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Anticoccidial Efficacy of Coccimuel-S composed with Diclazuril on Experimental and Field Coccidiosis in Broiler Chickens

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Abstract : The efficacy of water soluble formulation of diclazuril (Coccimuel-S 0.5%) was tested against *Eimeria* spp. infection broiler chickens. The experiment was performed both experimentally infection and in the field test. Coccimuel-S composed with diclazuril induced a marked inhibitory effect on the different stages of *Eimeria* life cycle in experimentally infected broiler chickens treated with the drug. The tested dosage levels of Coccimuel-S (0.5 ml/L, equivalent to diclazuril 2.5 ppm) in drinking water showed the significant effect compared with the control group in controlling coccidial infection and reducing the total oocyst numbers, lesion and fecal scores ($p < 0.001$). In addition, testing of Coccimuel-S (0.25 and 0.5 ml/L) in naturally infected poultry farms (1,200 broiler chickens), showed the significant anticoccidial effect compared to control ($p < 0.001$). In conclusion, addition of Coccimuel-S at the dose of 0.25 and 0.5 ml/L in the drinking water, induced efficacious effect for the treatment of coccidiosis in naturally coccidia infected broiler chickens.

Key words **:** coccidiosis, *Eimeria* spp., diclazuril, broiler, Coccimuel-S.

Introduction

In the poultry industry, coccidiosis is one of most economically important diseases, which is caused by seven species of intracellular protozoan parasites of the genus *Eimeria*: *Eimeria tenella* (*E. tenella*), *Eimeria acervulina* (*E. acervulina*), *Eimeria maxima* (*E. maxima*), *Eimeria necatrix* (*E. necatrix*), *Eimeria brunette* (*E. brunette*), *Eimeria praecox* (*E. praecox*) and *Eimeria mitis* (*E. mitis*) (5,20). In the world, the annual economic losses due to poultry coccidiosis were estimated at about $800 million (19). According to the epidemiological study by Lee *et al*. (10), the prevalence of *Eimeria* spp. infection in Korea was 78.7% of 356 randomly selected broiler or layer farms in Korea, and *E. acervulina* was the most prevalent species.

As *Eimeria* spp. invades cells of the intestinal epithelium and causes destruction of the infected cells, the infected chickens were reduced feed conversion, body weight gain, egg production, and an increase of morbidity and mortality (11-13).

In spite of anticoccidials addition in poultry feed, there are many reports of clinical and subclinical coccidiosis occurring as a result of insufficient effective medication with conventional anticoccidials (2). Thus, effective therapeutic agents from new classes of chemical compounds are required as supplements to current treatments or as alternatives to them. Such new substances must have a high efficacy against all developmental stages of *Eimeria* species in the infected poultry and they did not simultaneously interfere with the immune response of the host during and after treatment of coccidial infections (6). With the consideration of this respect, Coccimuel-S (Dae Han New Pharm Company, Seoul, Korea) is a suitable anticoccidial product composed with diclazuril which is a chemical substance synthesized from the benzeneacetonitrile class of compounds, developed successfully as anticoccidial agent for poultry (4,17).

Previous studies demonstrated activity of diclazuril against asexual and sexual reproduction cycles of *E. tenella*, asexual later schizonts of *E. acervulina* and against sexual, zygote for *E. maxima* and gametocytes for *E. brunette* (1,6). Recently, a new formulation of diclazuril for drinking water administration was introduced in some countries (6).

The present study is carried out to evaluate the therapeutic capacity of the water-soluble formulation of Coccimuel-S composed with diclazuril both experimentally and in the field.

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Materials and Methods

Drugs and Chickens

Coccimuel-S (diclazuril, 0.5%) was obtained from Dae Han New Pharm Company. It was administered at various concentrations in drinking water.

A total of fifty 2-day-old Ross breed broiler chicks, were obtained from a private poultry farm (Chinju, Korea) and used in the trial. Chickens were allocated based on a random block design into six groups per 30 birds at 6 rice hull-littered floor pens. Experimental diets (Cargill Agri Purina Korea Inc., Seoul) and tap water were provided for consumption *ad libitum*. Continuous lighting was provided throughout the experimental period and the room temperature was at 32 ± 1°C.

All procedures were approved by the Animal Care and Welfare Committee of Gyeonsang National University (GNU-LA-2011-103).

Coccidial infections

The challenge oocysts were isolated from the intestines of naturally infected chickens. The oocyst was separated by using sieving and sedimentation techniques (16). The collected oocysts were allowed to sporulate at room temperature in 2.5% potassium dichromate solution. The sporulated oocysts were cleared and counted per 1.0 ml of the solution using the McMaster technique (16). The different species of *Eimeria* present in the used inoculums were identified according to difference in size from each size group. They could be identified as *E. acervulina* (36.5%), *E. maxima* (17.5%) and *E. tenella* (46.0%). The stock solution contained the sporulated oocysts were orally administered 0.5 ml (oocysts 220,000/ml) to 20-day-old chickens for the induction of experimental infection.

Testing the anticoccidial efficacy of Coccimuel-S

A total of fifty broiler chickens were divided into six equal groups (Control, group I, group II and group III) each consisting of ten broiler chickens. Control was infected and non-treated. From on fifth day after experimental infection, group I, II and III were treated with Coccimuel-S 0.5, 1.0 and 2.0 ml/L in drinking water during two days.

Field testing the curative capacityof Coccimuel-S

Three broiler farms (Chinju, Korea) suffering from severe intestinal coccidiosis and high morbidity, was selected. The flock of each broiler farm was allocated into four equal groups and one group was consisted with a hundred 20-day-old broiler chickens.

Control group was not treated with Coccimuel-S, and group I, II and III were treated with 0.25, 0.5 and 1.0 ml/L in drinking water, respectively. All treatments were administered for 24 h per day for two successive days.

Evaluation of the efficacy for Coccimuel-S

Fresh fecal dropping were collected on 1st and 2nd day after administration of Coccimuel-S. The mean number of oocysts per gram feces for each group was counted by the Mc-Master technique (16).

The daily number of dead chickens as a result of coccidial infection and mean body weight were recorded and calculated for each group. For determination of lesion score, five chickens from each group were randomly picked up for post-mortem examination at day 1 and 2 after medication as well as all chickens that died during the experiment. Lesion scores were determined by macroscopic examination of the small intestine (upper, middle and low) and cecum of each chicken (9). Lesion score was 0 when no evident lesions were detected while a score of 4 referred to the severely affected chicken.

A fecal score was recorded for each group of broiler chickens. Fecal score was calculated by observing the feces voided each day; a rating of 1 indicating normal feces, through 4, denoting the presence of severe diarrhea and/or a profuse amount of blood (14).

The efficacy of Coccimuel-S was determined by comparing the mean number of oocysts shed, lesion and fecal scores as well as daily number of dead chickens in treated and non-treated control group.

Statistical analysis

The data were analyzed by a one-way analysis of variance (ANOVA) (15), followed by the results expressed as mean ± standard deviation (SD). The means were compared for significance by Student's *t*-test at $p < 0.05$.

Results

The efficacy of Coccimuel-S in experimentally infected broilers

Although the body weight of broiler chickens in groups treated with Coccimurel-S were increased dose-dependent higher than that of control group, there was no significant difference (Table 1). Similarly, all groups treated with Coccimuel-S (0.5, 1.0 and 2.0 ml/L) successfully reduced the shedding of oocysts after the appearance of the blood in feces of the experimentally infected broiler chickens as given in Table 2. The reduction of fecal oocyst in all treated-groups was significantly different compared with that in the non-treated group ($p < 0.001$). All concentrations tested appear to be very efficacious in treating symptoms associated with experimental infection with mixed *Eimeria* species (Table 3). At the end of administration of Coccimuel-S, lesion scores of intestine in groups treated with drug were significantly decreased compared with that in control group as given in Table 3 ($p < 0.001$).

Field testing the curative capacity of Coccimuel-S

The results for the number of death and fecal score in broiler chickens naturally infected with with *Eimeria* spp. were shown in Table 4. Although all of groups treated with Coccimuel-S in three farms was decreased depending on time the number of death in broiler chickens infected with *Eimeria* spp., the number of death in control group of all farms was

Table 1. The change of body weight in broiler chickens challenged with *Eimeria* spp. after administration of Coccimuel-S via drinking water

Group	Treatment	Body weight (g)		
		0 day [a]	1st day	2nd day
Control	Infected non-treated	732.7 ± 13.2	763.4 ± 20.1	806.5 ± 18.3
Group I	Coccimuel-S 0.5 ml/L drinking water	730.4 ± 12.5	766.3 ± 21.5	815.1 ± 23.2
Group II	Coccimuel-S 1.0 ml/L drinking water	733.3 ± 11.4	764.5 ± 18.4	817.4 ± 20.1
Group III	Coccimuel-S 2.0 ml/L drinking water	728.8 ± 13.3	768.2 ± 19.7	820.1 ± 19.6

[a]day after administration of Coccimuel-S.

Table 2. The number of fecal oocyst in broiler chickens challenged with *Eimeria* spp. after administration of Coccimuel-S via drinking water

Group	Treatment	Number of total oocyst ($\times 10^3$/g)		
		0 day[a]	1st day	2nd day
Control	Infected non-treated	36.2 ± 2.95	35.4 ± 2.17	34.9 ± 2.48
Group I	Coccimuel-S 0.5 ml/L drinking water	37.0 ± 3.13	15.6 ± 1.25*	1.2 ± 0.10*
Group II	Coccimuel-S 1.0 ml/L drinking water	35.9 ± 2.87	12.4 ± 0.79*	1.0 ± 0.08*
Group III	Coccimuel-S 2.0 ml/L drinking water	38.3 ± 3.26	7.8 ± 0.24*	0.8 ± 0.06*

[a]day after administration of Coccimuel-S.
*$p < 0.001$, significantly difference compared to control.

Table 3. The lesion score analysis of intestine for broiler chickens challenged with *Eimeria* spp. after administration of Coccimuel-S for 2 days via drinking water

Group[a]	Small intestine			Cecum	Average
	Upper	Middle	Low		
Control	2.68 ± 0.29	2.80 ± 0.57	2.28 ± 0.26	3.20 ± 0.45	2.74 ± 0.38
Group I	0.50 ± 0.31	0.88 ± 0.38	0.22 ± 0.22	0.36 ± 0.21	0.49 ± 0.28*
Group II	0.36 ± 0.42	0.68 ± 0.29	0.18 ± 0.19	0.24 ± 0.35	0.37 ± 0.22*
Group III	0.20 ± 0.27	0.40 ± 0.42	0.22 ± 0.20	0.18 ± 0.22	0.25 ± 0.10*

[a]Control, infected non-treated; Group I, Coccimuel-S 0.5 ml/L drinking water, Group II, Coccimuel-S 1.0 ml/L drinking water, Group III, Coccimuel-S 2.0 ml/L drinking water.
*$p < 0.001$, significantly difference compared to control.

Table 4. The number of death and fecal score in broiler chickens naturally infected with *Eimeria* spp. after administration of Coccimuel-S via drinking water

Farm	Group[a]	Number of death			Fecal score		
		0 day[b]	1 day	2 day	0 day[b]	1 day	2 day
Farm 1	Control	8	7	8	3.8 ± 0.32	3.7 ± 0.21	3.9 ± 0.33
	Group I	9	5	3	3.9 ± 0.21	2.6 ± 0.22	1.5 ± 0.09*
	Group II	8	3	0	3.7 ± 0.34	1.7 ± 0.14	0.8 ± 0.10*
Farm 2	Control	11	7	8	4.1 ± 0.34	3.9 ± 0.29	4.0 ± 0.33
	Group I	8	6	4	3.8 ± 0.29	2.5 ± 0.18	1.9 ± 0.11*
	Group II	9	2	1	4.0 ± 0.31	1.9 ± 0.13	0.9 ± 0.08*
Farm 3	Control	10	9	7	4.2 ± 0.27	4.3 ± 0.32	4.2 ± 0.28
	Group I	7	6	3	3.7 ± 0.32	2.9 ± 0.19	2.0 ± 0.14*
	Group II	11	3	2	4.2 ± 0.26	2.1 ± 0.17	1.2 ± 0.01*

[a]Control, non-treated; Group I, Coccimuel-S 0.25 ml/L drinking water; Group II, Coccimuel-S 0.50 ml/L drinking water.
[b]day after treatment of drug.
*$p < 0.001$, significantly difference compared with control group.

Table 5. The lesion score analysis of intestine for broiler chickens naturally infected with *Eimeria* spp. after administration of Coccimuel-S for 2 days via drinking water

Farm	Group[a]	Small intestine			Cecum	Average[b]
		Upper	Middle	Low		
Farm 1	Control	2.73 ± 0.34	2.85 ± 0.59	2.34 ± 0.28	3.32 ± 0.58	2.81 ± 0.41
	Group I	0.72 ± 0.31	0.96 ± 0.35	0.45 ± 0.26	0.57 ± 0.32	0.67 ± 0.29*
	Group II	0.50 ± 0.43	0.81 ± 0.33	0.24 ± 0.16	0.40 ± 0.29	0.49 ± 0.25*
Farm 2	Control	3.12 ± 0.42	3.34 ± 0.66	2.52 ± 0.27	3.41 ± 0.48	2.98 ± 0.45
	Group I	0.61 ± 0.29	0.93 ± 0.41	0.51 ± 0.26	0.40 ± 0.34	0.61 ± 0.32*
	Group II	0.56 ± 0.38	0.85 ± 0.33	0.28 ± 0.21	0.32 ± 0.36	0.51 ± 0.26*
Farm 3	Control	2.89 ± 0.27	2.95 ± 0.58	2.43 ± 0.22	3.35 ± 0.43	2.91 ± 0.46
	Group I	0.66 ± 0.34	1.05 ± 0.41	0.34 ± 0.24	0.47 ± 0.51	0.63 ± 0.37*
	Group II	0.51 ± 0.38	0.83 ± 0.37	0.25 ± 0.17	0.40 ± 0.31	0.50 ± 0.24*

[a]Control, non-treated; Group I, Coccimuel-S 0.25 ml/L drinking water; Group II, Coccimuel-S 0.50 ml/L drinking water.
[b]Mean of lesion scores in the same row.
*$p < 0.001$, significantly difference compared with control group.

not much variation compared with that at day before treatment of drug. In addition, Coccimuel-S has the ability to reduce the mortality percentage resulting from coccidial infection. Also, fecal score in all treated-groups was significantly reduced compared with each control group ($p < 0.001$).

The results of intestinal lesion score analysis for broiler chickens naturally infected with *Eimeria* spp. were shown in Table 5.

The lesion score of intestine in all groups treated with Coccimuel-S during 2 days was significantly decreased compared with the control group ($p < 0.001$), but there was not shown significant difference between group I and group II in all farms.

Discussion

In the study of broiler chickens experimentally infection with the mixed *Eimeria* spp., the addition of Coccimuel-S to the drinking water of broiler chickens effectively reduced the number of fecal oocyst of *Eimeria* spp. and lesion score of small intestine and cecum. The results of this study agree with those obtained previously for inclusion of diclazuril in feed and drinking water of broiler (4,6,17).

In the field trial test, addition of Coccimuel-S in drinking water was found to be highly efficacious in controlling coccidiosis as shown by mortality prevention, reduction in the total of oocyst shed, the lesion and fecal scores. The results indicated that Coccimuel-S induced an effective activity in disrupting the cycle of *Eimeria* development within broiler chickens administered with drug especially in early infection. As Coccimuel-S significantly reduced the total oocyst number ($p < 0.001$), lesion score ($p < 0.001$) and increase the survival percentage in comparison with non-treated chickens, all tested dosage level of in drinking water (0.25, 0.5 and 1.0 ml/L) showed the same effect for the control of coccidial infection in naturally infected broiler chickens.

Conway *et al*. (3) reported that the use of 1 ppm diclazuril in feed during 42 days was highly efficacious against a mixed inoculum of *Eimeria* spp. in comparison with nicarbazin, the combination of narasin and nicarbazin, and zoalene in starter diets and salinomycin, monensin, and lasalocid in grower diets. According to the previous researches (1,18), diclazuril breaks down all intracellular developmental stages of the reproduction cycles of *Eimeria* spp.

The effect of Coccimuel-S medicated for 2 days on treating naturally infected broilers (1,200 chickens) was also proved in this study. This result showed that addition of Coccimuel-S (0.25 ml/L) in drinking water of birds naturally infected with coccidia induce the same effect of 2.5 ppm toltrazuril in drinking water for the treatment of coccidial infection indicated by decreased total oocyst number, the fecal and lesion scores of treated groups (7,8).

The suitability of Coccimuel-S composed with diclazuril for administration via drinking water and its good efficacy after only 2 days of treatment showed that the product is highly appropriate for use in therapy and intermittent treatment of *Eimeria* spp. infected broiler chickens.

In conclusion, Coccimuel-S, diclazuril water-soluble formulation, is very efficacious and more effective than feed additive form in controling symptoms of poultry coccidiosis. In addition, Coccimuel-S is suitable at the concentration of 0.25-0.5 ml/L drinking water (equivalent to 1.25-2.5 ppm diclazuril) as 2-day medication for the treatment of coccidiosis in broiler chickens.

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References

1. Brander GC, Pugh DM, Bywater RJ, Jenkins WL. Vet-

erinary applied pharmacology and therapeutic. 5th ed. London, Bailliere Tindall. 1991: 552-554.
2. Chapman DH. Control of E. tenella, partly resistant to monensin, by including toltrazuril discontinuously in the drinking water of chickens. J Comp Path 1987; 97: 21-27.
3. Conway DP, Mathis GF, Johnson J, Schwartz M, Baldwin C. Efficacy of diclazuril in comparison with chemical and ionophorous anticoccidials against Eimeria spp. in broiler chickens in floor pens. Poult Sci 2001; 80: 426-430.
4. Conway DP, Mathis GF, Johnson J, Schwartz M, Baldwin C. The use of diclazuril in extended withdrawal anticoccidial programs: 1. Efficacy against Eimeria spp. in broiler chickens in floor pens. Poult Sci 2002; 81: 349-352.
5. Costa CA, Gomes RF, Melo MN, Ribeiro MF. Eimeria parasites of domestic fowl: genetic relationships of different isolates estimated from random amplified polymorphic DNA. Parasitol Res 2001; 87: 459-466.
6. El-Banna HA, El-Bahy MM, El-Zorba1 HY, El-Hady M. Anticoccidial efficacy of drinking water soluble diclazuril on experimental and field coccidiosis in broiler chickens. J Vet Med A 2005; 52: 287-291.
7. Haberkorn A, Stoltefuss J. Studies on the activity spectrum of toltrazuril, a new anticoccidial agent. Vet Med Rev 1987; 1: 22-32.
8. Harder A, Haberkorn A. Possible mode of action of toltrazuril: studies on two Eimeria species and mammalian and Ascaris suum enzymes. Parasitol Res 1989; 6:8-12.
9. Johnson J, Reid WM. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Exp Parasitol 1970; 28: 30-36.
10. Lee BH, Kim WH, Jeong J, Yoo J, Kwon YK, Jung BY, Kwon JH, Lillehoj HS, Min W. Prevalence and cross-immunity of Eimeria species on Korean chicken farms. J Vet Med Sci 2010; 72: 459-466.
11. McDonald V, Shirley MW. Past and future: vaccination against Eimeria. Parasitology 2009; 136: 1477-1489.
12. Min W, Dalloul RA, Lillehoj HS. Application of biotechnological tools for coccidia vaccine development. J Vet Sci 2004; 5: 279-288.
13. Morris GM, Gasser RB. Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in Eimeria. Biotechnol Adv 2006; 24: 590-603.
14. Park ES, Jo S, Seong JK, Nam TC, Yang IS, Choi MC, Yoon YS. Effect of acupuncture in the treatment of young pigs with induced Escherichia coli diarrhea. J Vet Sci 2003; 4: 125-128.
15. SAS Institute, SAS/STAT User's Guide. Version 6, 4th ed. Cary; SAS Institute Inc. 1999.
16. Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. 6th ed. London; Bailliere and Tindall. 1978: 594-605.
17. Vanparijs O, Marbsboom R, Desplenter L. Diclazuril, a new broad spectrum anticoccidial drug in chickens. Poult Sci 1989; 68: 489-495.
18. Verheyen A, Maes L, Coussement W, Vanparijs O, Lauwers F, Vlaminckx E, Borgers M, Marsboom R. In vivo action of the anticoccidial diclazuril (Clinacox) on the developmental stages of Eimeria tenella: an ultrastructural evaluation. J Parasitol 1988; 74: 939-949.
19. Williams RB. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int J Parasitol 1998; 28: 1089-1098.
20. Yim D, Kang SS, Kim DW, Kim SH, Lillehoj HS, Min W. Protective effects of Aloe vera-based diets in Eimeria maxima-infected broiler chickens. Exp Parasitol 2011; 127: 322-325.

Diclazuril을 주성분으로 하는 콕시멸-에스의 육계에 대한 실험실 및 야외적용 실험에서의 항콕시듐 효과

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요 약 : 디클라쥬릴의 음수형 제제인 콕시멸-에스의 항콕시듐 효과를 *Eimeria* spp. 감염 육계를 이용하여 수행하였다. 본 실험은 실험실 및 야외적용시험으로 나누어 진행하였으며, 실험실 실험에서는 *Eimeria* spp.를 인공 감염시킨 육계를 대상으로 콕시멸-에스 0.5 ml/L을 음수로 투여한 군에서 대조군과 비교하여 콕시듐 치료, 콕시듐 충란수의 감소, 그리고 장상해도 및 분변 설사지수 등에 있어서 유의성 있는 효과를 나타내었다($p < 0.001$). 또한, 야외적용실험에서는, 콕시듐증에 걸린 육계를 대상으로 콕시멸-에스를 0.25와 0.5 ml/L로 각각 음수로 투여한 결과, 대조군과 비교하여 모두 유의한 콕시듐 치료효과를 나타내었다($p < 0.001$). 이상의 결과로부터, 콕시멸-에스 0.25와 0.5 ml/L를 음수로 콕시듐증에 걸린 육계에 투여할 경우, 콕시듐증 치료에 매우 효과적일 것으로 판단된다.

주요어 : 콕시듐증, *Eimeria* spp., 디클라쥬릴, 육계, 콕시멸-에스