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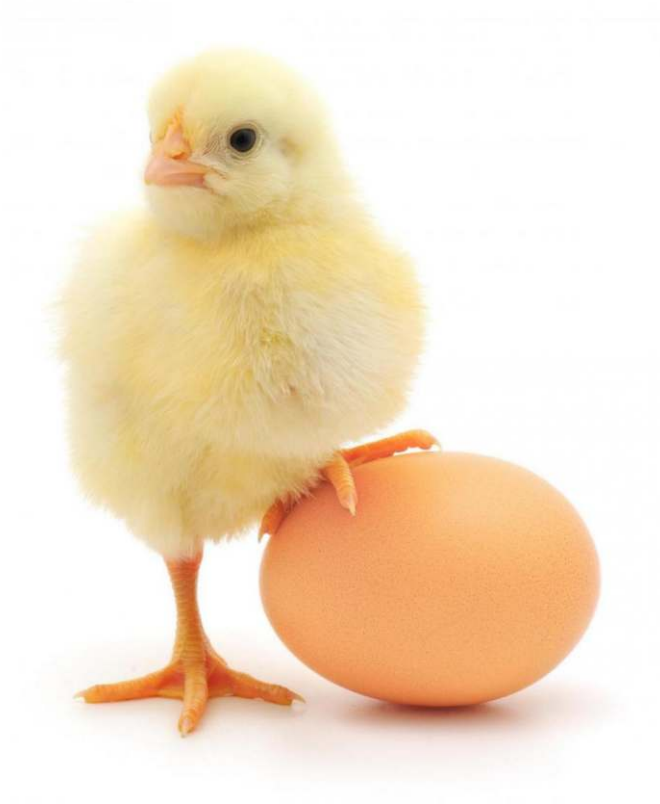

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Research Article
Effects of *Areca catechu* Seed and *Anredera cordifolia* Leaf on *Ascaridia galli* Infection in the Domestic Chicken (*Gallus gallus domesticus*)

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Abstract

Background and Objective: *Ascaridia galli* (*A. galli*) infection can depress the body weight and egg production of domestic chickens. Synthetic anthelmintics are usually used as a treatment but *A. galli* resistance to synthetic anthelmintics is rising. The aim of this research was to understand the effects of combinations of *Areca catechu* seed and *Anredera cordifolia* leaf on the number of eggs per gram (EPG) of feces, body weight, number of *Ascaridia galli* worms, histopathology and immunoexpression of CD4+lymphocytes in the intestine. **Materials and Methods:** Three groups of fifteen chickens infected with *A. galli* were used in this research. For seven days, group 1 was orally administered a combination of 2 mg kg^{-1} b.wt., of *Areca catechu* and 1 mg kg^{-1} b.wt., of *Anredera cordifolia*, group 2 was given a combination of 5 mg kg^{-1} b.wt., of *Areca catechu* and 2.5 mg kg^{-1} b.wt., of *Anredera cordifolia* and group 3 was not treated. This research had ethical clearance No. 00040/04/LPPT/IV/2017. On the 10th day, the chickens were euthanized and examined for the number of EPG of feces, body weight, number of *A. galli*, histopathology and immunoexpression of intestinal CD4+lymphocytes. The data were analyzed statistically and the histopathological changes were analyzed descriptively. **Results:** The results showed that the combination of *Areca catechu* and *Anredera cordifolia* significantly reduced EPG of feces and eliminated *A. galli* in 60% of the chickens in group 1 but there was no significant difference in body weight among groups. In group 2, the alkaloids and saponins contained in *Areca catechu* and *Anredera cordifolia* display antagonistic activity. Thus, high doses do not reduce EPG levels in group 2. Immunohistochemistry showed that wound healing is improved in the treated groups. **Conclusion:** *Ascaridia galli* infection might be reduced by a combination of *Areca catechu* and *Anredera cordifolia*.

Key words: Chickens, *Areca catechu*, *Anredera cordifolia*, *Ascaridia galli*, CD4+lymphocyte

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The domestic chicken is widely reared in traditional farms. These chickens are the most important source of protein and economic income for rural people[1-3]. Intestinal parasitic nematodes cause severe diseases in poultry and decrease the productivity of poultry farms in developing countries, including Indonesia[4]. *Ascaridia galli* is a nematode commonly found in chickens that causes economic losses. Infection by *Ascaridia galli* can occur in chickens of all ages, but the greatest degree of damage is often found in young birds under 12 weeks of age[5,6]. Heavy infection by *Ascaridia galli* is characterized by retarded growth, emaciation, anorexia, anemia, diarrhea, dehydration and decreases in body weight and egg production[7-9].

Ascaridia galli infection is most commonly treated using synthetic anthelmintics, but these anthelmintics have many problems. Such problems include the high cost of the anthelmintics, the negative impacts of carcinogenic drug residues in poultry meat and the development of anthelmintic resistance in helminths. Salam[10] revealed that the main treatment for *Ascaridia galli* infection in native chicken was using synthetic anthelmintic. If the anthelmintic used for the worm treatment is of the same type and used for a long time, it may cause resistance to the worms[11]. Drug resistance causes the effectiveness of therapeutic drugs to be suboptimal[3,12]. The development of drug resistance in helminths to chemotherapeutical products has drawn attention to the need for alternative treatments. Anthelmintics from natural sources could play a key role in the treatment of parasitic infections. One of the plants that can be used as an anthelmintic is *Areca catechu*. *Areca catechu*, or betel nut, belongs to the family Arecaceae. *Areca catechu* contains active substances such as flavonoids, tannins, saponins, monoterpenes, sesquiterpenes, phenols, quinones and alkaloids (arecoline and arecaidine)[13,14]. *Anredera cordifolia* is a plant that can be used to improve the wound-healing process[15]. Infection by *Ascaridia galli* causes histopathological change. Immature *Ascaridia galli* penetrate intestinal mucosa and cause severe hemorrhagic enteritis[16]. The active substances in *Anredera cordifolia* such as saponins, alkaloids and flavonoids were capable of promoting wound-healing processes[15]. Considering the above facts, the present study was performed to evaluate the effect of *Areca catechu* seed and *Anredera cordifolia* leaf on the number of *A. galli* eggs per gram (EPG) of feces, body weight, number of *A. galli* in the intestine, histopathology and immunoexpression of intestinal CD4+lymphocytes. Research on the potential of *Areca catechu* powders to work as anthelmintics for local chickens *in vivo* has also been reported by Tangalin[17] and Ozaraga *et al*.[3]. *Areca catechu* powder is able to decrease the number of EPG of feces by 66.51%[3]. *Anredera cordifolia* also shows potential as an anthelmintic that causes paralysis in the worms[18].

MATERIALS AND METHODS

The study was carried out at the Department of Veterinary Pathology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Powdered *Areca catechu* seed and *Anredera cordifolia* leaf seeds of *Areca catechu* and leaves of *Anredera cordifolia* were collected from the field. The collected seeds and leaves were dried in the shade at the ambient air temperature of the surrounding area, minced to powder using an electrical blender and stored[17].

Sample size and experimental animals: Young domestic chickens were used in this research. Fifteen infected chickens were divided into 3 groups of 5 chickens each: Group 1, group 2 and group 3. Group 1 was orally administered a combination of 2 mg kg^{-1} b.wt., of *Areca catechu* seed and 1 mg kg^{-1} b.wt., of *Anredera cordifolia* leaf extract, group 2 was given a combination of 5 mg kg^{-1} b.wt., of *Areca catechu* seed and 2.5 mg kg^{-1} b.wt., of *Anredera cordifolia* leaf extract and group 3 was not given any treatment. Treatments were given for seven days[19] and all chickens were then euthanized on the 10th day. This research had ethical clearance No. 00040/04/LPPT/IV/2017.

Fecal egg count (FEC): Fecal samples were collected from the initially selected birds on day 0 (for pretreatment FECs) and again on the 10th day of treatment. Fecal egg count was calculated as the number of eggs per gram (EPG) of feces using the McMaster technique[20].

Number of *A. galli* in the intestine: Chickens were euthanized on the 10th day. During necropsies, all visible *A. galli* were collected. The number of worms in each part of the intestine (duodenum, jejunum and ileum) was counted. The efficacy of the treatments was determined by comparing the number of worms in the control and treated groups.

Body weight: The body weight of chickens was measured. Every chicken was examined on day 0 (pretreatment) and 10. The effect of *Areca catechu* and *Anredera cordifolia* was determined by comparing body weight in the control and treated groups.

Histological evaluation: The histological change of the intestine was evaluated using immunohistochemistry (IHC).

RESULTS AND DISCUSSION

Most medicinal plants show antiparasitic characteristics and have usually been applied as a remedy against parasites[21]. Many researchers demonstrated the effectiveness of numerous plants, including *Areca catechu*, against *A. galli* infection in poultry and some other animals[3,13,17,22]. The results of the present study revealed that, on day 0 (pretreatment), the EPG of groups 1, 2 and 3 were 570, 230 and 240, respectively. On the 10th day, the EPG in group 1 was significantly lower (Table 1). In group 2, these results illustrate that the combination of *Areca catechu* and *Anredera cordifolia* produces a negative effect. *Areca catechu* seeds contain active ingredients such as flavonoids, saponins, monoterpenes, sesquiterpenes, phenols, quinones and alkaloids such as arecoline, tannin and arecaine[13,14], while *Anredera cordifolia* contains active ingredients on leaves such as saponins, flavonoids, triterpenes, steroids, quinones, monoterpenoids and sesquiterpenes[23-26]. According to Milugo *et al.*[27], alkaloids and saponins can display antagonistic activity, as evidenced by the inhibition activity testing at each fraction of 80%, which reduced to 53% after merging the two fractions. Thus, high doses do not result in decreased EPG levels compared to low doses. Further studies on the combined antagonistic effects of both active ingredients that do not produce toxic results are needed[28].

This result showed that powdered *Areca catechu* and *Anredera cordifolia* were effective against *A. galli* in chickens. Tangalin[17] used *Areca catechu* extract and reported a decrease in *A. galli* EPG of feces in chickens. Present observations revealed the strong effectiveness of *Areca catechu* and *Anredera cordifolia* against *A. galli* infection and agree with the study of Ozaraga *et al.*[3], who demonstrated the successful control of helminths, including *A. galli*, through the use of *Areca catechu*. The successful control of *A. galli* using *Areca catechu* extract was also reported by Febriani *et al.*[13]. The seeds of *Areca catechu* are used as an anthelmintic because they contain alkaloids, arecoline and tannins. Arecoline is toxic to some worms and can paralyze them. Proanthocyanidins (a group of condensed tannins) can inhibit enzymes and degrade membranes. The inhibition of enzymes causes metabolic failure, which decreases energy production and leads to worm death. Tannins also act as anthelmintics through their ovicidal properties[29].

Table 1: Effect of treatment on EPG result

	EPG	
Groups	Before	After
1	570±585.88	140.0±147.48[a]
2	230±144.05	940.0±1320.70[b]
3	240±163.55	1030.0±1061.01[b]

Same row with different superscripts are significantly different, $p<0.05$

Table 2: Number of *A. galli* in intestine

Groups	Number	Duodenum	Jejunum	Ileum
1	1	-	-	-
	2	-	-	-
	3	-	2	7
	4	-	-	-
	5	1	2	5
2	1	-	-	3
	2	4	6	-
	3	-	-	-
	4	9	5	-
	5	-	-	-
3	1	-	-	13
	2	4	35	-
	3	-	1	-
	4	1	6	-
	5	-	13	6

Table 3: Effect of *Areca catechu* seed and *Anredera cordifolia* leaf on body weight of chicken

	Body weight (g^{-1})	
Groups	Before	After
1	0.67±0.145	0.71±0.208
2	0.52±0.133	0.83±0.396
3	0.74±0.135	0.88±0.134

Post-mortem parasite counts indicated the anthelmintic activity of *Areca catechu* and *Anredera cordifolia*. The number of *A. galli* did not differ significantly between the untreated group and groups treated with *Areca catechu* and *Anredera cordifolia*, although *A. galli* was absent in 60% of the chickens in group 1, 40% of the chickens in group 2 and 100% of the chickens in group 3. This anthelmintic property represents a promising alternative to chemical treatments. This would be of great importance for poultry farms and will contribute to sustainable agriculture. Furthermore, the active compounds in plants are diverse and stable natural compounds with low molecular weights, which can prevent the development of anthelmintic resistance[30]. The numbers of *A. galli* in the intestine are shown in Table 2.

Body weight increased in all groups, but these increases were not significantly different among treated and untreated groups (Table 3). Body weight increased in all groups because the movement activity of chickens was lower, which was probably a direct effect of the increased amount of time used for food intake. The total time used for resting behavior in treated groups was similar to that in the control group.

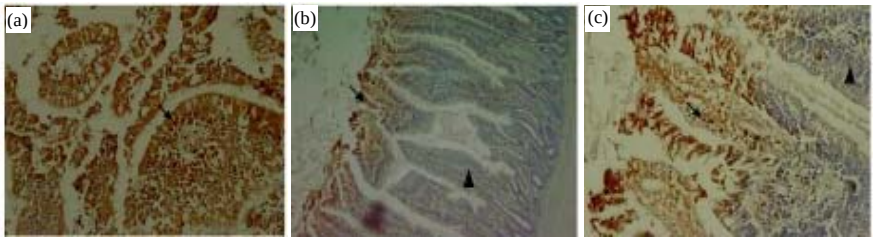

Fig. 1: Expression of CD4+lymphocytes in jejunum (arrows, A = group 1), (B = group 2) and (C = group 3). Wound healing in process, normal tissues (arrow heads). Scale bar: 20 μm

Because basic activities were similarly reduced among groups, these changes were probably caused in part by reduced energy availability or modulation of the neuroendocrine system that allowed body weight to increase[31]. This increase in body weight in the treated groups may have compensated for negative effects of *A. galli* on body weight throughout development. Some of the many sensory, neural and hormonal factors that modulate food intake in healthy animals are known to be affected by certain host-parasite relationships[32].

The results of immunohistochemistry in the intestine indicated that powdered *Areca catechu* and *Anredera cordifolia* improve wound-healing processes (Fig. 1). Lymphocytes in the jejunum are dark brown in color. The number of lymphocytes increased with treatment in group 1 but decreased in group 2, which was treated with a higher dose of *Anredera cordifolia* and had normal intestinal villi. These findings proved that *Anredera cordifolia* can improve healing processes in the intestine. CD4+lymphocytes stimulate the differentiation of B cells that then secrete antibodies and these lymphocytes can also secrete cytokines that activate macrophages to phagocytize antigens[33]. CD4+lymphocytes are activated when an antigen is processed by an antigen presenting cell (APC). When an antigen is transported through lymphoid organs by APC cells with MHC class II molecules, the number of activated CD4+lymphocytes increases until the antigen is eliminated and the number of lymphocytes then decreases[33-35]. *Anredera cordifolia* contains substances that can improve the wound-healing process and it has also antimicrobial and anti-inflammatory effects. Saponins have an antimicrobial effect that disturbs bacterial membranes. Saponins can increase TGF-β1, which increases inflammatory cells in the wound area and can accelerate the inflammation process. Saponins are also used as agents of angiogenesis[36]. Miladiyah and Prabowo[15] revealed that flavonoids and polyphenols can also be used to improve wound healing by inhibiting peroxidase lipids and antioxidants. The inhibition of peroxidase lipids increases the production of collagen, fibrin and blood vessels.

Previous studies have shown the effectiveness of *Areca catechu* in controlling *A. galli* infection in poultry[17,22] and of *Anredera cordifolia* in accelerating wound-healing processes[15,36]. In the present study, powdered *Areca catechu* seed and *Anredera cordifolia* leaf were proven to be an effective treatment for *A. galli* infection. These findings agree with Ozaraga *et al.*[3], who tested various medicinal plants against *A. galli* and demonstrated that the best control occurred with *Areca catechu*. Febriani *et al.*[13] also reported that *Areca catechu* is effective against *A. galli* eggs.

CONCLUSION

It is concluded that, treating chickens infected by *A. galli* using a combination of *Areca catechu* and *Anredera cordifolia* significantly reduced the EPG of feces and eliminated *A. galli* in 60% of the chickens in group 1, but there was no significant difference in body weight among groups. CD4+lymphocytes were expressed in the intestine, which showed that wound healing improved in the treated groups.

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