Original Article

Efficacy of Salinomycin, Robenidine and Decoquinate Against Infection with *Eimeria* Species Field Isolate in A Densely Populated Broiler Farm in Thailand

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Abstract

Avian coccidiosis is caused by intracellular protozoan parasites in the genus *Eimeria*. It has a significant economic impact on the poultry industry and is highly prevalent in chicken producing countries worldwide. Current control measurements including good animal husbandry, chemoprophylaxis, and vaccination cannot effectively control the disease. It is well understood that parasites repeatedly exposed to the same anticoccidial drug is a major cause of resistance. Therefore, it is critical to monitor parasite response to anticoccidial drugs in different field settings. The aim of this experiment was to investigate responses of a local field isolate of *Eimeria* species to commonly used anticoccidial drugs which were salinomycin, robenidine and decoquinate at different concentrations and their combinations. All groups of chickens receiving in-feed medication showed significant reduction in average lesion scores compared to the infected unmedicated control group (p<0.05). The group of chickens receiving robenidine at 33 ppm showed the highest reduction in lesion score in this investigation. Our findings serve as a key step forward for poultry industries as well as drug suppliers to adjust and forecast anticoccidial use strategies.

Keywords: anticoccidial drug, coccidiosis, decoquinate, *Eimeria,* robenidine, salinomycin

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Introduction

Global poultry consumption has tripled in the past two decades and the world's chicken flock is estimated at nearly 21 billion, producing 68 million tons of eggs and approximately 87 million tons of meat annually (FAO, 2009). Thailand is a leading country that exports chicken products to feed consumers mostly in Japan, EU countries and China (Naranong, 2007). Avian coccidiosis is a major disease that causes economic loss in poultry production worldwide including Thailand. The global economic impact of coccidiosis is uncertain but has been estimated to be over 3 billion US dollar a year owing to production losses combined with costs of prevention and treatment (Williams, 1999). An estimation made by Bera et al. (2010) revealed that commercial broiler sector was a major sufferer due to coccidiosis wherein 95.61% of the total economic loss occurred due to the disease. It is well understood that coccidia oocysts are heavily resistant to harsh environment. The sporulated oocyst efficiently propagates in the bird's gastrointestinal tract, leading to devastation of epithelial cells responsible for feed use. Even though the bird survives, in case of mild infection, the oocysts have already undergone propagation and contaminated the area. Based on this fact, coccidiosis poses a serious threat to a secure production of poultry derived food products. Preventive measures to cope with this threat rely on a few options. Good husbandry plays a major role in limiting oocyst sporulation and recycling in the farm. However, in fact, husbandry alone is inadequate. The main alternative option for coccidiosis control is vaccination. Vaccine is effectively used to protect egg-laying and breeder chickens. However, it is applied rarely within a majority of broiler sector. Another crucial measurement to deal with coccidiosis is using anticoccidial drugs. Salinomycin sodium is an active ingredient classified as monocarboxylic polyether ionophore with known antimicrobial, anticoccidial and anti-cancer effects (Huczynski et al., 2012; Zhou et al., 2013). Robenidine is a feed additive intended for the control of coccidiosis, a debilitating protozoal infection in poultry and rabbit (Joyner et al., 1983; Gerhold et al., 2011). It contains 6.6% of the active substance, the synthetic chemical robenidine hydrochloride. Decoquinate (Deccox®) is a coccidiostat currently authorized as a feed additive intended for the control of coccidiosis caused by *Eimeria* spp. in chickens for fattening (Williams, 1997; Guo et al., 2007). Its active substance is decoquinate produced by chemical synthesis. Salinomycin, robenidine and decoquinate are used routinely in broiler farms in Thailand without monitoring process.

The cost of the drugs per unit is less expensive than vaccination, but the major concern is that repeated use of them creates "reduced sensitivity" situation. In different broiler production areas around the world, frequency of drug used, number of drug available, period of time that drug is used as well as how veterinarians manage the use of drug are not publicly accessible and probably unrecorded or not routinely monitored in many farms. Drug efficacy to coccidia in one area may give some clues of the current situation in the area. Response to the anticoccidial drugs of different parasite isolates is dynamic and hardly predictable. Therefore, routine evaluation of parasites in the area is required. The aim of our study was to evaluate the efficacy of salinomycin, robenidine and decoquinate against infection with *Eimeria* species field isolate in Chonburi province for evidence of resistance to these commonly used anticoccidial drugs. The findings could serve as an idea for poultry industries in the area as well as drug suppliers to adjust their strategies concerning anticoccidial uses.

Materials and Methods

Experimental design: Nine experimental groups were conducted as summarized in Table 1. Group 1 was medicated with 70 part per million (ppm) or milligram of salinomycin per kilogram of feed, hereafter designated as "Sal 70". Group 2 (Sal 70 + Rob 16.5) was treated with a combination of salinomycin at 70 ppm plus robenidine at 16.5 ppm. Group 3 (Sal 70 + Rob 33) was treated with a combination of salinomycin at 70 ppm and an increased amount of robenidine at 33 ppm. Group 4 (Sal 70 + Dec 20) was given a mixture of salinomycin at 70 ppm and decoquinate at 20 ppm. Groups 5 (Rob 16.5) and 6 (Rob 33) were given 16.5 and 33 ppm of robenidine, respectively. Group 7 (Dec 20) was given a single anticoccidial drug of decoquinate at 20 ppm in feed. Group 8 was an infected unmedicated control (IUC) (served as a positive control group). And group 9 was an uninfected unmedicated control (UUC) (served as a negative control group). Level of robenidine used in this study followed the instructions from "opinion of the scientific panel on additives and products or substances in animal feed on a request from the commission on the re-evaluation of coccidiostat cycostat 66 g" by the European Food Safety Authority (2004[a]). Salinomycin use followed the recommendation of the European Food Safety Authority (2004[b]) in "opinion of the scientific panel on additives and products or substances used in animal feed on a request from the commission on the safety and the efficacy of product". Protocol for decoquinate use was conducted according to the "scientific opinion on the modification of authorization of Deccox® (decoquinate) as feed additive for chickens for fattening" published by European Food Safety Authority (2013).

Experimental animals, housing and feed: A total of 135, 1-day-old commercial broiler chickens were obtained from AKE Breeder hatchery (Chonburi Province, Thailand). The chickens were housed in the same environmental conditions until day 9, during which period they received feed without anticoccidial product. At day 10, a group of 5 chickens were allocated randomly to each experimental group in a triplicate manner resulting in a total of 15 chickens per experimental group. Starting from day 10 until the end of the experiment at day 21, all groups of chickens were medicated with anticoccidial drugs as described in Table 1. The chickens were allowed to freely access water and feed *ad libitum*. Weight gain and feed intake as a group and individually were monitored at days 1, 10, 15 and at the end of experiment (day 21) as well as mortality rate. The use of animals was reviewed and approved by Chulalongkorn University ethical committee (Approval No. 13310067). The care and use of experimental animals complied with animal welfare laws, guidelines and policies of the National Research Council of Thailand.

Coccidia oocyst inocula: Coccidia oocysts were collected in 2012 from a pool of infected broiler chicken's guts and fecal contents in Chonburi province, approximately 80 km away to the east of Bangkok where chicken farms are densely populated. The oocysts were scraped from caecal epithelial as well as caecal contents. Subsequently, the oocysts and other contents were passed through a 1 mm pore size sieve to get rid of unwanted materials. The oocysts then underwent sporulation process in 2.5% (w/v) potassium dichromate at room temperature and periodical aeration. The sporulated oocysts were then propagated in 5 of 15-day-old commercial broiler chickens, and their guts and fecal contents were harvested at day 6 after inoculation and underwent the same sporulation process as described above. The species of the coccidia oocysts were confirmed based upon the location and appearance of the lesion mainly at the cecum. Number of sporulated oocyst inoculum was calculated based on the number of unsporulated and sporulated oocysts observed under a microscope and counted for the number of total oocysts using McMaster counting chamber. The parasite inocula were washed 3 times with distilled water to remove potassium dichromate solution. At day 15 of the experiment, all groups except the UUC group were orally inoculated with 100,000 sporulated oocysts.

Table 1 Experimental groups and descriptions

Group	Description
1 (Sal 70)	salinomycin 70 ppm
2 (Sal 70 + Rob 16.5)	salinomycin 70 ppm + robenidine 16.5 ppm
3 (Sal 70 + Rob 33)	salinomycin 70 ppm + robenidine 33 ppm
4 (Sal 70 + Dec 20)	salinomycin 70 ppm + decoquinate 20 ppm
5 (Rob 16.5)	robenidine 16.5 ppm
6 (Rob 33)	robenidine 33 ppm
7 (Dec 20)	decoquinate 20 ppm
8 (IUC)	Infected, Unmedicated Control
9 (UUC)	Uninfected, Unmedicated Control

ppm = part per million or milligram per kilogram

Table 2 Lesion scores of experimental chickens medicated with different formulation of anticoccidial drugs

Treatment group	Individual lesion score	% Reduction	Classified as
1 (Sal 70)	0/0/0/1/1/1/1/1/1/0/1/0/0/1/3 [a]	69.58	S
2 (Sal 70 + Rob 16.5)	1/1/1/0/1/0/0/1/0/0/1/0/0/1/1 [a]	77.92	S
3 (Sal 70 + Rob 33)	0/0/1/1/0/0/1/1/0/1/1/0/0/1/0 [a]	85.83	S
4 (Sal 70 + Dec 20)	0/0/0/0/0/0/0/0/0/0/0/0/0/0/0 [b]	α	
5 (Rob 16.5)	0/0/0/0/0/0/0/0/1/0/0/0/0/0/0 [b]	97.08	S
6 (Rob 33)	0/0/0/0/0/0/0/0/0/0/0/0/0/0/0 [b]	α	
7 (Dec 20)	2/2/1/0/0/0/0/1/0/0/0/0/0/0/0 [a]	83.33	S
8 (IUC)	2/2/3/1/2/2/2/3/2/3/2/3/2/3/4 [ab]		
9 (UUC)	0/0/0/0/0/0/0/0/0/0/0/0/0/0/0 [b]		

Different superscripts within a group differ significantly ($p < 0.05$).
S = fully susceptible

Evaluation of lesion score: At day 21 of the experiment or day 6 after inoculation, the chickens in all groups were humanely euthanized by the cervical dislocation method and gut lesions were recorded. Lesion scoring of each experimental group was judged according to Johnson and Reed (1970). Evaluation of reduction in average lesion score of each medicated group was compared to the IUC group using the following formula: 100% - (mean lesion score of medicated group over mean lesion score of IUC) x 100%. The parasite response to each anticoccidial drug concentrations (single drug and combinations) was classified into 3 categories as previously explained by McDougald et al. (1986). A reduction in percentage in the medicated groups compared with the IUC group of 0-30, 31-49 and 50% or more was considered coccidial resistant (R), partially resistant (PR) and fully susceptible (S), respectively. Gross lesions produced by *E. acervulina* and *E. necatrix* in this study were excluded from the analysis because there were no clear differences in appearance between the IUC and medicated groups. Statistical analysis of lesion score, body weight gain and feed conversion ratio (FCR) was made with the One-Way ANOVA and Duncan Multiple Range tests for group comparison implemented in SPSS software. The lesion scores were analyzed by the Kruskal-Wallis and Mann-Whitney tests. A p value of ≤ 0.05 was considered as statistically significant.

Results

Lesion scores: A group of chickens receiving a high dose of parasite inocula at 100,000 oocysts without medication (IUC group) showed severe hemorrhage in the ceca with lesion scores mostly between 2 to 3 (min = 1, max = 4, average lesion score = 2.4) as shown in Figure 1 and Table 2. No coccidia lesion was observed in the UUC negative control group. The lesion scores in all medicated groups receiving different anticoccidial drugs were significantly reduced compared to those of the IUC group ($p < 0.05$). Reduction in percentage in the medicated groups compared with the IUC group was over 50% in Sal 70, Sal 70 + Rob 16.5, Sal 70 + Rob 33, Rob 16.5, and Dec 20 which is considered as fully susceptible. The group of chickens receiving salinomycin at 70 ppm plus decoquinate at 20 ppm in feed showed no gross lesion specific to coccidia infection, suggesting that the lesions in each chicken were reduced in a direction heading to 100%. The same findings were observed in group 6 (Rob 33). Surprisingly, the single regimen of salinomycin at 70 ppm resulted in insignificant difference in suppressing the coccidia lesion as compared with the combination of salinomycin at 70 ppm plus robenidine either at 16.5 ppm or 33 ppm. A combination of salinomycin at 70 ppm and decoquinate at 20 ppm in feed showed a better outcome equivalent to the UUC group compared with the combination of salinomycin with robenidine in this present study. On the other hand, the medication with decoquinate alone at 20 ppm was not as potent as the combination of salinomycin at 70 ppm plus decoquinate at 20 ppm. Interestingly, the single regimen of robenidine either at 16.5 or 33 ppm gave a significantly different outcome in reducing the lesion to the same level as the UUC group compared to the combination of robenidine and salinomycin at 70 ppm. Consequently, the efficacy of salinomycin, robenidine and decoquinate at the concentrations mentioned herein was classified as highly effective in reducing lesion produced by an overwhelming infection with 100,000 sporulated coccidia oocysts.

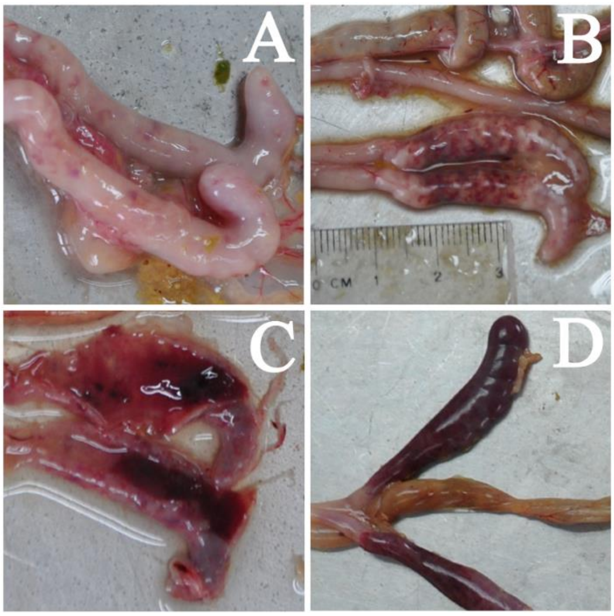

Figure 1 Representative images of lesions observed in this experiment. A to D are categorized as lesion scores +1 to +4, respectively, according to degree of infection as explained by Johnson and Reed (1970).

Table 3 Performance parameters of chickens

Treatment group	BWG (g/chicken) Day 0-15	BWG (g/chicken) Day 0-21	Feed intake (g/chicken) Day 0-21	Feed conversion (kg kg^{-1}) Day 0-21
1 (Sal 70)	280 ± 2.6^{a}	475 ± 9.0^{a}	746.67 ± 45^{ab}	1.57^{a}
2 (Sal 70 + Rob 16.5)	296 ± 2.1^{ab}	513 ± 6.4^{a}	800.00 ± 50^{a}	1.56^{a}
3 (Sal 70 + Rob 33)	287 ± 1.7^{a}	475 ± 8.5^{a}	780.00 ± 80^{a}	1.64^{a}
4 (Sal 70 + Dec 20)	284 ± 1.5^{a}	496 ± 7.6^{a}	793.33 ± 40^{a}	1.56^{a}
5 (Rob 16.5)	302 ± 1.6^{ab}	516 ± 6.3^{a}	793.33 ± 62^{a}	1.54^{a}
6 (Rob 33)	291 ± 9.4^{a}	492 ± 4.9^{a}	780.00 ± 55^{a}	1.58^{a}
7 (Dec 20)	285 ± 1.5^{a}	469 ± 6.4^{a}	800.00 ± 50^{a}	1.70^{ab}
8 (IUC)	294 ± 6.8^{ab}	437 ± 8.0^{a}	766.67 ± 90^{ab}	1.75^{ab}
9 (UUC)	283 ± 1.5^{a}	487 ± 4.1^{a}	800.00 ± 50^{a}	1.64^{a}

Means ± SD within column with different superscripts differs significantly ($p < 0.05$).
BWG = body weight gain

Body weight gain and feed conversion: The weight gain and feed intake as a group and individually were recorded at days 1, 10, 15 and 21. At day one of the study, the average body weight of the chickens in our study was 42.60 ± 2.2 g and increased to 200.59 ± 12.05 g at day 10. The average feed intake per chicken until day 10 was 297 ± 15.00 g (data not presented in the table). The chickens were randomly divided into 9 groups of 15 as mentioned earlier. The body weight gain at days 0-15 (before oocyst inoculation) and days 0-21 (after inoculation) were assessed in all groups as shown in Table 3. The average feed intake and feed conversion were analyzed at the end of the experiment. At days 0-15 of the experiment, when the chickens received different regimens of medication but were not challenged with the oocysts, the body weight gain in the UUC group was at the same level as most treatment groups. Group 2, group 5 and IUC showed statistically higher average body weight gain ($p < 0.05$) during the first 15 days of the experiment or 5 days of receiving the combination of salinomycin at 70 ppm plus robenidine 16.5 ppm, robenidine alone at 16.5 ppm and without medication, respectively. No statistically different in body weight gain was observed in the UUC group and the other treatment groups from days 0 to 21, when the chickens were allowed to take the different medications for 11 days and challenged with coccidia oocysts for 6 days. The average feed intake in the UUC group showed a similar level to most medicated groups. In contrast, the IUC group and group 1 of chickens receiving 70 ppm of salinomycin showed a statistically different reduction in feed intake compared to those of the UUC and other medicated groups. The feed conversion ratio observed in IUC and group 7 (Dec 20) from days 0 to 21 was statistically increased compared to those of UUC and the other medicated groups, suggesting a less efficient feed conversion.

Discussion

Efficacy study of anticoccidial drugs is crucial in terms of monitoring the response of the parasites against those drugs commonly launched in the area. Dose recommendation for prevention of coccidiosis in poultry according to Gerhold (2014) is 44-66 ppm for salinomycin, 33 ppm for robenidine and 30 ppm for decoquinate. In the present study, we evaluated the efficacy of salinomycin, robenidine and decoquinate against coccidia in a densely populated area of chicken farms in Thailand. We observed the outcome of a fix dose of salinomycin at 70 ppm. For robenidine, we assessed both at the recommendation dose and half of its concentration as well as in combination with 70 ppm of salinomycin. Decoquinate was observed either at 20 ppm alone or in combination with 70 ppm of salinomycin. Groups 4 (Sal 70 + Dec 20) and 6 (Rob 33) showed complete inhibition of lesion from infection with a relatively high dose of coccidia oocysts similar to that observed in China by Guo et al. (2007). An opposite outcome in the US was reported by Chapman and Hackerin in 1994 that intensive use of salinomycin, robenidine and decoquinate resulted in resistance of salinomycin or showed reduced sensitivity to robenidine and decoquinate. Another unsatisfactory outcome of decoquinate was reported in the United Kingdom (Williams, 2006) suggesting that repeated use of decoquinate should be aware of resistance. In the present study, both salinomycin alone at 70 ppm and the mixture of 70 ppm of salinomycin and 16.5 or 33 ppm of robenidine showed a significant reduction in lesion scores but not 100% complete. It is important to note that the combinations of Sal 70 plus Rob 16.5 and Sal 70 plus Rob 33 gave % reduction of lesion score of 77.92 and 85.83 similar to Dec 20 (83.33%). This might be due to a slight reduction in feed intake (as well as medication within the feed) in these groups. Regarding the responses of coccidia parasites against the drugs, it should be noted that parasites collected from different localities or countries as well as their previous history of drug exposure do matter. The recent findings of Gerhold et al. (2011) in North America, for example, demonstrated that excellent to good efficacy was found in decoquinate (30 ppm), robenidine (33 ppm) while moderate to high resistance was found using salinomycin (60 ppm). Additionally, salinomycin was found partially resistant in the Middle East (Arabkhazaeli et al., 2013). Taken together, the overall response of the parasites against salinomycin, robenidine and decoquinate in this study was considered as highly susceptible for the time being. However, further evaluation of the parasites nationwide as well as other anticoccidial drugs will give a bigger picture of the effectiveness of drugs in the region. Our findings serve as a record of drug efficacy and could be a guidance for deploying salinomycin, robenidine and decoquinate or bringing in a new product into the area. Moreover, further investigation will allow generation of comparable data to compare situations in different regions or countries to consolidate results at national, regional and international level as suggested by Franklin et al. (2001). Moreover, our parasite collection could be a reference parasite isolate that has already been evaluated for their responses to anticoccidial drugs mentioned herein.

In this study, the chickens receiving 70 ppm of salinomycin in feed showed a statistically different reduction in feed intake compared to those of the UUC and other medicated groups, which is in agreement with the findings observed by Czerwinski et al. (2012). This observation may be explained by the salinomycin supplementation suppressing the microbial activity and altering the microbial community structure mainly in ileum. The body weight gain at days 0 to 15 in all medicated groups (except Sal 70 + Rob 16.5) improved significantly ($p<0.05$). However, the chickens at days 0 to 21 that were fed on those supplements failed to show such improvement. The feed intake in the IUC and Sal 70 groups reduced significantly ($p < 0.05$). The feed conversion ratio (FCR) showed significant improvement in the infected chickens (except Dec 20), which indicates that feed supplemented with Sal 70, Sal 70 + Rob 16.5, Sal 70 + Rob 33, Sal 70 + Dec 20, Rob 16.5 and Rob 33 could reduce adverse effects after challenge with coccidia oocysts in 10-day-old chicks. Our findings were consistent with those of Bozkurt et al. (2014) that the feed conversion in chickens receiving salinomycin supplement was improved after coccidia infection. Consequently, our data indicate that the use of these anticoccidial supplements in broiler production can lessen lesions made by coccidiosis and improve feed conversion. Rational use of anticoccidial drugs and vaccines could prevent further drug resistance.

In conclusion, the inoculation of 100,000 sporulated oocysts in the IUC group created lesion scores mostly 2-3 in our present study. All medicated groups showed statistically significant result in preventing coccidia lesions made by the relatively high dose of parasite inoculum compared to the IUC group. The combination of Sal 70 plus Dec 20 and Rob 33 gave the most promising percentage of lesion reduction score in the present study. At the end of the experiment, the body weight gain in all groups showed no difference. The feed intake in IUC and group 1 (Sal 70) was statistically decreased compared to that of the UUC and other treatment groups. The feed conversion observed in IUC and group 7 (Dec 20) was significantly less efficient than that of the UUC and other medicated groups.

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บทคัดย่อ

ประสิทธิภาพของยา Salinomycin Robenidine และ Decoquinate ต่อการต้านเชื้อบิดที่แยกได้จากฟาร์มไก่เนื้อในประเทศไทย

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โรคบิดในไก่มีสาเหตุมาจากเชื้อโปรโตซัวในสกุลไอเมอเรีย เป็นโรคที่มีความสำคัญทางเศรษฐกิจ และพบได้ทั่วโลก มาตรการควบคุมโรคต่างๆ เช่นการบริหารจัดการฟาร์ม การใช้ยา และวัคซีน ยังไม่สามารถควบคุมโรคนี้ได้อย่างถาวร ในบางกรณีการใช้ยาชนิดเดิมซ้ำๆอาจส่งผลให้เกิดปัญหาเชื้อดื้อยาได้ ดังนั้นการวางแผนการใช้ยา หรือเปลี่ยนชนิดของยา หรือการทดสอบประสิทธิภาพยาอย่างสม่ำเสมอจึงมีความสำคัญเพื่อหลีกเลี่ยงปัญหาดังกล่าว วัตถุประสงค์ของการทดลองนี้เพื่อศึกษาประสิทธิภาพยาต้านบิด 3 ชนิดคือ salinomycin robenidine และ decoquinate ซึ่งเป็นยาที่ฟาร์มนำมาใช้เป็นประจำโดยการผสมลงไปในอาหารไก่เนื้อ จากการศึกษาในครั้งนี้พบว่า ไก่กลุ่มที่ได้รับยาทุกกลุ่มแสดงรอยโรคลดลงอย่างมีนัยยะสำคัญทางสถิติ โดยกลุ่มที่ได้รับยาในขนาด 33 ส่วนในล้านส่วน (ppm) ให้ผลการยับยั้งรอยโรคดีที่สุดในกลุ่มการทดลองครั้งนี้ ผลการศึกษาครั้งนี้น่าจะเป็นประโยชน์ต่อผู้ที่ทำงานเกี่ยวข้องกับการผลิตไก่เนื้อ ในการวางแผน หรือปรับขนาดยาให้เหมาะสมต่อไป

คำสำคัญ: ยาต้านบิด โรคบิดไก่ decoquinate เชื้อบิด (*Eimeria*) robenidine salinomycin

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