

## Ovicidal Effects of Pineapple Peel Juice (*Ananas comosus L.*) as a Control of Ascaridians in Chicken (*Gallus Domesticus*) In Vitro

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### Abstract

The aim of this research was to know whether pineapple peel juice can be used as an ovicidal of *in vitro* *A. galli*. This study used eggs of *A. galli* worms obtained by collecting adult *A. galli* worms from the small intestines of fresh local chickens purchased from traditional markets in Surabaya. The research design used was a completely randomized design (CRD) with four treatments, the concentration of pineapple peel juice was 0% (Control), 5%, 10%, and 20%. Each treatment consisted of six replications, with observations on days 5, 10, 15 and 20. Developed eggs identified with the presence of larvae within egg and the movement of larvae. The data obtained analyzed with the ANOVA Statistical Test with the SPSS device and then followed by the Duncan test. The results of statistical analysis showed that pineapple peel juice had a significant effect on the development of *A. galli* worm eggs *In Vitro*. The number of damaged *A.galli* eggs increased as they given higher pineapple juice concentration. This proves that pineapple peel juice has higher anthelmintic effect with increasing concentration given. Pineapple peel juice 20% is the best juice concentration.

**Keywords:** Ascariasis, Ovicidal effects, *Ascaridia galli*.

### Introduction

Pineapple is widely cultivated as garden plants and plantation cultivation in Indonesia since brought by the Spanish in the 15<sup>th</sup> century originated from Brazil (Prihatman, 2000). The fruit contains useful substances, such as vitamins, carotenoids, flavonoids, bromelain enzymes etc (Salasa, 2017). Unfortunately, the fruit is not well positively appreciated, with most people only take the fruit's flesh in the consumption while the peel is discarded as a waste. One of the substances, Bromelain enzymes, is a proteolytic enzyme which able to hydrolyze other proteins and act as an anthelmintic, to destroy the worm egg wall and kill the larvae (Christy, 2012; Wiyati, 2018). However, there is no research on pineapple peel as ovicidal.

Ascariasis is an infection that attacks poultry and cause intestinal damage, decrease egg production and quality, and infect poultry if the ineffective eggs are ingested with contaminated food or water (Sonda *et al.*, 2018; Zalizar and Satrija, 2009; Zalizar *et al.*, 2007). The infection caused by *Ascaridia galli* worms.

The use of Anthelmintics is to treat and prevent infection with *A.galli* worms by killing larvae (as larvicidal), adult worms (vermicidal), and inhibiting the development of worm eggs (ovicidal) as the mechanism of action in controlling helminth infection (Ardana *et al.*, 2012). However, the substance is mostly used from synthetic chemical, such as Albendazole, which is expensive and could cause resistance and adverse side effects such as diarrhea on the livestock health (Ardana, 2007; Walter, 2008). Therefore, medicinal plant, such as pineapple, become one of the alternatives as the substitute of the chemicals that contains anthelmintic (Astri and Sukohar, 2019). However, these studies mentioned it only effectively impacted in adult worms, with no ovicidal effect of the herb have been reported. Therefore, an *in vitro* study of the ovicidal effect of pineapple peel conducted to control Ascariasis chickens.

In a conclusion, this study aimed to determine whether pineapple peel juice can be used as an ovicidal of *in vitro* *A. galli*. Further benefits expected to provide scientific reference in increasing the quality to prevent the ascariasis



infection in chickens and reduce the dependency of chemical substance in daily use.

### Materials and Methods

In order to test the effectiveness of pineapple peel against *Ascaridia galli* eggs in vitro, this study used a Completely Randomized Design (CRD) with four treatments and six repetitions for each treatment.

Determination of the number of samples based on Federer:

$$(t-1)(n-1) \geq 15$$

t = number of treatment

n = number of repetition,

Based on the formula, the sample in this research were obtained 4 samples distributed into 24 petri dishes, which focus on the development of *Ascaridia galli* worm eggs influenced by the concentration of pineapple juice. The ovicidal effect of pineapple peel juice can be seen from the number of broken worm eggs counted in each observation.

### Research Procedure

The eggs were collected from adult worms isolated from the intestines obtained from sellers in Surabaya traditional markets. The worms were put in a petri dish containing PBS solution. The eggs are removed from the female worm by dissection through the vulva. The treatment was given after being divided into 24 petri dishes of 50 worm eggs each. The method of counting 50 eggs was previously carried out in preliminary research experiments 3-4 times by counting the number of eggs from 10 mL of egg suspension in a glass object and counted under a microscope.

Pineapple peel was obtained from a honey pineapple seller. Pineapple peel was washed and then processed into fresh juice (Hossain *et al.*, 2015). For the production of fresh juice, the fresh peels were cut into small pieces and aquades was added at 1:1 ratio in a kitchen blender. Then juice were made by blending the peels for 2-3 minutes and stored in a refrigerator at 4°C to maintain the quality of active ingredients of juice.

Four treatments were made using pineapple peel juice concentrations of 0% (Control), 5%, 10%, and 20%. Each treatment with six repetitions contained 50 worm eggs. Each treatment with six repetitions contained 50 worm eggs. Pineapple juice solvent using PBS. Put pineapple juice with each concentration to a volume of 10 ml and add 1 ml of worm eggs into a Petri dish. The petri dish placed in a large tray and kept moist. Place it at room temperature for 20 days and observe the development of larvae in eggs on day 5, 10, 15, and 20 (Hossain *et al.*, 2015). The percentage of inhibition of hatchability of worm eggs calculated using the formula: the number of

undeveloped worm eggs divided by the total number of eggs before treatment multiplied by 100% (Hossain *et al.*, 2015).

### Data Analysis

The data obtained analyzed with the ANOVA Statistical Test with the SPSS device. The effectiveness between treatments compared by Duncan's multiple distance test.

### Result and Discussion

The research results over *Ascaridia galli* worm eggs were divided into 4 experiments of Pineapple peel juice Submersion with 0% 5% 10% and 20% concentrations. Based on the result, there is an average presentation of the highest breakage worm age in the 20% concentration. According to it, an average presentation of *A. galli* worm eggs that died all over the experiments are presented in Table 1.

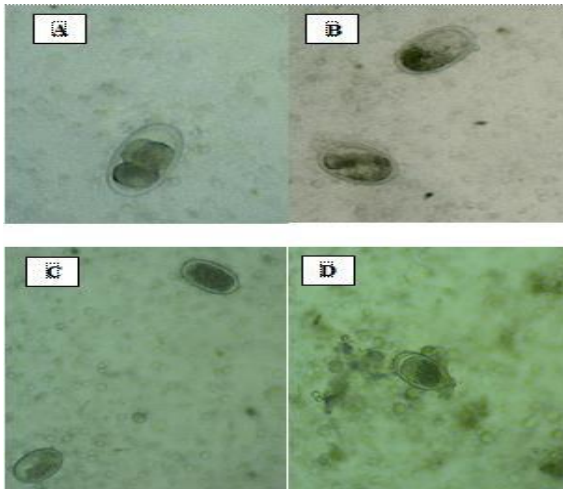
Concentration	Mean ± SD
0% (Control)	21,33 8,36 <sup>a</sup>
5%	42,33 7,941 <sup>b</sup>
10%	63,00 6,782 <sup>c</sup>
20%	71,67 3,445 <sup>d</sup>

**Table 1.** An Average Effectivity of Pineapple Peel Juice in Hindering the Development of *A. galli* Eggs.

Reviewed from an efficacy point of view, pineapple peel juice with a concentration level of 20% has the optimum capability in hindering *A. galli* worm egg development as per (72%), the concentration of 10% can hinder the development of worm eggs as per (63%). On the other hand, the concentration of 5% can hinder the development of worm eggs as per (42%), which is less effective in hindering the *A. galli* worm eggs compared to the first and second concentrations.

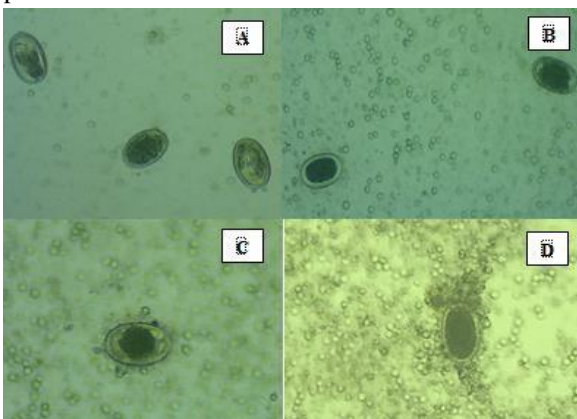
Observations done on the 5<sup>th</sup> day show that each treatment group, Po (control group), P1 (5%), P2 (10%), and P3 (20%), yields different effectiveness in hindering *A. galli* worm eggs development. Observations show that the Po and P2 groups are experiencing the early morula phase. Po, P1 and P2 appear to develop normally,

while the eggs in P<sub>3</sub> start to suffer from damage and perish.



**Figure 1.** Microscopic view of *A. galli* worm eggs on day 5 at 100x magnification. A. Developed *A. galli* worm eggs in PBS solution (control) B. Developed *A. galli* worm eggs in submersion of pineapple peel juice 5%. C. Developed and Undeveloped *A. galli* worm eggs in submersion of pineapple peel juice 10%. D. Undeveloped *A. galli* worm eggs in submersion of pineapple peel juice 20%.

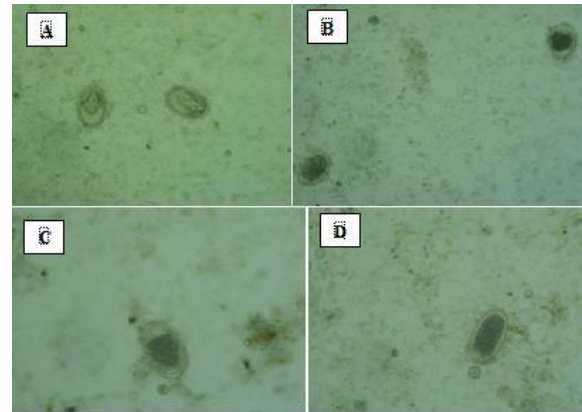
Observations done on the 10<sup>th</sup> day show that each treatment group, Po (control group), P<sub>1</sub> (5%), P<sub>2</sub> (10%), and P<sub>3</sub> (20%), also yields different effectiveness in hindering *A. galli* worm eggs development. Observations show that the eggs in the Po group are developing in the late morula and vermiform phases. In the P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub> group, some eggs are suffering from damage and perish.



**Figure 2.** Microscopic view of *A. galli* worm eggs on day 10 at 100x magnification. A. Developed *A. galli* worm eggs in PBS solution (control) B. Undeveloped *A. galli* worm eggs in submersion of pineapple peel juice 5%. C. Undeveloped *A. galli* worm egg in submersion of pineapple peel juice 10%. D. Undeveloped *A. galli*

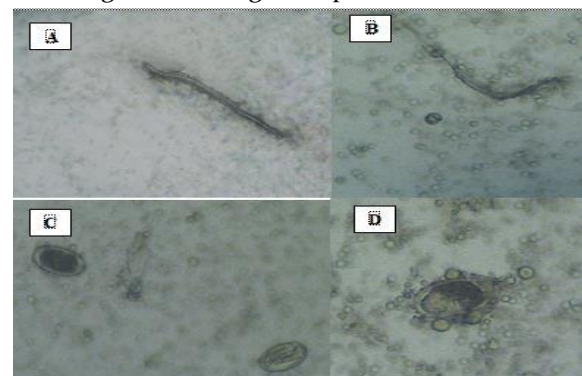
worm eggs in submersion of pineapple peel juice 20%.

Observations done on the 15<sup>th</sup> day show that each treatment group, Po (control group), P<sub>1</sub> (5%), P<sub>2</sub> (10%), and P<sub>3</sub> (20%), also yields different effectiveness in hindering *A. galli* worm eggs development. Observations show that the eggs in the Po group are developing into larva motile (L<sub>3</sub>). In the P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub> group, more eggs are suffering from damage and perish.



**Figure 3.** Microscopic view of *A. galli* worm eggs on 15<sup>th</sup> day at 100x magnification. A. Developed *A. galli* worm eggs in PBS solution (control) B. Undeveloped *A. galli* worm eggs in submersion of pineapple peel juice 5%. C. Undeveloped *A. galli* worm egg in submersion of pineapple peel juice 10%. D. Undeveloped *A. galli* worm eggs in submersion of pineapple peel juice 20%.

Observations done on the 20<sup>th</sup> day show that each treatment group, Po (control group), P<sub>1</sub> (5%), P<sub>2</sub> (10%), and P<sub>3</sub> (20%), also yields different effectiveness in hindering *A. galli* worm eggs development. Observations show that most eggs in the Po and P<sub>1</sub> group are developing into larva, while more eggs in the P<sub>2</sub> and P<sub>3</sub> group are suffering from damage and perish.



**Figure 4.** Microscopic view of *A. galli* worm eggs on day 20 at 100x magnification. A. *A. galli* Larvae in PBS solution (control) B. *A. galli* larvae in submersion of pineapple peel juice 5%. C. Developed and Undeveloped *A. galli* worm eggs

in submersion of pineapple peel juice 10%. D. Undeveloped *A. galli* worm egg in submersion of pineapple peel juice 20%.

From the observations done on the eggs, the most common damages to the *A. galli* worm eggs are changes in the egg to a dark black colour and damaged outer walls. The eggs contain no cells and have thin walls. On the next day, the egg walls appear to splay and disappear or dissolve, as if they have breaches and leaks. It suspected that the cause of the cells' deaths is the damaged cell membrane, induced by pineapple peel juice soaking.

### Conclusion and Suggestion

#### Conclusion

Based on the research, it concluded that pineapple (*Ananas comosus* L.) peel juice has ovicidal effects on *Ascaridia galli* worms in vitro.

#### Suggestion

Based on the results of this study, further research needed on the ovicidal effect of pineapple peel juice on the environment (soil) which aims to control the spread of ascariidiasis.

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