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RESEARCH COMMUNICATION

Anthelmintic efficacy of tuba (*Croton tiglium* L.) seeds on the gastrointestinal parasites of native chickens (*Gallus domesticus*)

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ABSTRACT

The efficacy of capsulized *Croton tiglium* L. (CCT) seeds on the gastrointestinal parasites of native chickens (*Gallus domesticus*) was tested in experiments. A total of thirty-six free-range native chickens naturally infected with gastrointestinal parasites were divided into four treatment groups (positive control of levamisole+niclosamide, 200 mg, 300 mg and 400 mg CCT seeds) following a completely randomized design (CRD). Prior to treatment and on the 3rd, 6th, 9th and 12th days after treatment, the fecal egg count per gram was measured using the mc master technique. The analysis of variance (ANOVA) was used to statistically analyze all the data obtained. Using Least Significant Differences (LSD), significant differences between treatments were compared. On the day twelve after treatment, percent efficacy of capsulized *Croton tiglium* seeds on *Ascaridia galli/Heterakis gallinarum* at 200 mg and 400 mg was highly effective. The comparative cost analysis of the four treatments showed that the use of *C. tiglium* seeds resulted in a lower cost compared to the commercial dewormer. Commercial anthelmintic was more costly compared to the cost of capsulized *C. tiglium* seeds on T_4 (400mg CCT) by 89. 67 %. The findings indicate the ability of *Croton tiglium* seeds in native chickens (*Gallus domesticus*) particularly against *Ascaridia galli/Heterakis gallinarum* as an alternative anthelmintic.

Introduction

In the agricultural economy, the Philippines' poultry industry plays an important role. Philippine native chickens are reared in rural communities as a rich source of protein in the form of meat and eggs for Filipinos. Due to its distinct flavor, leanness and pigmentation, its meat is highly favored by consumers (1). In rural communities, it can provide necessary protein source to people under minimal management and interventions. Moreover, minimal capital is needed to start the venture in native chicken giving farmers the advantage in starting this enterprise as inputs are readily available in the community. Rearing indigenous chickens may also produce income for other household expenses such as school fees and medicine (2, 3). The country's total inventory of chicken was estimated at 184.88 million birds as of April 2019, of which 83.66 million birds were native/improved species (4).

Despite these advantages of chicken raising, there are still major problems for the industry that require solutions and interventions. One of which is the high mortality rate of around 40 % due to different factors (5). One of the major causes of high mortality can be attributed to parasitic infection. In developed countries, intestinal parasitic nematodes cause significant poultry diseases (6). Considerable losses are caused by gastrointestinal parasitism due to weight loss, nutrient malabsorption and bird death. *Ascaridia galli* infected birds typically suffer from extreme diarrhoea, anaemia leading to body weight loss, resulting in substantial economic losses in poultry farming (7). In addition, the immune system of the infected bird may be significantly reduced, thus increasing its susceptibility to various diseases (8). The risk of high parasitic infection in free-range chicken can be attributed to its scavenging behavior, where it is possible to consume different infectious stages of parasites. Studies in other countries have found that the prevalence of parasitic infections in village chicken flocks is closed to 100 % and in most cases more than one form of parasite is harbored by individual birds (9). Therefore, it is important to develop control measures to prevent these parasites from having a negative economic effect.

One of the preventive measures used by poultry breeders to combat parasitic infection is the use of commercially available anthelmintic agents. With continued use, however, the parasites have established resistance to commercially available anthelmintics (10). In addition, the unavailability of treatment regimen and the cost of anthelmintic drugs impedes village raisers from controlling the impact of parasitism in their flocks.

Medicinal plants are excellent alternatives to replace the existing use of anthelmintics, as they are used especially in developing countries to cure helminth infections (11). It is considered a safe way to overcome drug resistance and the significant danger of drug residues in human foods (12). In addition, herbal treatments are cost-effective, have minimum toxicity and are readily available compared to prescription drugs with reduced health risks (13).

Traditionally, different types of *Croton* have been used to treat constipation, diabetes, stomach disorders, dysentery, external wounds, fever, intestinal worms, ulcers and weight loss. *Croton cajura* stem bark and leaves are used in South America in the form of tea or tablets to manage diabetes, elevated blood cholesterol levels and gastrointestinal disorders (14). Several other species, for example, *C. lechleri*, *C. palanostigmatism*, *C. draconoide* and *C. urucurana* contains red sap, which is highly valued for its capacity to speed up wound healing (15). *C. lechleri* is also used as a tonic and purgative and has a long history of medicinal usage by South American rainforest people (16). In Asia, seed and seed oil have long been used as solid purgative, cathartic and poisonous products. In Malaysia, *C. tiglium* is consumed by adults as a purgative. The seed oil is a potent vesicant but can be used as a counterirritant for different skin infections when diluted. The powdered seeds are eaten as purgative combined with dates and the leaves are used as poultice to treat snakebites (17). The objective of this study is to evaluate the anthelminthic efficacy of *Croton tiglium* seeds on naturally infected free-range native chicken and evaluate the cost of deworming native chicken compared to commercially available anthelmintic. The outcome could pave the way for promoting indigenous purgative in the production of native chicken to reduce the cost of production and endoparasite incidence.

Fig. 1. *Croton tiglium* plant.

Materials and Methods

The research was carried out at Quirino State University's Integrated Fowl Project from August to September 2019. Thirty six experimental birds were purchased in Diffun, Quirino, from backyard poultry raisers. It was selected based on age, preferably in the growing stage. Birds with fecal egg count (EPG) above 150 was included in the study (18). They were fed twice a day with commercial feed containing 19% crude protein and given clean water *ad libitum*. The birds were randomly divided into four treatments, each treatment with three replications at three experimental birds per replication following the Completely Randomized Design (CRD). Positive control of levamisole and niclosamide (T_1), 200 mg CCT (T_2), 300 mg CCT (T_3) and 400 mg CCT (T_4) was used as treatments. The fruit from *C. tiglium* L. was collected in Ifugao village, Diffun, Quirino by hand picking. The seed was manually removed from its shell and air dried for five days. To pulverize the seeds, an electric blender was used. Using an electronic weighing scale, the pulverized seeds were weighed according to the treatment dose and capsulized. The prepared CCT was administered orally and evaluated through fecalysis on the 3rd, 6th, 9th and 12th days after administration of the CCT.

Fig. 2. Administration of the CCT to the experimental bird.

Using the flotation method and McMaster Technique, parasite identification and egg count per gram (EPG) were determined.

Based on the following formula, anthelmintic efficacy was calculated:

$$\text{"Percent Efficacy"} = \text{("Pre-treatment Average EPG-Post treatment Average EPG" /"Pre treatment Average EPG")" x100"}$$

The anthelmintic effectiveness was based on the standard as follows: 81-100% reduction in count is highly efficient, 60-80% reduction in count is effective, less than 60% reduction in count is ineffective (19). All data collected was statistically analyzed using the Analysis Of variance (ANOVA). Using Least Significant Differences (LSD), significant differences between treatments were compared.

Table 1. Mean percent efficacy of the different treatments to *Ascaridia galli/Heterakis gallinarum*

Treatment	Days Post Treatment			
	3	6	9	12
T1 (Levamisole+niclosamide)	70.23	91.95	88.39	94.13^{a}
T2 (200 mg CCT)	75.45	87.73	80.91	85.00^{a}
T3 (300 mg CCT)	73.18	83.24	81.56	63.13^{b}
T4 (400 mg CCT)	77.42	77.42	65.52	91.94^{a}
	ns	ns	ns	0.05

ns=not significant, means with the same letter notation are comparable

Table 2. Cost comparison of commercial anthelminthic and treatment dose

Items	Quantity	Unit	Cost of Anthelmintic/Bird (Php)	No. of Birds	Total Cost (Php)
T$_1$ (Levamisole+niclosamide)	1	capsule	18.00	9	162.00
T$_2$ (200 mg)	1	capsule	0.93	9	8.36
T$_3$ (300 mg)	1	capsule	1.39	9	12.53
T$_4$ (400 mg)	1	capsule	1.86	9	16.71

Results

All the experimental birds harbored gastrointestinal parasites identified as *Ascaridia galli/ Heterakis gallinarum* through fecal flotation method and counted through mc master technique. *A. galli* and *H. gallinarum* were counted as one because differentiating the two parasites based on egg morphology is unreliable and difficult (20).

The data showed, according to standard (19), that all treatments were effective against *A. galli/H. gallinarum* on the third day after treatment. The results showed that T$_1$ (Levamisole+niclosamide), T$_2$ (200 mg CCT) and T$_3$ (300 mg CCT) were highy effective on the sixth and ninth days following treatment, and that T$_4$ (400 mg CCT) was effective. T$_1$ (levamisole+niclosamide), T$_2$ (200 mg CCT) and T$_4$ (400 mg CCT) were highly effective twelve days after treatment, while T$_3$ (300 mg CCT) was effective. Further analysis of the result using analysis of varience (ANOVA) showed that no significant difference in percentage efficacy were found during the third, sixth and ninth day post-treatment. T$_1$ (Levamisole+niclosamide), T$_2$ (200 mg CCT) and T$_4$ (400 mg CCT) were comparable to each other on the 12th day after treatment. T$_3$ (300 mg CCT) is significantly different with all of the treatments.

Commercial anthelmintic is more costly compared to the cost of capsulized *C. tiglium* seed on T$_4$ (400 mg CCT) by 89. 67 %.

Discussion

Croton tiglium seeds has many bioactive compounds such as saponins, tannins, flavonoids, alkaloids and phenols that has been linked to the ability of the plant material to initiate anthelmintic effect. Saponins affects the permeability of the cell membrane of parasites and cause vacuolization and disintegration of teguments (21). Phenolic compounds, on the other hand, interfere with the energy mechanism by uncoupling the oxidative phosphorylation and also interfere with the glycoprotein of the cell surface of the parasites causing death (22). Moreover, the presence of alkaloids may have acted on central nervous system and caused paralysis of worms. Alkaloids also serve as an antioxidant, capable of reducing the nitrate generation which can interfere in local homeostasis that is important in the development of helminths (23).

The presence of tannins disrupts the production of energy of the helminths through the uncoupling of oxidative phosphorylation (24, 25). Another mechanism of tannin is the ability to bind with the free protein in the digestive tract of the host animal or a glycoprotein on the cuticle of the worms which causes death. Some studies suggest that tannins found in the plant are able to increase the absorption of protein. Increase absorption of the protein in the host animal showed a decrease in the nematode worm infection rates (26), whereas the direct action of tannin on the nematode cuticle occurs through the hydrogen bonding. This reaction induces skin stiffness, resulting in paralysis and the death of nematodes ((27). This finding supports the claim of several studies that several plant species contain bioactive components necessary to elicit anthelmintic potential. *Carica papaya* L. latex has showed 77.7 % reduction of eggs per gm in feces of poultry to include *A. galli* and *Capillaria* spp. (28). This result confirms the research on the impact of papaya and neem seeds on *A. galli* infection in broiler chicken (29). *Sesbania grandiflora* L. (Fabaceae) leaves have historically been associated with selected helminth anthelmintic behavior (30). Results of the trial showed that flavonoids, phenols, tannins, alkaloids, saponins and acids that are present in aqueous extracts of *S. grandiflora* may act as an alternative, powerful helminthicide that can replace synthetic drugs. Aqueous extracts of *S. grandiflora* leaves showed that an increased concentration of extracts decreased the survival of *A. galli* at concentrations of 10-100 mg/100 ml of medium per 10 parasites (31).

This observation indicates that *C. tiglium* seeds have the ability to control *A. galli* and *H. gallinarum* twelve days after treatment in chickens. In addition, if the use of this plant material is to be adopted by a backyard raiser, the lower dosage should be used as it is shown to have a similar effect to the higher dose.

The cost analysis of the study supports the claim that indigenous medicinal plants can be a source of low-cost herbal anthelmintic as an alternative to chemical products (32). In addition, the source of herbal dewormer used in this research is available locally, making it more accessible to the community's backyard raisers.

Table 3. Cost analysis of Capsulized *Croton tiglium* seeds, 27 capsules

Particulars	**Unit**	**Quantity**	**Cost/ Unit(PhP)**	**Total (PhP)**
Variable Cost				
Seeds	kilogram	1	10	0.08
Capsule gel	piece	27	0.75	20.25
Labor	man-day	1	250	15.63
Electricity	Kwh	0.15	10	1.50
Total Variable Cost				37.46
Fixed Cost				
Depreciation Cost				
Blender				0.03
Analytical Weighing balance				0.11
Total Fixed Cost				0.15
Total Cost				**37.60**

Notes

Seeds (8.1 gms)

	Cost/Capsule (PhP)	**Cost/mg (Php)**	**Unit (mg)**	**Quantity**
T_2 (200 mg)	8.36	0.93	200	9
T_3 (300 mg)	12.53	1.39	300	9
T_4 (400 mg)	16.71	1.86	400	9
Total Cost	37.60			

Conclusion

The findings suggest that *C. tiglium* L. seeds can be used as an alternative anthelmintic at 400 mg dose against *Ascaridia galli/Heterakis gallinarum*. The comparative cost analysis showed that the use of *C. tiglium* resulted in a lower cost compared to the commercial dewormer. Further research on other parts of *C. tiglium* and other locally available plants can be studied for anthelmintic potential.

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Conflict of interests

Author does not have any conflict of interests to declare.

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