A Pontential Role of Papaya Latex As An Anthelmintic Against Patent Ascaridia Galli Infection In Chicken

ENDANG PURWATI MURSOF AND SIMON HE
FACULTY OF VETERINARY MEDICINE BOGOR AGRICULTUTAL UNIVERSITY
BOGOR, INDONESIA

ABSTRACT

The anthelmintic activity of papaya *(Carica papaya Linn)* latex against patent *Ascaridia galli* experimental infection with 1000 infective eggs in chicken was studied. One week after a single dose of papaya latex 20% treatment of infected chicken, their EPG counts decreased. There was a significant, strong positive correlation between the percentage decrease of EPG counts and the single doses of papaya latex 20% given to the birds (r = 0,98; P H 0.05). There was also a very high, negative correlation between adult *A. galli* numbers recovered from the experimental birds and the single doses of papaya latex 20% (r = 0.996; P = 0,067). A single dose of 1447.89 mg papaya latex 20% completely freed the birds from *A. galli* infection while the untreated, infection controls harboured a mean number of 50 adult worms. The 100% effective dose (ED100) of papaya latex 20% against the patent *A. galli* infection was a single dose of 1120 mg papaya latex 20% per bird. The egg- and body weights of treated, infected chicken improved to become similar to those of the untreated, unifected controls.

INTRODUCTION

The productivity of chicken is affected by various factors, one of them is disease. *Ascaridia galli* is a chicken parasitic nematode and cosmopolitant in nature. It causes chronic infection and decrease in egg production (Wehr 1972). In Bogor, the nematode infection ranks number 6 among poultry diseases (Gordon and Poernomo, 1974) and nationally its prevalence is 14.3% (Ginting, 1986).

The combination of farm management and anthelmintic treatment remains the best choice for the control of this nematode infection. However, all anthelmintics are still imported and moreover they are very expensive and are not readily available in the markets. On the other hand, Indonesia is rich in medicinal plants which have been used in tradisional medicine for centuries. Some of our medicinal plants are mentioned to possess anthelmintic activities without scientific proof.

This study is aimed to investigate the anthelmintic activity of papaya latex against patent infection of *Ascaridia galli* in chicken since it is rnentioned that papaya latex was used as an anthelmintic against this nematode (Brander and Pugh, 1971).

MATERILS AND METHODS

Parasite

A stock of *Ascaridia galli* infective eggs was obtained from macerated adult worms collected from naturally infected chicken and the collected eggs incubated in distilled water at ambient temperature for 30 days after added with Penicilline 1000 units/ml, Streptomycin 100 mg/ml and Bacitracin 100 units/ml to prevent the growth of other mocroorganisms (Mishra, Sen and Chatar, 1983). Infective egg dose was prepared from the stock immediately before infection of chicken. Ten alliquotes of 0.05 ml of the stock were taken and the numbers of infective eggs counted which should yield a mean and S.E. of $< 10\%$. The number of infective eggs per ml of the stock was adjusted to give 500 infective eggs per ml of distilled water

Host

Sixteen-weeks-old Hy Sex Brown pullets bought from a commercial poultry farm in Bogor were used. These chicken were examined for helminth infection and they were found free from helminths. They were kept in a closed area for two weeks before the infection was made when they were eighteen-weeks-old.

Papaya latex

Papaya latex was freshly collected from green papaya fruits by making small incisions from 2 – 3 mm in depth (Wijaya, 1977) on the skin and the latex collected into a known volume of distilled water in a beaker glass and then after finish the collection, the papaya latex content was adjusted with distilled water to give a final concentration of 20% (v/v).

Parasitological techniques

Faecal egg counts were done on individual chicken using a modified McMaster technique (Whitlock, 1948). At the end of the experiment, chicken were killed, the feathers unpluged, and the abdomen opened, the small intestine stripped from the mesenteries, opened with scissors and the worms removed and counted.

Experimental design

Sixty eighteen-weeks-old pullets were randomly divided into 15 groups of 4 pullets each. Thirteen groups were infected with 1000 *A. galli* infective eggs in 2 ml of distilled water per bird (Ackert, 1931). Ten groups of these were each treated with a single dose of papaya latex 20% when they were 31-weeks-old. The dose was incremented logarithmically starting with 2.25 mg as the second dose, while the first dose was 0.44 mg. One infected group was used for testing the ED100 of papaya latex. Two infected

groups were used as untreated infection controls. one of these was killed along with the ten groups of the treated. infected chicken and the other group was killed together with the infected group receiving the ED100 of the papaya latex. Two groups of the chicken were used as uninfected controls; one of them was killed along with the ten groups of the treated. infected chicken and the other one was killed together with the infected group receiving the ED100 of the papaya latex. Faecal egg counts were done on all birds every day from the beginning of the fifth week until the end of the eighth week after infection but afterword they were done twice weekly. All birds were weighed once every month and all their eggs were weighed individually.

Statistics

Analysis of variance and the T method for multiple comparison were used to determine the level of significance in differences between chicken groups. Correlation between characters was measured by linear regression analysis. ED100 of the papaya latex was calculated using probit analysis (Fisher and Yates. 1974).

RESULTS

Ascaridia galli infective egg dose and prepatent period

The *Ascaridia galli* egg dose contained 95% infective eggs with at least 5% infectivity in 10-weeks-old cockerels. The prepatent period of the *Ascaridia galli* experimental infection in the pullets was 6 weeks (Table 1).

The effect of *Ascaridia galli* infection on egg production

The uninfected chicken started laying at 20 weeks of age with a mean egg weight of 45 g and reached a maximum mean egg weight of 60 g at 27 weeks old and stabilised until 31 weeks old. The infected birds started laying when they were 23 – 24 weeks old with a mean egg weight of 40 g and reached a maximum mean egg weight of 47 g when they were 29 weeks old and stabilised until they were treated with papaya latex at 31 weeks old. The means weight of egg production from 20 weeks until 31 weeks of age were 2849 g and 4690 g for the infected and the uninfected birds, respectively, which differed significantly (mean difference 1841 g = 39.25%) (Table 2).

Anthelmintic activity of papaya latex

After the prepatent period has passed, the *A. galli* EPG counts increased steadily with the increased age of infection (Table 1) and was at peak when the chicken were 31 weeks old by which time they were treated with single doses of papaya latex. One week

following the papaya latex dose, the EPG counts decreased. The percentage decrease of EPG count was higher in chicken receiving higher dose or papaya latex (Table 3). There was a significant, strong positive correlation between percentage decrease of EPG counts and the dose of papaya latex ($r = 0.98$; P H 0.05).

There was a negative correlation between the number of adult *A. galli* recovered from the treated, infected birds and the dose of papaya latex ($r = 0.996$; $P = 0.067$). The uninfected control chicken did not harbour any worms. Infected chicken without papaya latex treatment had a mean worm number of 50 and so did those chicken given a single dose of 0.44 mg papaya latex 20%. While those chicken receiving a dose pf 1447.89 mg papaya latex 20% were clean from worm (Table 4). There was a significant and very strong correlation between the dose and the realtive efficacy of papaya latex againts adult *A. galli* ($r = 0.99$; P H 0.05).

Using probit analysis, the calculated ED100 of papaya latex 20% against paten infection of *A. galli* was 1120 mg per bird. It was then proofed that the calculated ED100 can clean out patent *A. galli* infection (Table 5).

On week following treatment with single doses of papaya latex 20%, the mean egg and body weight of the chicken increased to become close or similar to the mean egg weight of uninfected chicken (Tables 6 – 8).

Toxicity of papaya latex in chicken

With single doses ranged from 0.44 mmg to 1447.89 mg of papaya latex 20% per bird, no signs of toxicity in the chicken were observed.

Table 1. : Mean *Ascaridia galli* egg counts in experimental chicken infected with 100 infective eggs.

Age (in weeks) of		Mean egg count
chicken	infection	
24	5	0
25	6	137
26	7	531
27	8	601
28	9	831
29	10	1410
30	11	2100
31	12	3600

Table 2 : Mean egg weigth (MEW) of chicken infected with 1000 *Ascaridia galli* infective eggs.

Age of chicken (weeks)	Infected chicken		Uninfected chicken		Student's t test			
	N	MEW (g)	N	MEW (g)	Difft.	T	DF	P
20	40	–	4	45.0				
21	40	–	4	50.0				
22	40	–	4	52.0				
23	40	40.0	4	52.0	12.0	3.41	42	0.0078
24	40	42.8	4	55.0	12.2	12.31	42	0.0000
25	40	44.4	4	57.0	12.6	6.89	42	0.0005
26	40	45.0	4	59.0	14.0			
27	40	45.0	4	60.0	13.2	29.6	42	0.0000
28	40	46.0	4	60.0	14.0			
29	40	47.2	4	60.0	12.8			
30	40	47.4	4	60.0	12.6			
31	40	47.4	4	60.0	12.6			
Mean Total/bird		2849.0		4690.0	1841.0 (39.25%)			

Table 3 : The mean and percentage decrease of *Ascaridia galli* egg count of infected chicken, one week after treatment with single doses of papaya latex 20%.

No.	Dose (mg) of papaya latex 20%	Mean EPG	% Decrease of EPG
1	Untreated infection control	3600.0	
2	044	3570.0	0.833
3	2.25	3435.0	4.583
4	5.05	3037.5	15.625
5	11.39	2726.7	24.258
6	25.63	2465.0	31.528
7	57.57	1850.0	48.611
8	129.75	1161.7	67.731
9	291.93	722.5	79.931
10	656.89	482.5	85.597
11	1447.89	0.0	100.000

Table 4 : Adult *Ascaridia galli* worm numbers recovered from infected chicken one week after treatment with single doses of papaya latex 20% and the relative efficacy of papaya latex.

Dose (mg) of papaya latex 20%	Number of adult worms	Relative efficacy (%)
Untreated infection control	50	
0.44	50	0
2.25	42	16
5.05	36	28
11.39	30	40
25.63	25	50
57.57	20	60
129.75	15	70
291.93	11	78
656.89	6	88
1447.89	0	100

Table 5 : Mean egg counts and worm numbers of adult *Ascaridia galli*, one week after treatment of infected chicken with the calculated ED100 (1120 mg) of papaya latex 20%.

Infected chicken group	Mean EPG	Mean adult worm numbers
No papaya latex treatment	3600	50
Treated with ED100	0	0

Table 6 : Mean egg weight (g) of chicken infected with 1000 *Ascaridia galli* infective eggs, one week after treatment with single doses of papaya latex 20%.

Dose (mg) of papaya latex 20%	Before treatment (31 weeks old)		One week after treatment (32 weeks old)	
	Uninfected	Infected	Uninfected	Infected
ED 100	60		60	
0.44		50		50
2.25		49		49
5.05		50		50
11.39		49		49
25.63		45		53
57.57		50		60
129.75		46		52
291.93		45		55
656.89		47		55
1447.89		50		60

Table 7 : Mean egg weight (g) of chicken infected with 1000 *Ascaridia galli* infected eggs, one week after treatment with a calculated ED 100 of papaya latex 20%.

Bird No.	Infected birds		Untreated, uninfected birds
	Untreated	Treated with ED100 papaya latex 20%	
1	45	60	60
2	47	60	60
3	45	55	60
4	46	55	60
Mean	46.75	57.5	60

Table 8 : Mean body weight (Kg) of chicken infected with 1000 *Ascaridia galli* infective eggs, one week after treatment with a calculated ED100 of papaya latex 20%.

Bird No.	Infected birds		Untreated, uninfected birds
	Untreated	Treated with ED100 papaya latex 20%	
1	1.60	1.96	1.86
2	1.50	1.95	1.70
3	1.50	1.98	1.95
4	1.56	1.85	2.20
Mean	1.54	1.94	1.93

DISCUSSION

In this study, pullets over 3 months old instead of younger chicken were used in order to maintain patent *Ascaridia galli* infection while avoiding death of experimental birds since chicken over 3 months old are more resistant to *A. galli* infection (Deo and Srivastava, 1962). On the other hand, younger chicken are more susceptible and very often die from infection by this nematode (Ackert and Herrik, 1928; Todd and Hollingsworth, 1957). By maintaining patent infection in experimental birds during their productive age, the effect of infection on the productivity of the birds can be measured and further, anthelmintic activity of prospective chemicals and drugs can be assessed.

The prepatent period of *Ascaridia galli* in the experimental chicken was 6 weeks which is in good agreement with the general knowledge saying that *A. galli* infection has a prepatent period of 6 – 8 weeks (Card and Neshaim, 1973).

Infected chicken started laying at older age (23 – 24 weeks old) compared to uninfected controls which started laying at 20 weeks of age. This is probably due to lacking of vitamin B_{12}, since *A. galli* has a very high affinity for this vitamin. Interaction between vitamin B_{12} and vitamin A deficiencies tended to exaggerate the pathologic effect of *A. galli* infection (Gaafar and Ackert, 1953). It is also possible that the chicken lacked protein due to *A. galli* infection since chicken fed protein-enriched ration start

laying before 20 weeks old while birds fed ration with low protein content start laying at older age (Soeyoto and Rachman, 1983) although the former will usually produce smaller eggs. Results from this study support the notion that *A. galli* infection causes production loss and hence economic loss (Nilson and Alderin, 1989).

The strong anthelmintic activity of papaya latex against adult *A. galli* is thought to be due to proteolytic activity of proteolytic enzymes in papaya latex since papaya latex is know to contain papain, chymopapain and lysozyme (Winarno, 1983). *In vitro,* papaya latex completely hydrolysed adult *Ascaridia galli* incubated at ambient temperature but it did not hydrolyse fresh small intestine of chicken (Purwati and He, 1990). It has been shown that papain has stronger hydrolytic activity against muscle fibre compared to its action against collagen (Fogle, Plimpton, Ockerman, Jarenback and Person, 1982).

ABSTRAK

Aktivitas anthelmintik getah papaya *(Carica papaya L.)* terhadap infeksi paten cacing *Ascaridia galli* pada ayam petelur dewasa yang diinfeksi dengan 1000 telur infektif cacing tersebut telah diteliti. Satu minggu pasca pengobatan dengan getah papaya 20%, jumlah telur cacing dalam tiap gram tinja (ttgt) ayam menurun. Ada korelasi positif dan bermakna antara persentase penurunan ttgt dengan dosis getah papaya 20% (r = 0.98; P H 0.05). Juga ada korelasi negatif yang kuat antara jumlah cacing dewasa pasca pengobatan dengan dosis getah papaya 20% (r = 0.996; P = 0.067). Dosis tunggal 1447.89 mg getah papaya 20% mampu membebaskan ayam dari infeksi paten sedangkan kelompok ayam terinfeksi yang tidak diberi getah papaya 20% maupun yang diobati dengan dosis 0.44 mg getah papaya 20%, mengandung cacing dewasa dengan rataan masing-masing 50 ekor. Dosis infektif 100% (ED100) sebesar 1120 mg getah papaya 20% yang diperoleh dari analisis probit, ternyata membunuh semua cacing dewasa di dalam usus ayam terinfeksi. Rataan berat telur dan berat badan ayam terinfeksi meningkat mendekati atau menjadi sama dengan rataan berat telur atau berat badan ayam kontrol tanpa infeksi. satu minggu pasca pengobatan.

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