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In-vivo and *in-vitro* evaluation on the anthelmintic efficacy of sweet tamarind (*Pithecellobium dulce*) aqueous leaf extract against *Ascaridia galli* in Philippine native chicken

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Key words: Anthelmintic efficacy, *Pithecellobium dulce*, *Ascaridia galli*, *In-vivo* and *in-vitro* evaluation, Philippine native chicken

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ABSTRACT

This study aimed to evaluate the efficacy of *Pithecellobium dulce* aqueous extract against *Ascaridia galli* in native chicken using in-vivo and in-vitro conditions. The trials were carried out from October to December 2022. Sixty(60) Philippine native chickens were used in the *in vivo* experiment, while 60 adult *Ascaridia galli* worms were used in the *in vitro* experiment. A completely randomized design was used in the study. There were four treatment groups with five replicates in each treatment and each replicate has 3 animal samples each. Treatment 1 has 20% extract, Treatment 2 has 50% and the Treatment 3 has 100% extract. The fourth group constituted the control group where animals were dosed with a commercial dewormer, Levamisole Hydrochloride + Albendazole (Bastonero Plus), and was given using the manufacturer's recommendation. For the *in vivo* experiment, fecal samples were collected before and after treatment with the test drug at day 0, day 7 and day 14 and were analysed using the McMaster technique. The results obtained were recorded as egg per gram (EPG) of fecal sample. The percentage mean fecal egg counts reductions (FECR%) were used for the analysis of the data. It was observed that *Pithecellobium dulce* aqueous extract had the ability to reduce the fecal egg counts of *Agaridida galli* of chicken. In the *in vitro* experiment, it was found out that the extract also has the ability to induce paralysis in adult worms. Overall, there was a significant difference in the results when Treatment 1(20%) and Treatment 2(50%) are compared with the positive control group while Treatment 3(100%) and the positive control group has no significant difference, indicating that *Pithecellobium dulce* is as effective as the conventional dewormer when given in high concentrations. The secondary metabolites identified form t he leaves of *Pithecellobium dulce* extract mainly include alkaloids, phenols and most especially tannins. This diversity of compounds present in the leaves of *Pithecellobium dulce* has dealt great effects on the results of the experiment.

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INTRODUCTION

Poultry production is presently one of the fastest-growing industries in the Philippines. Chicken meat is becoming the most popular food commodity as source of protein in the human diet due to the effects of the current pork crisis in the world (Farrell, 2013). Compared to other livestock, the production cost of poultry raising per unit is low, and the return on investment is faster and higher (Ojo, 2003).

Despite the growing popularity and demand for poultry meat in the Philippines, the economics of chicken raising is still being slowed down by episodes of disease outbreaks happening all over the country. One of the major problems in poultry production today is the presence of gastrointestinal parasites (Poulsen, 2005).

Gastrointestinal nematode infections are very common in poultry all over the Philippines (Ybanez, 2018) and Cagayan is not an exception. Gastrointestinal parasitic infections of poultry is one of the major factors responsible for economic losses through reduction in productivity and increased mortality. These parasites cause the birds to be unhealthy and unthrifty, which may include the loss of appetite and weight, leading to significant profit loses. Due to parasitism, the poultry animals become susceptible to other health problems which can lead to high morbidity and mortality inside the farm. The most important GI nematode responsible for considerable production losses in poultry is *Ascaridia galli.*

Effective anthelmintics are needed for the implementation of integrated parasite control programs, which combine non-pharmacological methods with strategic use of drugs. Monitoring of Fecal Egg Count (FEC), the evaluation of treatment efficacy and the detection of Anthelmintic Resistance (AR) are becoming increasingly important for health programs of grazing livestock and poultry (Pena-Espinoza *et al.*, 2014). Resistance has now been reported to all of the broad spectrum anthelmintic types currently available, namely to the benzimidazoles, levamisole/morantel and to ivermectin. These three classes of anthelmintics are the most commonly used anthelmintics in the world today.

Many researches for prevalence rate of gastrointestinal parasites all over the world have been reported but researches for parasitic sensitivity from different anti parasitic drugs are low and in Cagayan the study on this particular area has not been done for a very long time.

Some traditional medicinal plants have been used to treat parasitism in the past. These plants are often given in the form of aqueous extracts. The leaves from plants belonging to the genus *Pithecellobium* were traditionally used as vermifuge drugs. This gave the researcher an idea in using this traditional plants as natural deworming agents against worms. In view of this, this study was conducted to investigate the anthelmintic efficacy of Kamatsile (*Pithecellobium dulce*) aqueous leaf extract against *Ascaridia galli* in native chicken.

MATERIALS AND METHODS

Materials

The following materials were used in the study: kamatsile leaves, sharp knife, chopping board, blender, gloves, native chicken, chicken feeds, feeding troughs, drinking troughs, cages, weighing scale, commercial anthelmintics, containers for fecal collection, light microscope, Mcmaster slide, test tubes, stirring rod, petri dishes, Whatman no.1 filter paper, stereomicroscope, sugar solution, syringes and stirring rod.

Gathering of plant material

Leaves of kamatsile were gathered from different places of Tuguegarao City and was authenticated by the Bureau of Plant Industry, DA R02.

Preparation of extract

Leaves were washed thoroughly with tap water and air dried. Dried leaves were cut into small pieces and ground coarsely into a uniform powder using a

blender. The powdered leaves of kamatsile were also sieved and were submitted at Cagayan State University- Andrews Campus for the processing of extract. Preparation of extract was done prior to the conduct experiment

Experimental animals

Sixty (60) infected native chickens (4 to 5 weeks old), regardless of sex was used in this study. Chickens infected with *Ascaridia galli* worms was placed in individual cages for 7 days before the administration of Kamatsile leaves aqueous extract.

Experimental design and treatments

The Complete Randomized Design (CRD) was used in the study to determine the efficacy of the treatments In Vivo and In Vitro. The experimental birds were randomly distributed into the treatments using 5 replicates with 3 birds per replicate. The treatments used are as follows:

Treatment 1- 1ml/kg of 20% kamatsile leaf aqueous extract
Treatment 2- 1ml/kg of 50% kamatsile leaf aqueous extract
Treatment 3- 1ml/kg of 100% kamatsile leaf aqueous extract
Treatment 4 – Commercial dewormer

Administration of kamatsile leaf aqueous extract

After one week of acclimatization, the experimental chickens were fasted prior to the day of administration of treatments. The birds were orally administered with 1ml/kg of the kamatsile leaf extract using disposable syringe following the formulation specified for each treatment while the commercial dewormer was based on the amount in the product indicated in the label. The administration of dewormer was done at 0 and 7 days of the experiment.

Collection of fecal samples

Prior to the administration of the kamatsile leaves extract as dewormer to the experimental birds, feces were collected early in the morning. Samples were placed in a plastic container with label and stored in refrigeration prior to fecal examination in the laboratory to determine the parasites and eggs count. Second and 3rd samples were collected after 7 days and 14 days. This was done to assess the egg reduction of *Ascaridia galli*.

Examination of feces

The eggs per gram feces (EPG) modified McMaster with the sugar flotation method was used based on FAO. Fecal Egg Reduction Test (FRCT) calculation was also used for the examination of feces (Coles *et al.,*). The Fecal Egg Reduction Test was the result of comparing the eggs per gram feces (EPG) before (pre-treatment) and after (post-treatment) the administration of dewormer.

Arithmetic means of pre-treatment and post-treatment fecal egg counts of giving of leaf extract and giving of Levamisole groups was used to calculate the percentage efficacy using the following formula (Coles *et al.,*): FECRT%=(T1-T2)/T1 × 100 where T1 is pre-treatment egg count and T2 is posttreatment egg count.

Examination of feces using Mc master technique

Three grams of feces of the sample from the experimental animals was suspended in 3.0 ml of sheather's solution. The suspension was mixed thoroughly using mortar. After the mixing, it was transferred to 100 ml beaker and let it stand for 5 minutes. Prior to laboratory examination, the solution was allowed to flow into the two chamber of McMaster slide with the used of pipette.

***In vitro* experiment**

For the *in vitro* experiment, the study consisted of 1ml of 20%, 50% and 100% kamatsile leaf extract to evaluate the efficacy of the plant against live nematodes of native chickens. Levamisole (T4) was used as positive control in the study. Each treatment was composed of five replicates and for each replicate was 3 sub replicates. A total of 60 nematodes of

native chickens were used and was randomly divided into 4 treatment groups. The result of the study after laboratory examination of the feces was used in identifying and ascertaining count of eggs.

During the administration of treatments, observation of gastrointestinal nematodes was done with agitation using thumb forceps during the entire session. The gastrointestinal nematodes that are being subjected in different concentrations of leaf extract showed signs of continuous motility in the first minutes of the experimentation with varying duration. As length time increases, paralysis and death are being observed.

Statistical analysis

The raw data was analyzed using the Statistical Tool for Agricultural Research (STAR). The Least Significant Difference (LSD) at 0.05 and 0.01 level was used to compare the significance of the different treatment tested.

Data gathered

1. Fecal egg count pre-treatment (day 0)- this was done by assessing the egg per gram of Ascaridia galli through fecal analysis. This was also the basis in the administration of dewormer.
2. Fecal egg count at 7 days post-treatment- this was undertaken by computing the amount of Ascaridia galli egg reduced a week after the administration of dewormer. This data was expressed in terms of percentage e.g. initial egg count minus egg count 1 week after administration divided by the total initial egg count multiplied by 100.
3. Fecal egg count at 14 days post treatment- this was undertaken by computing the amount of Ascaridia galli egg reduce 2 weeks after the administration of dewormer. This data was expressed in terms of percentage e.g. initial egg count minus egg count 2 weeks after administration divided by the total initial egg count multiplied by 100.
4. Time of paralysis of live worms- this was done by determining the time from the administration of dewormer up to the time were Ascaridia galli worms are no longer moving.

RESULTS AND DISCUSSION

General observation

The study was conducted under laboratory condition at Cagayan State University Carig campus to assess the efficacy of kamatsile against nematodes of native chicken. Fecal examination was done to assess the egg count under pre-treatment and post-treatment evaluation of kamatsile leaves extract. During the evaluation, internal parasites such as *Hetarakis gallinae*, *Ascaridia galli,* and *Capillaria spp* was identified. However, the study shall focus mainly on the effect of kamatsile in treating *Ascaridia galli*.

Initial egg count before administration of treatments

Fig. 1 shows the initial egg count of *Ascaridia galli* on native chickent. It was found out that egg count ranges only from 503 to 613 since no significant difference existed among the different treatments based on the analysis of variance.

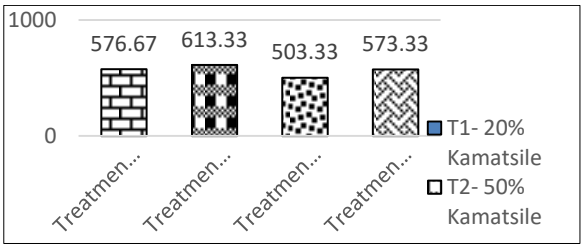

Fig. 1. Initial egg count of *Ascaridia galli* on native chicken

Egg count after administration of treatments

Seven (7) days after the administration of dewormer, significant result could be observed when Treatment 4 and Treatment 3 were compared to Treatment 1 and Treatment 2 with respective means of 376.67 and 310.00 which is shown in Fig. 2. It is evident from the result that the application of commercial dewormer is still the best in controlling A*scardaia galli*, however, the use of 100% kamatsile has comparable impact on the commercial dewormer. This could be explained by Kaushik *et al.*,(2018), who claimed that kamatsile leaf extract has active component known as tannin which paralyzes worm to death. Phytochemical analysis on kamatsile leaves revealed the presence of tannins. In other studies conducted, tannins present in kamatsile

leaves were shown to produce anthelmintic activities at 100% concentration (Shrestha Bhupendra *et al.,* 2009). Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Mali R.G.2007).

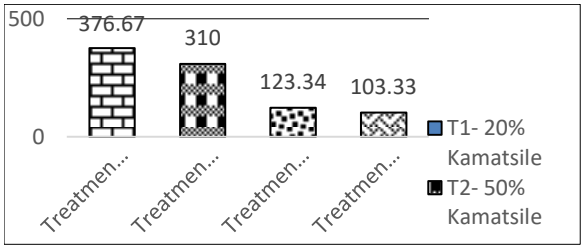

Fig. 2. Egg reduction of *Ascaridia galli* 7 days after the administration of dewormer

Results and gathered data were presented on the figure below focusing on the above- mentioned parasites reduction with regards of fecal egg count.

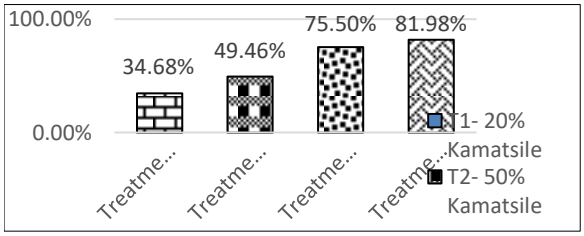

Fig. 3. Mean fecal count reduction % at day 7 post treatment

As reflected in Fig. 3 above, the highest percentage reduction % of EPG on day 7 after deworming was noted among birds in T4 (Positive control) with 81.98%, followed by birds assigned in T3 (100% kamatsile leaf extract) with 75.50% egg count reduction, followed by T2 (50% kamatsile leaf extract) with the efficacy to eliminate 49.46% of ova, and lastly T1 (20% kamatsile leaf extract) with a little effect in eliminating nematodes with 34.68% egg count reduction. This biological response from the nematodes may be due to the active component of the treatment present in the plant which is tannin whose action is to bind with the cuticle of the parasite and paralyze the worms which leads to death.

Fig. 4 below shows the egg reduction of *Ascardia galli* 14 days after the administration of dewormer. Analysis of variance revealed that there exists highly significant difference among the treatment tested. On comparison among treatment means, highest reduction of egg per grams was observed still in Treatment 4 (control) with a mean of 36.67. The ranking was followed by Treatment 3 and Treatment 2 with respective means of 60.67 and 104 egg per grams since no significant difference existed among the said treatments. Lowest reduction of egg per gram was observed in Treatment 1 with a mean of 183.33. This means that kamatsile leaf extract exhibits anthelmintic activity in a dose-dependent manner with the greatest effect at 100% concentration.

Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou *et al.*2001) or glycoprotein on the cuticle of the parasite (Thompson and Geary *et al.*1995) and may cause death. The more tannins available would mean more parasite can be affected.

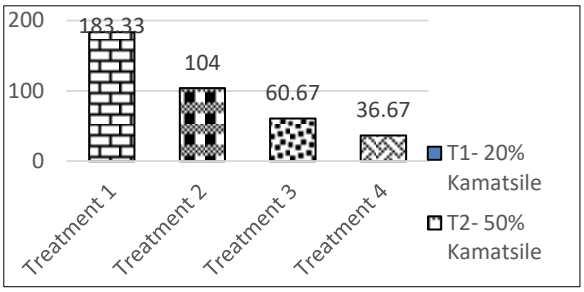

Fig. 4. Egg reduction of *Ascaridia galli* 14 days after the administration of dewormer

Fig. 5 below shows the fecal egg count reduction % of eggs after administration of kamatsile leaf extract and the commercially available dewormer at day 14 of administration. As shown on the table it has the highest reduced number of eggs in T4 (positive control) at 93.95%, 2nd highest was T3 which has 87.95% ability to eliminate ova, 3rd was T2 with 83.04% efficacy, and 4th was T1 with poor efficacy at 68.21%.

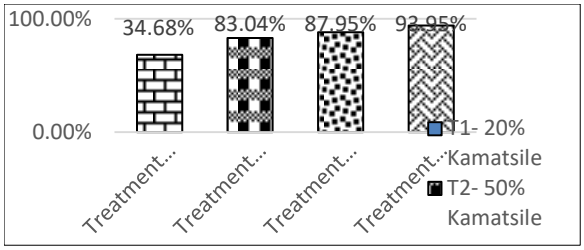

Fig. 5. Mean fecal egg count reduction % at day 14 post treatment

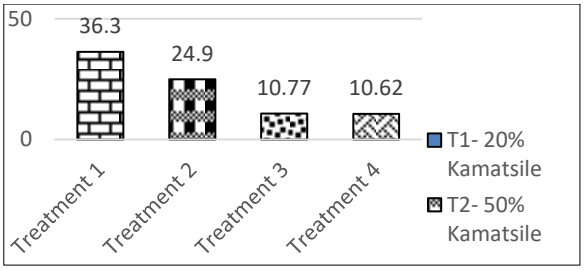

Fig. 6. Length of time for Ascaridia galli to be paralyzed under *in-vivo* environment

It can be deduced from the two raw data above that the kamatsile leaf extract has a biological ability to eliminate nematodes in native chickens. This response of the native chicken worms may be due to the active component of the treatment present in the plant which is tannin whose action is to bind with the cuticle of the parasite and paralyze which leads to death of the worms preventing further production of nematode eggs.

Length of time for paralysis

Fig. 6 shows the average length of time for *Ascaridia galli* to be paralyzed under in-vitro environment. Analysis of variance revealed that there exist highly significant difference among treatments tested. *Ascaridia galli* applied with 100% kamatsile and commercial dewormer recorded to have the fastest time of paralysis with a mean ranging from 10.62 to 10.77. The ranking was followed when worms were applied with 50% kamatsile with a mean of 24.90 minutes while the longest time of paralysis was recorded when worms are treated with 20% kamatsile. This only means that the higher concentration of kamatsile could be at par with the effect of commercial dewormer which paralyzes worm at almost equal time. The elicited observations may be attributed to different metabolites like phenols and tannins present in kamatsile leaves which are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation. Thus, blocking ATP synthesis in helminth parasite then causing paralysis and death (Athanasiadou *et al*,.2001). The higher the concentration of the extract the parasites are exposed to, the higher the effect it will elicit.

CONCLUSION

Based on the results of the experiment, kamatsile leaf extract at 100% can be used as an alternative anthelmintic in native chickens. Higher amount of kamatsile aqueous extract can lower the fecal egg count of native chicken and it can also cause paralysis to worms which is comparable with a standard dewormer.

RECOMMENDATION

Based on the result of the study, the following recommendations were drawn:

1. Use of 100% kamatsile leaf extract can be used as an alternative dewormer for chicken.
2. Follow up study higher than 100% solution may be adopted to determine the tolerable level as dewormer for chicken.
3. It is also recommended to use said dose to other experimental animal to assess its efficacy.

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