

The response of red ginger (*Zingiber officinale* var *rubra*) with various processing in broilers were infected by *Eimeria tenella*

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Abstract. Red ginger contains high antioxidants and have anti –inflammatory properties. Ginger also has the ability to treat kimatif, antiemetic, antinausea, and antiparasitik. The aim of this experiment was identified the response of red ginger in broilers were infected by *Eimeria tenella*. This research used Completely Randomized Design (CRD) with 5 treatments and 4 replications. *Eimeria tenella* were infected by 10.000 oocysts/ head and red ginger solution were applied with 1% concentration. The treatments consist of KP (positive control), KO (coccidiostat), K1 (red ginger powder), K2 (extracted red ginger by ethanol) and K3 (extracted red ginger by water) . The results showed that the treatment of red ginger was significant effect ($P<0.05$) to lower oocyst production in broilers were infected by *Eimeria tenella*. The comparison between extracted red ginger by ethanol is better than by water or in powder form to decreased. The utilization of red ginger showed the percentage of heterophile and eosinophile close to normal when compared with positive control. Assesment of caecum lesion score was not significant ($P>0.05$) different effect between all the treatments. It is concluded that the treatment by red ginger better than coccidiostat and positive control.

1. Introduction

The poultry breeding sector in Indonesia is experiencing rapid progress due to the increasing demand for animal protein. In order to maintain poultry production, it is necessary to conduct regular monitoring efforts to check emerging diseases in animals. One of the most common diseases in broilers is coccidiosis. Coccidiosis is a parasitic disease in broilers that cause many losses, with the result of decreasing the efficiency of feed use and growth restriction, to death [1]. The disease is easy to develop in Indonesia because it is in accordance with the optimum temperature for the development of *Eimeria* is 21°C- 32°C, and enough moisture so that oocyst can sporulate. Sporulated oocyst can infect host [2].

Since the monetary crisis that occurred in Indonesia until now the price of manufactured medicines (imports) is very expensive, so it is not affordable by the breeders, especially breeders in the middle to lower scale [3]. In addition, most of the Indonesian population resides in the countryside, so the problem of distribution, lack of communication makes it difficult for the area to be reached by modern medicine and veterinary power [4]. Based on this the breeders are trying to find another alternative by utilizing some medicinal plants as traditional medicines called animal herbs that can be given in the form of solution through drinking water and or in the form of simplicia (flour) mixed into the ration as "feed additive" or "feed supplement" [3].



One of these medicinal plants is ginger. Ginger (*Zingiber officinale*) is a herb and medicine that has long been known to the public. Besides being used as a seasoning and traditional herbs, this plant also became a commodity trade as a material industry of drugs, cosmetics, beverages, snacks and kitchen needs. Ginger rhizome is known to contain oleoresin and essential oils of 1-3%. Ginger in the form of powder of 10 mg / kg body weight of chicken can decrease the result of coccidiosis disease [5].

This study aims to determine the response of red ginger for broilers which infected by *Eimeria tenella*, includes the value of cytotoxic injury, oocyst production, and differentiation leucocyt in blood.

2. Materials and Methods

The study was conducted in January 2015. The preparatory phase was held in early January 2015 at Pharmacy Laboratory of Faculty of Pharmacy, University of Sumatera Utara, Medan. Furthermore, the implementation stage of the research was conducted for five weeks at the experimental cage of Livestock Biology Laboratory, Faculty of Agriculture, University of Sumatera Utara Medan and Parasitology Laboratory of Balai Veteriner Medan.

2.1 Phase I. Propagation of Isolate *Eimeria tenella*

After obtaining *E. tenella* isolate, then it is proportionalized with addition of 2-2.5% potassium bichromate solution (5-10 times from sample) for 1-2 days in room temperature (26°C - 28°C). Cover the Petridis / glass cup slightly with open air to give chance. For propagation, *E. tenella* isolates were inoculated in 2-week-old coccus-free chickens, 6 days post-inoculation was harvested as previously worked and then stored in the refrigerator until use.

2.2 Phase II. Making Red Ginger Solution

In making red ginger solution, this study uses 3 kinds of ginger processing which will be used as a solution. The form of ginger processing in question is ginger powder, ginger extract using ethanol, and ginger extract using water.

2.2.1 Ginger Powder. Red Ginger is obtained from Pasar Medan. The fresh red ginger is washed and then slashed thinly and dried in an oven at 37°C for 48 hours until dry, then ground powder [1]. The solution was prepared in the manner indicated in the Indonesian Pharmacopoeia, to obtain a solution of 1 g of dried powder in 1 ml [1].

2.2.2 Ginger Extraction Using Ethanol. The freshly cleaned red ginger rhizome is dried with oven blower (40-60°C) for 30-36 hours to obtain dry ginger with 8-11% water content. The dried ginger is milled and then filtered to produce 30 mesh ginger powder. A total of 250 grams of ginger powder in the extract 4 times by using ethanol solvent (500 ml). The extract obtained was filtered with filter paper under vacuum. The obtained liquid is fed into a weighed rotary tube, then distilled with a rotary vacuum-evaporator. The distillation is stopped after the solvent stops dripping, it is obtained oleoresin consisting of semi-solid colored light brown to dark brown. Further weighing the oleoresin produced in the rotavapor flask. To homogenize the results of ginger extract with water at the time of preparation of the solution it is necessary to add 1% solution of CMC (Carboxyl Methyl Cellulose). Comparison of powder dosage with extract result is 1: 5. So Red ginger extract solution using ethanol made with concentration of 1% (2mg ginger / ml).

2.2.3 Ginger Extraction Using Water. Red ginger extraction uses water as the extracting solution. Ginger extraction is done on ginger powder. Every 25 g of ginger powder requires 125 ml of water. Extraction is done 4 times. To obtain the ginger extract, the filtrate is dried so that the solvent and water evaporate. Red ginger extract solution using water is made with a concentration of 1% (2mg ginger / ml).

2.3 Phase III. In Vivo Test

In this study, 80 chickens aged one day (DOC) Cobb 500 were randomized into 5 treatments (KP, KO, K1, K2, K3) with 4 replications, and each replication consisted of 4 chickens. At the age of 23 days, five chicken treatments (KP, KO, K1, K2, K3) were infected with *Eimeria tenella* each of 10.000 oocysts / oral. Five (5) days post infected *Eimeria tenella*, given the treatment of ginger solution as much as 1ml / head per oral (K1, K2, K3), and aquadest (KP) for 3 days, rest for 2 days, then given again for 3 days (system 3-2-3), as well as Coxymas coccidiostat administration (as directed by PT.Mensana).

2.4 Determination of the Number of Oocysts per Gram of Excreta

Six (6) days post infected *Eimeria tenella*, chicken excreta accommodated every day. In each treatment was taken excreta as much as 2 sample of cage, then collected and separated based on each treatment for 1 week. Excreta weighed 1 gram which was then dissolved into 29 ml of saturated salt solution, centrifuged for 10 minutes at 1500 rpm. After a centrifuge, the top of the supernatant (clear) is taken with a Pasteur pipette then dripped on both sides of the McArm's count chamber. Further observed under a microscope with a magnification of 10 x 10 [6].

2.5 Determination of SLS (Score Lesion Score)

The caecum lesion score (SLS) was determined based on pathological-anatomical changes in the degree of damage from infected chicken according to the Johson and Reid (1970) method.

2.6 Leucocyte Differentiation Examination

Blood sampling was done 3 times (age 27 days, 30 days, and 35 days). In each treatment 2 samples were taken per plot. To make blood slides and blood sampling according to the Piatina (2001) method [7].

3. Results and Discussion

3.1 Oocyst per Gram Excreta

Oocyst is the result of fertilization of microgamet and macrogamet in the sexual stage. After zygote fertilization will form oocyst. Oocyst *E. tenella* will come out with the excreta in a sporulated state, and will sporulate within 1-2 days of obtaining oxygen, suitable temperature, and humid environment [8]. The average production of oocysts per gram of excreta that is stored for seven days in *E.tenella* infected chickens can be seen in Table 1.

Table 1. Average production of Oocyst Per Gram (OPG) excreta were infected by *E.tenella* and giving red ginger solution (*Zingiber officinale* var *Rubra*)

Treatment	Observation on day-to-day (post infection)						
	6	7	8	9	10	11	12
KP	9425 ^a	9463	12725 ^a	12950 ^a	11163 ^a	8600 ^a	6363 ^a
KO	3925 ^b	8138	9000 ^a	7800 ^{bc}	5175 ^b	3350 ^b	2988 ^{ab}
K1	7888 ^{ab}	8475	12725 ^a	7950 ^{bc}	5400 ^b	3675 ^b	4338 ^{ab}
K2	4025 ^b	4988	3838 ^b	3488 ^c	2875 ^b	3250 ^b	1625 ^b
K3	3725 ^b	7575	9900 ^a	9980 ^{ab}	7188 ^{ab}	4675 ^b	3313 ^{ab}

Description: Superscript with different letters in the same column states differ significantly at the level ($P < 0.05$)

The result of statistical analysis using Anova (Analysis Varian) showed that giving 1% red ginger solution gave a significant effect ($P < 0.05$) to lowering the production of oocysts in broiler were infected by *E. tenella*. This can be seen from the treatment of K1, K2, K3 < positive control (KP).

While the treatment of coccidiostat (KO) gave no significant difference ($P > 0,05$) with 1% red ginger solution ($KO = K2 = K3$).

Furthermore, at the 7th and 8th days post infection, there was no significant difference ($P > 0.05$) between treatment of KP, KO, K1, K3. The number of oocyst of these four treatments has increased. Maudya (1994) stated that on the 6th and 7th days after the oocyst production infection will increase again because on that day the bleeding has somewhat decreased. However, all four treatments (KP, KO, K1, K3) were significantly different ($P < 0.05$) by treatment of K2 (on day 8). K2 treatment produces fewer oocysts than other treatments.

On the 9th and 10th day post-infection showed that there was a significant difference ($P < 0.05$) between the KO, K1, K2 treatment with positive control (KP). On that day seen the treatment of K3 still continue to increase the number of ookista. And slowly decline until the 12th day. However, statistically, the treatment showed no significant difference ($P > 0.05$) with positive control treatment (KP).

On the 11th day post infection showed no significant difference ($P > 0.05$) among all treatments (KP, KO, K1, K2, K3). The peak of oocyst secretion / expulsion on day 8 and the number of oocysts will decrease on the 9th day after infection. It will gradually decline by the 11th day, the oocyst remains small but may still be present in the stool for several months after infection. Oocyst left in the chicken body will serve as a stimulant to form immunity. Conversely high doses of 50,000 oocysts *E.tenella* will cause an outbreak of disease [9].

On the 12th day post infection showed no significant difference ($P > 0,05$) between coccidiostat treatment (KO) with treatment 1% red ginger solution (K1, K2, K3) to decreasing oocyst production. While K2 treatment was significantly different ($P < 0,05$) with positive control treatment (KP). The treatment of K2 (red ginger solution of ethanol extract 1%) tends to decrease oocyst production from day 8 to day 12. The decrease of oocyst production by giving the red ginger solution of ethanol extract of 1% indicates the existence of antioxy activity. The antioxy activity of ginger extract depends on the processing and chemical content [10].

Red ginger contains bioactive components such as oleoresin and gingerol. Gingerol is a phenol-derived compound that interacts with protozoan cells through an adsorption process involving hydrogen bonding. Low levels of phenol interact with proteins form the phenol protein complex. The bond between protein and phenol is a weak bond and immediately decomposes. The free phenol penetrates into the cells, causing precipitation and protein denaturation. At high levels of phenol causes coagulation of proteins so that cell membranes undergo lysis. The antioxy power of *E.tenella* oocysts occurs through its lysis of the *E.tenella* cell. Oleoresin and gingerol are anti-inflammatory and anti-bacterial. The consistency of the presence of chemical compounds in an herbal origin preparation is influenced by many factors such as where to grow, harvest time, extraction and solvent used. Treatment given antioxy will work faster to reduce oocyst production. Red ginger can improve digestion, the process that occurs is arousal intestinal stomach and intestinal stimuli by ginger oil. In addition, red ginger will stimulate and launch blood circulation.

3. 2. SLS (Score Lesi Scores)

Coccidiosis is an intracellular protozoal infection. Infection by *Eimeria tenella* as the cause of coccidiosis will always have implications for epithelial cell tissue damage, particularly in the mucosal and submucous parts of the caecum [5].

The result of statistical analysis using Anova showed that red ginger solution gave an unreal effect ($P > 0,05$) in decreasing the score of caecal lesions in broiler infected with *E.tenella*. The treatment that showed the highest score of the caecal lesion was the treatment of KP (positive control) and K1 (1% powdered ginger solution) of 0,56%, while the mean score of the lowest (mild) Treatment K2 (ginger solution ethanol extract 1%) that is equal to 0,37%. This means that the administration of red ginger is effective and responses in preventing tissue / bleeding damage to broiler caecum.

In addition, the way of processing herbs and active compounds oleoresin, essential oils, and gingerol contained in herbal raw materials of red ginger can work effectively in suppressing *E.tenella*

infection. Compounds contained in red ginger has been widely known to streamline the absorption of food into the body with high antioxidant content and strong anti-inflammatory properties [9]. This is also in accordance with the opinion of Iskandar *et al* (2000) which states that the infusion of red ginger as much as 1% can be useful as coccidiostat in broiler [1].

Based on the degree of tissue injury and bleeding in chicken caecum, the lesion score for all treatments is still relatively mild. It's because the resulting lesion score still ranges from 0-1. If sorted by the score of the lowest (mild) to the highest, the following sequence is K2, KO, K3, K1, KP. K2 treatment is lighter when compared with the treatment of KO, K3, K1, KP.

This suggests that red ginger solution effectively treats lesions in chicken caecum. KP treatment showed no significant difference ($P > 0,05$) with K1, K2, K3 and KO treatment because the amount of oocyst was not enough to give effect to broiler. In addition, it is possible chicken body response immediately form antibodies that can prevent tissue damage and bleeding in broiler caecum. The severity of coccidiosis attack is influenced by the number of attacking parasites (dose of infection), immune power, and age of host.

3.3 Leukocyte Differentiation

Leukocytes or white blood cells are cells that have nuclei and organelles. Leukocytes are active unit of the body's defense system. Leukocytes serve to protect the body from infection and cancer, and help the healing process [11]. The granulocyte group is characterized by the presence of granules in the cytoplasm, while the agranulocyte group has no granules [12]. The results obtained after the calculation of leukocyte differentiation are as follows:

Table 2. Mean percentage of heterophils, basophile, eosinophile, lymphocytes, and monocytes in broilers were infected by *E.tenella*

Leucocyte Differentiation (%)		Treatment				
		KP	KO	K1	K2	K3
After Treatment :						
Day (0)	Heterofil ^{ns}	30.63	33.75	28.00	30.75	44.50
	Basofil ^{ns}	9.54	7.50	8.25	8.62	7.75
	Eosinofil ^{ns}	10.00	9.00	6.50	7.13	6.50
	Limfosit ^{ns}	47.34	47.13	55.00	50.13	39.12
	Monosit ^{ns}	3.12	2.62	2.25	3.37	2.13
Day (3)	Heterofil	16.17 ^b	22.13 ^{ab}	28 ^a	27.37 ^a	26.50 ^a
	Basofil ^{ns}	6.00	5.75	5.62	4.50	5.50
	Eosinofil	10.63 ^a	8.12 ^b	4.25 ^c	4.50 ^c	5.88 ^{bc}
	Limfosit ^{ns}	65.00	61.75	59.63	60.63	59.25
	Monosit ^{ns}	2.25	2.25	2.50	3.00	2.87
Day (8)	Heterofil ^{ns}	30.50	35.75	35.00	25.63	36.37
	Basofil ^{ns}	7.87	4.25	4.00	2.75	5.25
	Eosinofil ^{ns}	9.87	6.87	5.00	5.50	6.25
	Limfosit ^{ns}	49.88	50.38	54.50	64.50	50.38
	Monosit ^{ns}	1.88	2.75	1.50	1.62	1.75

Description : ns = not significant

Table 3. The percentage of normal differentiation leucocytes in blood cell

Differentiation Leucocytes	(%) Normal in Blood Cell
Heterofil	27.20
Basofil	1.7
Eosinofil	2-8
Limfosit	59.1–64.6
Monosit	8.9-10.2

Scurkie (1976)

After Treatment (Day 0), the results of statistical analysis using Anova showed that the mean percentage of heterophils, basophils, eosinophils, lymphocytes, and monocytes were not significantly different ($P > 0.05$) in each treatment (Table 2). After Treatment (Day 3), the results of statistical analysis using Anova showed that the mean percentage of basophils, lymphocytes, and monocytes were not significantly different ($P > 0.05$) in each treatment (Table 2). There was a noticeable change where the positive control decreased heterophils count. While in the treatment of KO, K1, K2, K3 has slightly decreased the number of heterophils with the lowest percentage at KO (22.13%). The amount of heterophils in the treatment of red ginger (K1, K2, K3) tends to approach the normal value. This occurs because the heterophils are mobilized to enter the bloodstream from the bone marrow. Another possibility is the presence of antioxidant content in ginger is efficacious as anti-inflammatory. The mean percentage of eosinophil was significantly different ($P < 0.05$) between KP treatment with KO, K1, K2, K3. Treatment treated with coccidiostat and red ginger solutions are still able to maintain normal eosinophil count in blood despite being infected by *E. tenella*. The content of antioxidants in ginger can stimulate the intestinal mucous membrane to reduce inflammation. After Treatment (Day 8), the results of statistical analysis using Anova showed that the mean percentage of heterophils, basophils, eosinophils, lymphocytes, and monocytes were not significantly different ($P > 0.05$) in each treatment (Table 2). The anticoxy activity of ginger extract depends on the way processing and chemical content [10]. Red ginger contains bioactive components such as oleoresin and gingerol. Gingerol is a phenol-derived compound that interacts with protozoan cells through an adsorption process involving hydrogen bonding. Then when the chickens are able to survive past the 8th day and the 9th where oocyst production reaches its peak, then the chickens will go to healing by itself [13]. Some leucocyte have started normal.

4. Conclusion

The treatment which given red ginger solution by extract ethanol 1% (K2) was effective in decreasing oocyst production and showed the best result compared to other treatment. The administration of coccidiostat and red ginger solution showed not significant difference in reducing the caecum lesion value. The percentage of heterophils and eosinophils on the 3rd day post treatment tended to be more stable (normal) in the treatment of red ginger than the treatment of coccidiostat (KO) and positive control (KP).

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