simple Test Tube Flotation

The simple test tube flotation technique is a qualitative test for the detection of nematode and cestode eggs. This is a useful method to use in preliminary surveys to establish which parasite groups are present.

Procedure

Approximately one (1) gram of fecal sample was placed in a vial containing twenty (20) ml sucrose solution. An applicator stick was used to mix the solution thoroughly and to make the suspension homogenous. Additional sucrose solution was added to the suspension up to the rim of the vial. A cover slip was placed on the top of the vial for ten to fifteen (10-15) minutes. After which, the cover glass was lifted and place on the glass slide for microscopic examination under low and high power magnification.

McMaster Technique

The McMaster technique uses a counting chamber which enables a known volume (2 x 0.15 ml) of fecal suspension and was examined microscopically. Thus, if a known weight of feces and a known volume of flotation fluid are used to prepare suspension, then the number of eggs per gram of feces (EPG) was calculated.

Procedure

An approximately one gram (1 g) of crushed samples and 28 ml of saturated sugar solution was mixed vigorously in the beaker until an even emulsion is obtained. The solution was placed into the McMaster counting chamber. The slide was left for 5-10 minutes to allow the eggs to float. The number of eggs per gram was calculated by counting the number of eggs within the grid of each chamber, ignoring those outside the squares. The total number of eggs counted was multiplied by 200. This yielded the data on eggs per gram (EPG) of feces.

<u>On the Management of Experimental Birds</u>

Housing

The ranging area measured thirty feet (30n ft.) in length, width of twelve feet (12 ft.) and nine feet (9 ft.) high. It was divided into twelve (12) divisions and each division has a length and width of 30 ft. and 4ft., respectively. The range area was partitioned using bamboo sheets housing three experimental birds for every division.

Feeding and watering

Feeders were made up of bamboo and waterers were made up of empty bottles of mineral water. The experimental birds were fed four times a day at 7:00 and 11:00 in the morning, and 2:00 and 5:00 in the afternoon with crumble ration. Feeds were given at one- time for 10-15 minutes to avoid feed spoilage when left long in feeders. Potable water was given following the time of feeding. Feeders and waters were cleaned every day to avoid the prevalence and growth of bacteria.

Data Gathered

Initial egg per gram (EPG), per experimental unit are gathered from day 0 prior to the administration of slender carpet leaf extract as the baseline data of the study. EPG was gathered again at day 7, 13, 19, and 25 post-administration of slender carpetweed plant extract. Fecal egg reduction was noted to get the percentage efficacy following the formula Al-Shaibani et al., (2009):

$$\% \text{ Efficacy} = \frac{\text{Pre} - \text{administration EPG} - \text{Post administration EPG}}{\text{Pre} - \text{administration EPG}} \times 100$$

Data Analysis

Prior to administration of slender carpetweed extract fecal samples were collected and the eggs per gram (EPG) are determined using the fomula below:

EPG =total number of eggs x 200

Data gathered were analyzed using the Analysis of Variance (ANOVA) in Completely Randomized Design (CRD). Least Significant Differences was used to test the level of significance among treatment means.

Results and Discussion

On the Average Eggs per Gram (EPG)

Table 1 shows the summary of egg per gram before (Day 0) and after (7, 13, 19 and 25) the administration of treatments. Results revealed that the initial EPG mean count of birds under T1 (water, negative control) which is 7,088.67 has increased to 8,711.00 on day 7 and eventually decreased to 6,889.00 on day 13, 7,066.67 on day 19, and 6,133.33 on day 25, respectively. The observed egg counts, as expected, were relatively bigger than the rest of the treatments including the positive control since no deworming agents were given to birds under this treatment. In T2 (positive control), the initial EPG mean count was 9,044.33 but it decreased eventually in the succeeding day post-treatment with registered average EPG of 5,733.33 at day 7, 2,178.00 at day 13, 1,533.33 at day 19, and 244.33 at day 25, respectively. The average EPG difference recorded in T2 after 25-day post treatment was 8, 800.00 accounting to 97% of the total EPG count as observed from the experimental birds before the experimentation. Treatments 3, 4, and 5 also registered low averages of EPG after 25-day post-treatment with registered means of 911.00, 244.33, and 244.33, respectively, following their EPG means from Day 0 to Day 19. A decreasing trend of values in average EPG was also observed in all treatments including the positive control but not to the negative control which showed fluctuating values of EPG from Day 0 to Day 25.

Significant differences were also observed in all data except from the EPG values in Day 0. In Day 7, both the positive and negative controls are comparable with each other signifying similar effects. Meanwhile, all treatments including the positive control are significantly different to the negative control in all registered data from Day 7 to Day 25. Surprisingly, the data on Treatments 3, 4, and 5 are comparable with the positive control.

This means that the significant variation in all registered EPG averages in these treatments is statistically the same. Identified parasites in EPG records of this experiment were *Ascaridia spp., Heterakis gallinarum., Capillaria spp., Railietina spp* as confirmed by a scientific expert.

Table 1. Summary of egg per gram (EPG) of intestinal parasites found in native chickens before and after administration of treatments

Treatment	Day 0	Day 7	Day 13	Day 19	Day 25	Difference (Day 0-Day 25)
T1 (negative control	7088.67	8711.00^{a}	6889.00^{a}	7066.67^{a}	6133.33^{a}	947.34
T2 (positive control)	9044.33	5733.33^{ab}	2178.00^{b}	1533.33^{b}	244.33^{b}	8,800.0
T3 0.5 ml (SCE)	5822.00	4733.33^{b}	3066.67^{b}	1911.00^{b}	911.00^{b}	4,911.0
T4 1ml (SCE)	4577.67	3000.00^{b}	1755.67^{b}	911.00^{b}	244.33^{b}	4,333.3
T5 1.5 ml (SCE)	5111.00	2844.33^{b}	1866.33^{b}	1044.33^{b}	244.33^{b}	4,866.6

Note: Means with the same letter superscript are not significantly different.
CV= 17.00%

Based on this study, slender carpetweed plant extract showed reduction effects on the number of eggs of intestinal worms in native chickens, with 1.5 ml slender carpetweed plant extract administered revealing the strongest effect for egg reduction from day 7 to day 25 as shown in Fig 1. The results may be attributed to the bioactive compounds present in slender carpetweed such as alkaloids, flavonoids, glycosides, saponins, sterols, tannins, terpenes, and other metabolites with antioxidant activity. Various reports have also shown that many of these phytochemical compounds possess antibacterial, antifungal, antiviral, antiprotozoal, antihelminthic, antidiarrhoeal, anticarcinogenic, anti-inflammatory, antiatherosclerotic and antidiabetic activities. Tannins for instance, interact with protein excreted by nematodes in gut. Tannin can also bind to glycoprotein on the cuticle of the parasite and may cause death (Juliana *et al.,* 2015; Dutta *et al.,* 2012).

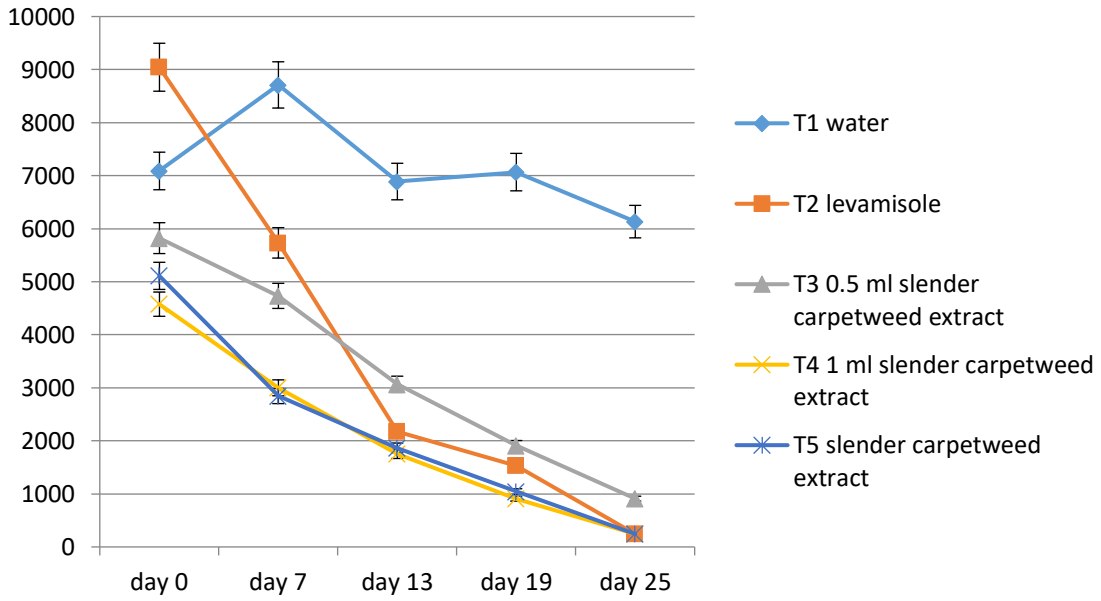

Figure 1. The graphical presentation on the Egg per gram (EPG) of intestinal parasites found in native chick before and after administration of slender carpetweed plant extract and levamisole. Error bars indicate percentage error of the data calculated based on their mean differences.

On the Percentage efficacy of slender carpet weed extract in intestinal worms of native chicken

Table 2 shows the percentage efficacy of slender carpet weed extract, levamisole against intestinal worms in native chicken. In T1, from -23 efficacy in day 7, the percentage efficacy increased to 3% in day 13, 0.3% in days 19 and 13% in days 25. In T2, from 37% efficacy in day 7, it increased to 75%, 83% and 97% in day 13, 19 and 25, respectively. T3 registered 19% efficacy in day 7, 47% in day 13, 67% in days 19 and 84% in days 25. In T4, from 34% efficacy in day 7, it increased to 62% in day 13, 80% in days 19 and 95% in days 25. T5 also registered an increasing efficacy trend like T2, T3, and T4 from Day 7, Day 13, Day 19 and Day 25 with efficacy percentages at 44%, 63%, 80%, and 90%, respectively.

According to the study of Puzon and Rivera (2015) Slender Carpetweed plants have anthelmintic efficacy due to the components, such as alkaloids, flavonoids, glycosides, saponins, sterols, tannins, terpens, and other metabolites with antioxidant.

Table 2. Percentage efficacy (%) of slender carpetweed extract (SCE) and levamisole as anthelmintic agents in native chicken

Treatments	Day 7 (%)	Day 13 (%)	Day 19 (%)	Day 25 (%)
T1 (negative control)	-23[a]	3[a]	0.3[a]	13[a]
T2 (positive control)	37[ab]	75[ab]	83[b]	97[b]
T3 0.5 ml (SCE)	19[b]	47[b]	67[b]	84[b]
T4 1ml (SCE)	34[b]	62[b]	80[b]	95[b]
T5 1.5 ml (SCE)	44[b]	63[b]	80[b]	95[b]

Note: Means with the same letter superscript are not significantly different.
CV= 18.6%

Figure 2 shows the percentage efficacy throughout the days of observation (day 7, 13, 19 and 25). As shown in the graph, T4 and T5 had reached the highest efficacy for about 95% in days 19 and 25 of study. As expected, lowest efficacy percentages were observed in T1 since no deworming agents were administered. This means that birds which remained untreated have significant number of intestinal worms which could possibly affect their health as they grow. In the research conducted by Juliana et al (2015) phytochemical screening of slender carpetweed states that the plant is rich in alkaloids, saponins, and terpenoids in excess together with tannins, glycosides, steroids, and flavonoids.

Hence, these results show further suggest that slender carpetweed extract have anthelmintic property when used in native chickens. Slender carpetweed extract 1 to 1.5 ml can achieve higher efficiency when used continuously for at least 25 days of treatment.

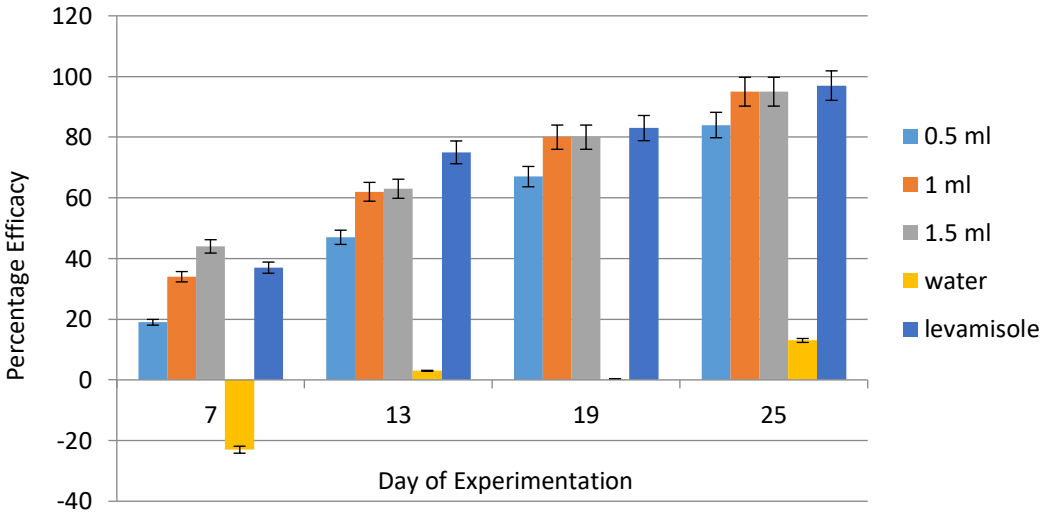

Figure 2. Graph representation of percentage efficacy of slender carpetweed plant extract and levamisole against intestinal worms of native chicken. Error bars indicate percentage error of the data calculated based on their mean differences.

Summary

The study was conducted to determine the optimum volume of slender carpet weed extract as possible deworming agents against intestinal worms of native chicken. Forty-five (45) native chickens, regardless of sex and weight, ranging from 4-6 months of age, randomly distributed in five (5) treatments were used in this study following the Completely Randomized Design (CRD). Each treatment was replicated thrice with three (3) birds per replication. Varying volumes of slender carpet weed

extract were administered as dewormer specified as treatments: Treatment 1 – control 1m water; Treatment 2 – positive control (levamisole); Treatment 3 – 0.5 ml of Slender carpetweed extract; Treatment 4 – 1 ml of Slender carpetweed extract; and Treatment 5 – 1.5 ml of Slender carpetweed extract.

Simple Test Tube Flotation was used to establish which parasite groups are present in the fecal samples. The McMaster technique was also used as a counting chamber which enables a known volume (2 x 0.15 ml) of fecal suspension to be examines microscopically. The number of eggs per gram was calculated by counting the number of eggs within the grid of each chamber, ignoring those outside the squares. The total number of eggs counted was multiplied by 200. This gives the data on eggs per gram (EPG) of feces.

The results revealed in T4 (1ml) slender carpet weed extract and T5 (1.5ml) have anthelmintic potentials when used in native chickens. Thus, slender carpet weed extract is an effective deworming agent.

Conclusion

Following the results of this study, the following conclusions can be drawn:

1. Slender carpet weed extract is effective in lowering the egg per gram of intestinal parasites found in native chickens.
2. The optimum volume to ensure effectivity is 1.5 ml which revealed the strongest effect in reducing intestinal worms.

Based on findings of this study, it is recommended that slender carpet weed extract can be used as an alternative anthelmintic agent in native chicken due to its bioactive components (alkaloids, flavonoids, glycosides, saponins, sterols, tannins, terpenes). It was also noted in that nematodes were found to be the most vulnerable to slender carpet weed extract.

Since the study is limited to native chicken species, a similar study may be conducted in other species of animals using this herbal medicine and determine their anthelmintic effects.

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