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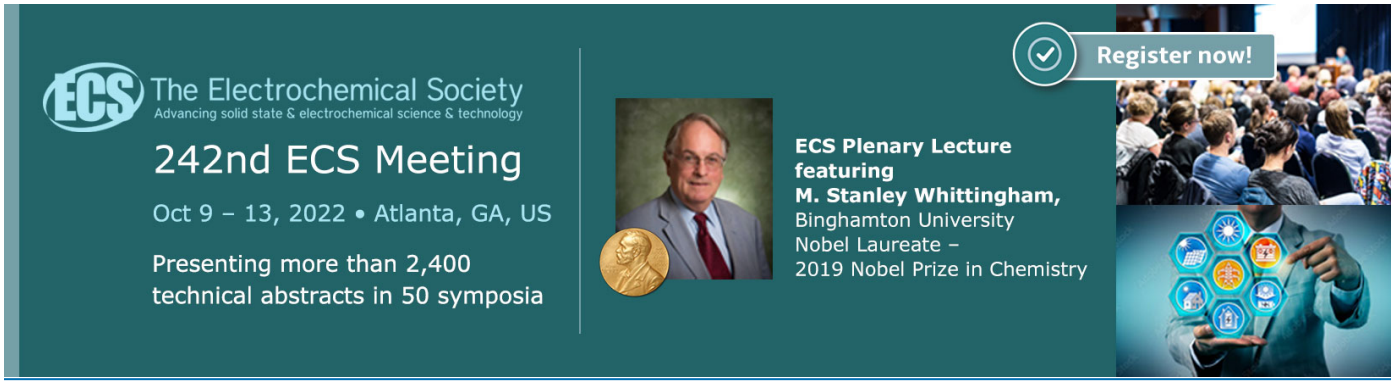

Treatment of Rabbit Coccidiosis with Combination of Herbal Extract II toward Oocysts Excretion and Hematology Parameters

D Indrasanti[1], M Indradji[1], E Yuwono[1], M Samsi[1], P V Sundari[2], M N Ichwan[2], E S Anengseh[2], M N Hatmadifia[2] and T N Hidayat[2]

[1]Animal Health Departement, Faculty of Animal Science, Universitas Jenderal Soedirman, Jl. Soeparno No. 60. Karangwangkal, Purwokerto, Indonesia 53123
[2]Graduate Student of Animal Science Faculty Universitas Jenderal Soedirman, Jl. Soeparno No. 60. Karangwangkal, Purwokerto, Indonesia 53123

E-mail: dianaindrasanti@gmail.com

Abstract. This study aims to determine oocysts excretion and hematological profile in coccidiosis rabbits given a combination of herbal extract II. Hematological profiles observed were red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), granulocytes, eosinophils, monocytes, lymphocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). This study used 40 rabbit coccidiosis material with ± 3 months age of ± 650 g weight, a combination of herbal extracts consisting of banana stem extract (BSE), papaya seeds (PSE) and garlic (GE), a set of tools and materials for rabbit maintenance and a set of hematological examination tools. The research method was carried out experimentally using a Completely Randomized Design (CRD). The analysis used variance analysis followed by Honest Real Difference (HRD). The combination of herbal extract II consists of BSE: 40 mg; PSE: 20 mg; GE: 40 mg. Rabbits were divided into 8 treatments with 5 replications, namely giving a combination of herbal extracts 0 mg (D0), 10 mg (D1), 20 mg (D2), 40 mg (D3), 80 (D4) mg, 100 mg (D5) and the comparison are used herbal extract I (consist of BSE: 33 mg; PSE: 2 mg; GE: 65 mg) as much as 100 mg (D6) and Aquaprime® (D7). Blood collection is carried out through the heart on the 14th day after treatment. The combination of herbal extract II had a very significant effect on oocysts excretion, but did not have a significant effect on all hematology parameters. Hence, a combination of herbal extracts can be used as an alternative to reduce the number of oocysts in rabbits coccidiosis.

Keywords: Rabbit coccidiosis, herbal extract, oocysts, hematology

1. Introduction

Rabbit's coccidiosis is a common disease in rabbits caused by *Eimeria spp*. This disease usually attacks the digestive system and liver with adverse effects in the form of watery diarrhea, decreased growth rates, anorexia, debilitation and coarse hair fur [1]. Coccidiosis in rabbits can cause changes in blood

parameters, decreased appetite, decreased body weight, and found coccidia oocysts in their feces [2]. Coccidiosis control can be carried out using vaccines and coccidiostat. However, since the rabbit coccidiosis vaccine is still being researched, control is done through improved maintenance management and use of prophylactic drugs in feed or drinking water [3, 4]. However, the use of chemical drugs that have been widely used anticoccidiosis has led to residues in meat, animal resistance, environmental contamination, as well as high costs and a long time for the development of new drugs [4, 5, 6, 7].

Coccidiosis control using herbal plants has begun even though the use in the field is not yet popular. Botany antioxidant's (saponins, flavonoids, tannins and aromatic compounds and plants that are rich in phytochemical compounds) role are increasing in controlling coccidiosis. Several studies, including banana, garlic and papaya stems have been carried out [8]. In vitro, we have examined each plant that is suspected to be used as a control of coccidiosis, including banana stems, garlic and papaya seeds [9]. Then, the extract of each plant was examined for its effect on rabbits infected with coccidiosis [2, 10, 11]. The use of herbal extracts as a control of coccidiosis is expected to reduce the amount of oocysts excretion while at the same time improves the physiological conditions of rabbits. So, this time we provided a combination of herbal extract II from a combination of banana, garlic and papaya seed extracts to give to rabbits infected coccidiosis from a breeder. Parameters observed were oocysts excretion and hematological profile after treatment.

2. Methodology

The material used in this study was banana stem extract (*Musa paradisiaca*), garlic extract (*Allium sativum*) and papaya seed extract (*Carica papaya*), 40 weaning crossbreed Rex rabbits infected with coccidiosis field isolates from breeder, age ± 3 months, weight ± 650 g. The tools used ware a set of tools for rabbit's maintenance, and a set of tools for making herbal extracts. The feed given were concentrate feeding, pellet and forage. While, drinking water is given ad libitum (boiled water). Making extracts on banana stems, garlic and papaya seeds was done separately by the maceration method according to our previous study [2]. Furthermore the extract is combined with a dose of banana stem extract (BSE): 40 mg; papaya seeds (PSE): 20 mg; garlic (GE): 40 mg. Rabbits were divided into 8 treatments with 5 replications. The administration of a combination of herbal extract with various doses were 0 mg (D0), 10 mg (D1), 20 mg (D2), 40 mg (D3), 80 (D4) mg, 100 mg (D5) and the comparison is used herbal extract I (consist of BSE: 33 mg; PSE: 2 mg; GE: 65 mg) as much as 100 mg (D6) and Aquaprime® (D7) (Romindo production), which is a diarrhea drug that is commonly used by breeder at a dose of 0.1 ml / kg body weight. The rabbits were divided evenly into treatments based on the number of oocysts in *Eimeria spp*, score 1: oocysts 0 to 250, score 2: oocysts 251 to 500, score 3: oocysts 501-1000, score 4: oocysts 1001-2000, score 5: 2001-4000, score 6: 4001-8000 (modification [12]).

Samples were rabbit feces before treatment and 14 days after treatment as well as blood samples taken through the heart 3 ml. Blood is taken with a syringe containing 10% ethylene acid diamin tetra acetate (EDTA). *Eimeria spp* oocysts examination and counting in feces refers to the Whitlock Method [13]. Whereas blood data were obtained using Sysmex XP-100 hematological analysis tools [14, 15]. The hematological profile observed were red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), granulocytes, eosinophils, monocytes, lymphocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). The research method was conducted experimentally using a Completely Randomized Design (CRD). The analysis is used Variance Analysis, continued by Honest Significant Difference (HSD) [15]. The study was conducted in several laboratories, namely the UNSOED Animal Health Laboratory, the UNSOED Research Laboratory, the Type B Animal Health Laboratory, Purwokerto, and the UNSOED Experimental Farm.

3. Result and Discussion

Blood parameters examined were red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), granulocytes, eosinophils, monocytes, lymphocytes, Mean Corpuscular Volume

(MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) and oocysts excretion. The results of measurement of herbal extract II for blood parameters and oocysts excretion can be seen in **Table 1** and **Table 2**.

Table 1. Measurement results of RBC, WBC, HGB, HCT, granulocytes and eosinophiles from rabbit given herbal extracts of various doses

Treatment	Parameters					
	RBC ($x10^6$/µl)	WBC ($x10^3$/µl)	HGB (g/dl)	HCT (%)	Granulocytes (%)	Eosinophils (%)
D0	4.63±2.42	8.92±13.65	8.66±6.26	30.30±63.41	73.76±230.34	3.52±3.89
D1	4.73±0.17	17.50±667.39	8.94±0.63	30.56±11.59	61.76±195.55	5.08±10.91
D2	3.86±0.48	9.14±12.74	7.32±5.14	25.60±67.14	61.52±231.09	6.98±33.34
D3	3.99±0.56	7.084±5.12	7.80±2.83	26.84±28.65	73.24±197.95	7.68±11.63
D4	4.42±0.24	7.56±6.95	8.64±0.70	30.36±11.43	55.78±651.73	9.08±13.13
D5	4.07±0.37	9.48±25.51	7.78±0.88	26.80±12.25	61.50±158.54	8.96±13.60
D6	5.45±2.36	10.18±10.82	10.50±8.64	35.24±119.38	63.28±421.49	7.64±57.71
D7	3.28±2.55	9.40±12.68	7.92±6.82	21.44±158.34	69.96±42.26	8.16±23.41

Herbal extract II: 0 mg (D0), 10 mg (D1), 20 mg (D2), 40 mg (D3), 80 mg (D4), 100 mg (D5), herbal extract I 100 mg (D6) and Aquaprime (D7).

Table 2. Measurement results of monocytes, lymphocytes, MCV, MCH, MCHC and oocytes from rabbit given herbal extracts of various doses

Treatment	Parameters					
	Monocytes (%)	Lymphocytes (%)	MCV (fL)	MCH (pg)	MCHC (%)	Oocytes* (opg)
D0	2.20±1.37	20.52±170.79	66.64±41.35	18.92±3.49	28.42±2.40	1.80±3.20ab
D1	2.52±2.42	30.64±302.75	64.60±16.44	18.94±0.97	29.36±1.15	-2.60±2.30ab
D2	3.02±4.51	28.48±237.98	70.28±32.39	20.08±1.56	28.60±0.36	-2.20±3.70ab
D3	2.92±0.77	16.14±117.07	67.26±35.06	19.50±2.08	28.98±1.31	-0.60±1.30a
D4	3.30±0.37	21.38±41.28	69.14±83.65	19.66±5.18	28.50±0.31	0±3.50ab
D5	3.42±0.22	26.12±84.64	66.06±19.39	19.18±0.90	29.10±1.18	-0.80±1.20ab
D6	3.16±1.19	25.94±149.06	64.20±21.99	19.34±1.48	30.20±3.11	-1.40±5.30ab
D7	2.64±0.60	19.24±6.36	61.46±1122.05	20.80±18.68	28.54±1.77	1.20±9.20b

Herbal extract II: 0 mg (D0), 10 mg (D1), 20 mg (D2), 40 mg (D3), 80 (D4) mg, 100 mg (D5), herbal extract I 100 mg (D6) and Aquaprime (D7). Oocysts difference *: the number of oocysts after treatment is reduced by the number of oocysts before treatment. Opg: oocytes per gram feces. The number followed by the same letter is not significantly different at HRD 5%.

Antimicrobial resistance (AMR) to effective treatments caused by bacteria, parasites, viruses and fungi over the past several decades has increased. Antimicrobial Resistance (AMR) results in the reduced efficacy of antibacterial, antiparasitic, antiviral and antifungal drugs, making patient care more difficult and expensive. The impact on patients who are very vulnerable is to produce prolonged disease and increased mortality [16]. Herbal extracts are widely studied to control coccidiosis in farm animals. This is due to the suspicion of anticoccidiosis resistance that has been used, namely diclazuril, amprolium + etopabate and salinomycin. Research shows that no field isolate is fully sensitive to selected anticoccidials. All isolates showed decreased sensitivity or resistance. The decreasing effectiveness of anticoccidials will increase the cost of using anticoccidial in the field. So that, a change in anticoccidial prevention and rotation strategy with better efficacy, will prevent further economic losses caused by coccidiosis [17, 18].

Research of herbal anticoccidiosis in the form of herbal extracts, especially garlic, has shown satisfying results which can reduce diarrhea, improve livestock performance and production, and reduce

the number of oocysts. So it can be used as an alternative therapy and prophylactic against coccidiosis [19, 20, 21]. The combination of herbal extracts used in this study was banana stem extract, garlic extract and papaya seed extract. In vitro studies showed that the administration of three extracts separately showed significant results in reducing the number of oocysts [9]. The average oocysts excretion and erythrogram parameters can be seen in **Table 1** and **Table 2**. Red blood cells and normal white blood cells in male rabbits, respectively were $5.3 \pm 0.4x10^6$ /µl; $9.7 \pm 3.3x10^3$ /µl [22]. Hematocrit and normal hemoglobin in male rabbits were 34 ± 2%; 11.2 ± 0.7 g/dL [22]. Normal standards of granulocytes, eosinophils, monocytes, lymphocytes were 30%; 1%; 3.1%; 60%. The normal standard MCV, MCH and MCHC are 65 ± 3 fL; 21 ± 1 pg; 33 ± 1%, respectively [22]. All treatments showed levels of RBC, HGB and HCT less than normal unless the D6 was within the normal range. All treatments of normal WBC levels, except for D1 which showed very high WBC. MCH and MCHC that were close to normal were also found in the D6, whereas for MCV, the treatments that were close to normal were D2 and D7. Granulocytes and eosinophils in all treatments were very high. Whereas monocytes that are close to normal are D2, D3 and D6. Lymphocytes in all treatments are below normal. In oocysts excretion, D1 until D6 showed a decreased oocysts compared to before treatment.

Blood profile can be beneficial for diagnosing and preventing liver coccidiosis in rabbits. The research showed that *E. stiedai* infection in rabbits caused significant changes in the parameters of the profile of red and white blood cells. Coccidiosis can decrease in erythrocytes, hemoglobin, hematocrit, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and also an increase in the average concentration of white blood cell hemoglobin, leukocytes, granulocytes, monocytes, eosinophils, lymphocytes and alkaline phosphatase (ALP) [23, 24, 25].

The results of variance analysis showed that administration of herbal extract II significantly decreased oocysts excretion, but did not show significant differences in hematologic parameters. The HRD test for oocysts excretion did not show different results between treatments (**Table 2**). Herbal extracts consisting of banana stem, garlic and papaya seeds have active substances that can potentially control coccidiosis in rabbits [9]. The most optimal combination of herbal extracts in this study was D6, namely the administration of herbal extract I. where the levels of hematologic parameters were near normal standard and oocysts excretion decreased. So that, the administration of a combination of herbal extract I (D6) 100 mg is likely to be more potent in controlling coccididosis than other treatments. Provision of oral herbal extracts by mixing rabbit feed was more practical to do, but there are fears of ineffective extracts that enter the rabbit's body when compared to being fed. That was because the amount of wasted food due to the habit of rabbits in scavenging food and the content of herbal extracts that taste bitter can reduce the palatability of rabbit. The use of herbal extracts for field rabbits has varied responses. This was probably due to the time of entry of the infection, the endurance of each individual rabbit and different maintenance management so that the immune response of rabbits will also vary.

4. Conclusion

Giving a combination of herbal extracts can reduce oocysts excretion in feces significantly. However, more in-depth research needs to be done about its effects on hematological parameters.

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