Reduction of fecal parasites by *Arecha catechu* L. seed and *Anredera cordifolia* (Ten) Steenis leaves powder in laying hens

Endang Kusumanti1, Retno Murwani1,2*

1 Laboratory of Physiology and Biochemistry-Faculty of Animal and Agriculture Science, Diponegoro University, Jl. Prof. Soedharto, SH, Tembalang, Semarang
2 Natural Product Laboratory, Centre of Research and Services, Diponegoro University. Jl. Prof. Soedarto, SH, Tembalang, Semarang, Central Java, Indonesia.

*rmurwani@gmail.com

Abstract. *Arecha catechu* L (bettle nut) seed and *Anredera cordifolia* (Ten) Steenis (“Binahong”) have been shown to have anti helmintic and wound healing activities respectively. Their combine use as phytogenic additives in layers have been shown to reduce serum transaminase. Further study was conducted to evaluate the effect of *A. catechu* seed and *A. cordifolia* leaves powder supplementation on fecal parasites number and the performance of laying hens obtained from outdoor small scale layer farmers. Forty eight of 42 weeks old laying hens were allocated randomly into 4 treatment groups i.e. no supplementation (T0), supplemented with 0.025% *A. catechu* seed and *A. cordifolia* leaves powder (T0.025%), 0.05% *A. catechu* seed and *A. cordifolia* leaves powder (T0.05%), 0.1% *A. catechu* seed and *A. cordifolia* leaves powder (T0.1%). Each treatment consisted of six replicates with two hens per replicate. Supplementation were carried out by administering alternately *A. catechu* seed powder for 3 days followed by *A. cordifolia* leaves powder for another 3 days. The alternate supplementation for each groups was conducted for 18 days. Feed consumption, egg production, egg weight, hen day production (HDP) were recorded daily. Parasites counts were sampled and enumerated at the beginning and at the end of supplementation. The result showed that alternate supplementation of 0.0.025% *A. catechu* seed and *A. cordifolia* leaves powder up to 18 days to 42 weeks old laying hens reduced fecal parasites without affecting performance.

Key words: heat stress, microbial load, *A. galli*, coccidia, *Eimeria* sp., antibiotic free

1. Introduction

A large number of small scale layer farmers in tropical country like Central Java Indonesia used commercial laying hens to produce commercial eggs. The hens start to produce eggs at about 16 to 20 weeks of age depending on the strain. Egg production peaks of about 24 to 30 weeks after which production gradually declines. When production declines and no longer profitable, the hens will be sold as meat chicken. Small scale layer farmers often extend production time which consequently extend environmental and disease challenges such as worm *Ascardia galli* and coccidia. For feed, farmers commonly use commercial complete feed as it is readily available and it has included antibiotics and coccidiostat to prevent endo-parasites infestation [1]. The present climate change such
as daily sudden change between rainy and hot-dry weather has added environmental stress and disease challenge which may no longer be able to be prevented by the existing in feed medication. In order to maintain the efficacy the existing one and prevent increasing the dosage of in feed medication, phytogenic additives can be used to help to maintain poultry productivity and health [2, 3, 4, 5]. Areca catechu has been shown to have antimicrobial, antihelminthic, and antioxidant activity and Anredera cordifolia leaves are known to assist wound healing [6, 7, 8, 9]. Their activities therefore could be beneficial to reduce endoparasites and ameliorate parasite associated internal wound. Earlier study using their combine powder in laying hens reduced serum transaminase which indicated reduced liver cell damage [10]. Therefore we studied further supplementation of A. catechu seed and A. cordifolia leaves powder in laying hens to reduce endoparasite number and maintain performance.

2. Material and Methods

2.1. Collection of plant material and preparation of powder
Fresh dry A. catechu seed were collected from local market and the pericarp was discarded. Fresh A. cordifolia leaves were obtained from local farmers and air dried. Each of the dried plant materials were ground to a coarse powder and filtered through 25-50 mesh screen to obtain a homogenous powder. The dried plant powder was stored in refrigerated container for further use.

2.2. Supplementation of A. catechu seed and A. cordifolia leaves powder in laying hens
Laying hens used for this study was obtained from a small scale layer husbandry farmers. The hens were raised in an open battery housing and they were selected randomly on the basis of body weight and age. Forty two weeks old of 48 Isa Brown laying hens with an average body weight of 1.82 ± 0.17 kg were allocated randomly into 4 treatment groups i.e. T0: control with no supplementation; T0.025%: supplemented with 0.025% A. catechu seed and A. cordifolia leaves powder; T0.05%: supplemented with 0.05% A. catechu seed and A. cordifolia leaves powder; T0.1%: supplemented with 0.1% A. catechu seed and A. cordifolia leaves powder. Each group consisted of 6 replicates with two hens per replicate. Each hen was given 120 g commercial layer diet (17% protein from Charoen Poppand) per day and free access to drinking water. Supplementation in each group was carried out by mixing it in the diet. A. catechu seed powder was supplemented for 3 days followed by A. cordifolia leaves powder for 3 days, and the alternate administration was conducted for 18 days. Egg production were recorded daily from all birds in the groups.

2.3. Collection of fecal samples, parasites identification and enumeration
On day 7 fecal samples were collected from fresh drops from one bird from each replicates. The samples were immediately preserved in 10% formalin and taken to Laboratory of Animal Health Service, Office of Animal Husbandry and Health, Semarang-Central Java Province for parasite identification and enumeration. The parasite determination was done by on duty laboratory staff expert. In brief, three gram of feces were dissolved in aquadest, stirred to homogenize, filtered to separate dirt and debri, and the filtrate was collected in plastic tubes. The filtrate in plastic tubes was stood at room temperature for about 15 minutes to let the sample settling in the bottom. The upper part of aquadest was then partly discarded carefully. The remaining sample in the tube was stirred to homogenize, and finally transferred into petri dish to be examined under microscope [11]. The microscope was equipped with camera and connected to computer screen and moved horizontally and vertically to scan the whole sample for parasites identification and enumeration.

2.4. Experimental design and statistical analysis:
A Completely Randomized Design with 4 treatments and six replicates was employed. Each replicate consisted of 2 hens. Parasites data from each supplemented group were analyzed by simple grouping into parasites types and number and analyzed descriptively. Statistical analyses were assisted using Excel. Feed consumption, egg number and weight, and hen day production (HDP) were analyzed by
ANOVA and when means were significantly different (p<0.01) Duncan’s multiple range test was carried out.

3. Results and Discussion

The results of fecal parasites types and number before and after 18 days supplementation of A. catechu seed and A. cordifolia leaves powder were shown in Table 1. It showed that before supplementation the types of internal parasites found were A. galli, T. americana sp., S. trachealis, and Eimeria sp, and Ascaridia sp. The number of each types of fecal parasites varied in each group. For A. galli, the highest number was found in T₀.05% (46) followed by T₀ (31), T₀.1% (17), and the lowest in T₀.025% (5). For T. americana sp or Ascaridia sp they were found only in T₀.025% (2). For S. trachealis, they were found only in T₀ (5) and T₀.025% (3). For Eimeria sp, they were found only in T₀.025% (8) and T₀.05% (1). Before supplementation T₀.025% was the only group infested with all types of parasites, albeit in relatively low number.

Table 1. Total number and types of fecal parasites after 18 days supplementation of A. catechu seed and A. cordifolia leaves powder alternately in laying hens.

| Endoparasites                  | T₀    | T₀.025% | T₀.05% | T₀.1% |
|--------------------------------|-------|---------|--------|-------|
| **Before supplementation:**    |       |         |        |       |
| Ascaridia galli                | 31    | 5       | 46     | 17    |
| Tetrameres americana sp        | 0     | 2       | 0      | 0     |
| Syngamus Trachealis            | 1     | 3       | 0      | 0     |
| Eimeria sp                     | 0     | 8       | 1      | 0     |
| Ascaridia sp                   | 0     | 5       | 0      | 0     |
| **Total**                      | 2 types | 5 types | 2 types | 1 type |
| **After supplementation:**     |       |         |        |       |
| Ascaridia galli                | 2     | 5       | 9      | 2     |
| Tetrameres americana sp        | 0     | 0       | 0      | 0     |
| Syngamus Trachealis            | 0     | 0       | 0      | 0     |
| Eimeria sp                     | 0     | 0       | 0      | 0     |
| Ascaridia sp                   | 0     | 0       | 0      | 0     |

Reduction of endoparasites (%):

| Parasite                     | Reduction |
|------------------------------|-----------|
| A. Galli                     | 93,5      |
| Tetrameres americana sp      | No change (0) | 100 |
| Syngamus Trachealis          | 100,0     |
| Eimeria sp                   | No change (0) | 100 |
| Ascaridia sp                 | No change (0) | 100 |

After 18 days alternate supplementation of A. catechu seed and A. cordifolia leaves powder, only A. galli were persistently found in all groups in a relatively low number i.e 2 (T₀), 5 (T₀.025%), 9 (T₀.05%), and 2 (T₀.1%) or equivalent to reduction of 92.3%, 0% (no change), 80.4%, and 88.2% respectively. The increasing dosage of supplementation appeared to be unrelated to the percentage of reduction. For example the initial A. galli in T₀ was 31 with no supplementation, and it was reduced to 2 after 18 days. On the other hand higher dosage of supplementation in T₀.1% and T₀.05% only reduced to 88.2% and 89.4% respectively, which were lower than control group. Such result could be due to the severity and the health status of individual hens being studied which can not be controlled. The hens for this study were obtained from local farmers and the hens were raised in an open (outdoor) battery housing. They were selected randomly on the basis of body weight and age. Thus the severity...
and parasites infestation could not be controlled. The persistent finding of fecal *A. galli* at the end of supplementation albeit in low number may reflect that this infestation is not easily eliminated in laying hens which are producing reaching 45 weeks. This was supported by the fact that T\(_{0.025}\%\) which had the lowest number of *A. galli* (5) before treatment remained the same after 18 days treatment. *Ascaridia galli* infestation occur worldwide and are very common in chicken and much more abundant in traditional farming (open cage house) than in industrial production facilities (close house). Birds become infected after eating infective eggs from contaminated feed, water, equipment, or through infected earthworms [12]. *Ascaridia galli* larvae enter and stays in gastrointestinal tissue and harms the lining of gastrointestinal. Their number in intestinal lumen could be fewer and therefore the excreted number in feces may not reflect the actual total number.

From all groups, T\(_{0.025}\%\) was the only group with four other types of parasites i.e. *Tetrameres americana* sp, *Syngamus trachealis*, *Eimeria* sp, and *Ascaridia* sp. They were initially present as many as 8 before supplementation and they were all eliminated after 18 days supplementation. *Eimeria* sp in T\(_{0.05}\%\) (higher dosage) which was initialy present as many as 2 (lower than T\(_{0.025}\%\)) was also eliminated. The data suggested that lower dosage was sufficient to eliminate *Eimeria* sp. It indicated that these phytogenic additives have anti coccidia activity. Further study is needed to provide more evidence.

Production performance of the hens were presented in Figure 1 and statistical analyses results showed that there was no effect of 18 days supplementation on feed consumption, egg weight and number, and HDP.

![Production performance of 42 weeks laying hens after 18 days alternate supplementation of Arecha catechu L. seed and Anredera cordifolia (Ten) Steenis leaves powder](image)

Figure 1. Production performance of 42 weeks laying hens after 18 days alternate supplementation of *Arecha catechu* L. seed and *Anredera cordifolia* (Ten) Steenis leaves powder

The supplementation appears to be safe to be adminstered to older laying hens without affecting production performance. Further study to dissect the mechanism of *A. catechu* seed and *A. cordifolia* leaves powder supplementation on parasites reduction using histopathology is needed. In situ outdoor layer farming study will be benefecial for local farmers.

4. Conclusion
Supplementation of *A. catechu* seed and *A. cordifolia* leaves powder up at 0.025% reduced fecal parasites types and number without affecting egg production performance.
References

[1] Murwani R and Bayuardhi B 2007 Broilers serum cholesterol and glutamic oxaloacetic transaminase and their relation to antibiotic in feed and medication programs in four broiler producers in Semarang region-Central Java, Indonesia. International Journal of Poultry Science, Vol. 6, No.4, pp. 266-270. Retrieved September 9th, 2016, from http://scialert.net/abstract/?doi=ijps.2007.266.270

[2] Murwani R and Murtini S 2009 Effect of chlortetracycline additive in broilers fed local diets on antibody titers to NDV vaccine. International Journal of Poultry Science, Vol. 8, No.8, pp. 755-759. Retrieved September 9th, 2016, from http://scialert.net/abstract/?doi=ijps.2009.755.759

[3] Murtini, S., Murwani, R., Satrija, F. and Handaryani, E., 2010. Anti Marek’s Disease Virus Activity of Scurrula oortiana (Tea Mistletoe) Stem Extract in Embryonic Chicken Eggs. International Journal of Poultry Science, 9 (9): 879-885.

[4] Murwani R, Indriani A, Wihardani K, Wahyuningsrum M A, Tawakal N R, Mulyono and Kusumanti E 2011 Blood biochemical indices and productivity of broilers on diet supplemented with mannan oligosaccharide, baker yeast, or combined baker yeast and Noni leaves extracts. International Journal of Poultry Science, Vol.10, No.12, pp. 990-997. Retrieved September 9th, 2016, from http://scialert.net/abstract/?doi=ijps.2011.991.998

[5] Tanod, W.N.L., Murwani, R., Susanti, S., Kusumanti, E. 2015 Addition of cashew (Annacardium occidentale) apple powder into diet can increase body weight and intestinal relative weight in broiler. Pakistan Journal of Nutrition 14(9), pp. 629-631

[6] Lim S K, Hong S P, Jeong S W, Kim B, Bak H, Ryoo H C, Lee S H and Ahn S K 2007 Simultaneous effect of ursolic acid and oleanolic acid on epidermal permeability barrier function and epidermal keratinocyte differentiation via peroxisome proliferator-activated receptor-α. The Journal of Dermatology, Vol. 34, pp. 625-634.

[7] Tangalin, M.G.G. 2011. Anthelmintic Effect of Processed Mature Betel Nut as Dewormer to Native Chicken and Small ruminant (Sheep and Goat). Asian Journal of Health.Vol.1.No.1., pp. 230-243.

[8] Prasetyo A T and Herihadi E 2013 The application of moist exposed burn ointment (MEBO) and binahong leaves in treating partial thickness burn : A case report. Jurnal Plastik Rekonstruksi (The Indonesian Journal of Plastic Reconstructive and Aesthetic Surgery), Vol. 3, pp.142-146. Retrieved September 9th, 2016, from http://www.jprjournal.com/index.php/jpr/article/view/178/139

[9] Baby A A and Raphael K R 2014 Potential antimicrobial, anthelmintic, and antioxidant properties of Areca catechu L. Root. International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 6, No.6, pp. 486-489. Retrieved September 29th, 2016, from www.ijppsjournal.com/Vol6Issue6/9731.pdf

[10] Marlani, H.P., Kusumanti, E., Murwani, R. 2017. Arechaatechu L. Seed and anredera cordifolia (ten) steenis leaf powder supplementation reduced serum transaminase in laying hens. Livestock Research for Rural Development, 29(5), from http://www.lrrd.org/lrrd29/5/rmur29094.html

[11] Parfitt, J. W. and Banks A. W. 1970. A Method for Counting Fasciolu Eggs in Cattle Faeces in The Field. Vet. Rec. 87 : 180-182.

[12] Désirée S. Jansson, Ann Nyman, Ivar Vågsholm, Dan Christensson, Magnus Göransson, Oddvar Fossum & Johan Höglund 2010. Ascarid infections in laying hens kept in different housing systems. Avian Pathology Volume 39, - Issue 6