Arab Gulf J. Scient. Res., Agric. Biol. Sci., **B6** (1), pp. 129-137 (1988)

Evaluation of Two Insect Chemosterilants (Thiotepa and 2,5 Dichloro - 2,4 Dinitro Benzenesulfonanilide) for Their Potential as Coccidiocidal Agents in Chickens

Mikky A. Amoudi[1]

Department of Biology, Utah State University, Logan, Utah, 84321, U.S.A.

ABSTRACT. A total of 33 chickens was used; each chick was inoculated with approximately 12,000 to 15,000 sporulated *Eimeria tenella* oocysts. Chickens were used in nine groups, four treated with thiotepa, and four treated with 2,5 dichloro-2,4 dinitro-benzenesulfonanilide, while the remaining group served as a control. Groups, two, four, six and eight were given doses of thiotepa at levels of 1.54 mg/bird, 3.08 mg/bird, 6.16 mg/bird and 9.24 while groups three, five, seven and nine were given doses of 2,5 dichloro-2,4 dinitro-benzenesulfonanilide at levels of 1.54 mg/bird, 3.08 mg/bird, 6.16 mg/bird and 9.24 mg/bird. Both groups treated with both drugs showed a significant decreases in oocyst production with increase of drug concentration.

The most serious pathogenic species in poultry are *E. meleagridis* Tyzzer, 1927, in Turkey and in chickens, *E. tenella* (Raillet et Lucet, 1891); *E. maxima* Tyzzer, 1929; *E. brunetti* Levine, 1942; and *E. acervulina* Tyzzer, 1929. *E. tenella* is one of the most important among the nine species that parasitize chickens. Losses from coccidiosis in chickens include death during severe infection, or morbidity in sublethal infections. The pathological effects of *E. tenella* are produced by a sexual stages located in the caecal epithelium. The severity of the infection is related to the number of organisms initiating the infection. Clinical signs of caecal coccidiosis are variable; the feces are blood-streaked, the birds become somnolent, hardly move, and stand for a long time with eyes closed. Appetite falls off, but thirst increases.

Since it has been shown that an organism may develop resistance to a particular pesticide after a long exposure to it (LeBrecque and Smith 1968),

1 *Present address:* Department of Zoology, College of Science, King Saud University, P.O. Box 2455 Riyadh 11451, Saudi Arabia.

industry is always searching for newer pesticides. Recently, a newer class of insecticides (chemosterilants) has been used successfully as an alternative for controlling insects and other invertebrates. The role of chemosterilants as control agents, especially for protozoa, has been studied by many investigators. In addition to their role in insect control, Lindquist (1961) suggested that they might also affect the pathogen within the body of the carrier, thus a more effective control of the disease could be accomplished. Dame *et al.* (1964) demonstrated that after the injection of aqueous solutions of metepa and apholate into the pronotum of *Glossina morsitans* males, they were still capable of transmitting *Trypanosoma congolense*, the causative agent of the nagana in cattle. They found that when flies were treated before exposure to the *Trypanosoma congolense*, 50% of the hosts became infected; when flies were treated after being exposed, only 17% of the flies became infected. The results of their work suggest that these two chemosterilants may have an effect on protozoan parasites.

Several attempts have been made to determine whether the insect host or the parasite would exhibit differential susceptibility to chemosterilants (Altman 1963, Bertram 1964 and Bertram *et al.* 1964). Some reduction in transmission of *Plasmodium gallinaceum* (a malaria parasite of birds) by tepa or thiotepa sterilized mosquitoes was noted, but the insects were more easily sterilized than was the parasite (Altman 1963).

Materials and Methods

Selection and Management

Chickens are the specific host for *E. tenella*. Coccidia-free chicks were obtained by laboratory hatching. A total of 33 chicks were divided into nine groups. Chicks ranged from 2 to 4 weeks in age and weighed 250-500 g and each group was maintained in a separate cage - measuring 50 cm × 38 cm × 36 cm high. Oily surface paper was place underneath for collecting feces. Chicks were kept at about 22°C and supplied with corn mash and water twice a day.

Preparation of Inoculum

Oocyst inoculum was prepared from fecal material collected from infected chickens. The fecal material was strained through two sieves. After straining the suspension was allowed to undergo sedimentation, then siphoned off. The sediment was washed and then suspended in 2.5% potassium dichromate solution to inhibit bacterial growth, then stored in a refrigerator until used.

Treatment of Animals

Fresh preparation of thiotepa and 2,5 dichloro-2,4 dinitro-benzenesulfonanilide were prepared daily from refrigerated stock for each experiment, Amoudi (1985).

Fecal Sample

Five g fecal samples of each group were collected in small bottles every morning, beginning 7-9 days after inoculation and continued until the end of the experiment. The feces were then examined macroscopically for the presence of blood, mucus and tissues. The concentration of oocysts in the fecal samples was determined with the aid of a McMaster chamber. Five g of feces were mixed with 45 ml of water; 1 ml of this dilution was then mixed with 2 ml of Sheather's solution (Levine 1973). A drop of this mixture was then placed in the McMaster chamber with a syringe, allowed to stand for approximately 15 min. then examined microscopically with 10X objective and 10X ocular lenses. The number of oocysts was counted with 1 cm^2 grid and this count was multiplied by 200 to give the total number of oocysts present in 1 g of feces (number of oocysts within 1 $cm^2 \times 200$).

Results

Experiment I

The birds in this experiment were randomized into three groups, each having five chickens of 30 days mean age (25-35) at the time of inoculation. Each of the chicken was inoculated with approximately 15,000 sporulated oocysts of *E. tenella*.

The results are summarized in Table 1 and 2 and in Figs. 1 and 2 indicating that the discharge of oocysts was much heavier in the control groups than in the groups treated with low doses (1.54 mg/chick) of both drugs for 6 days, starting one day before infection. The differences in oocysts production by both treated and non-treated birds were judged by the number of oocysts discharged as shown in Figs. 1 and 2.

Experiment II

In this experiment, the birds were randomized into six groups, each containing three chickens which with a mean age of 15 days (12-18) at the time of inoculation. Each of the chickens in these groups was given approximately 12,000-15,000 sporulated oocysts of *Eimeria tenella*. The results of this experiment are likewise summarized in Tables 1 and 2, and in Figs. 1 and 2.

The results indicated that groups treated with different levels of thiotepa, particularly 6.16 mg/bird and 9.24 mg/bird, showed significant decreases in the number of oocysts discharged (Table 1 and Fig. 1). Similar results were obtained in groups treated with the levels of 2,5 dichloro-2,4-dinitrobenzenesulfoanilide (3.08 mg/bird, 6.16 mg/bird, 9.24 mg/bird). All 3 treatment groups showed a significant decrease in the number of oocysts discharged compared to the control group, at all time points (Table 2 and Fig. 2). Increasing the concentration of both drugs, good protection against infection was provided.

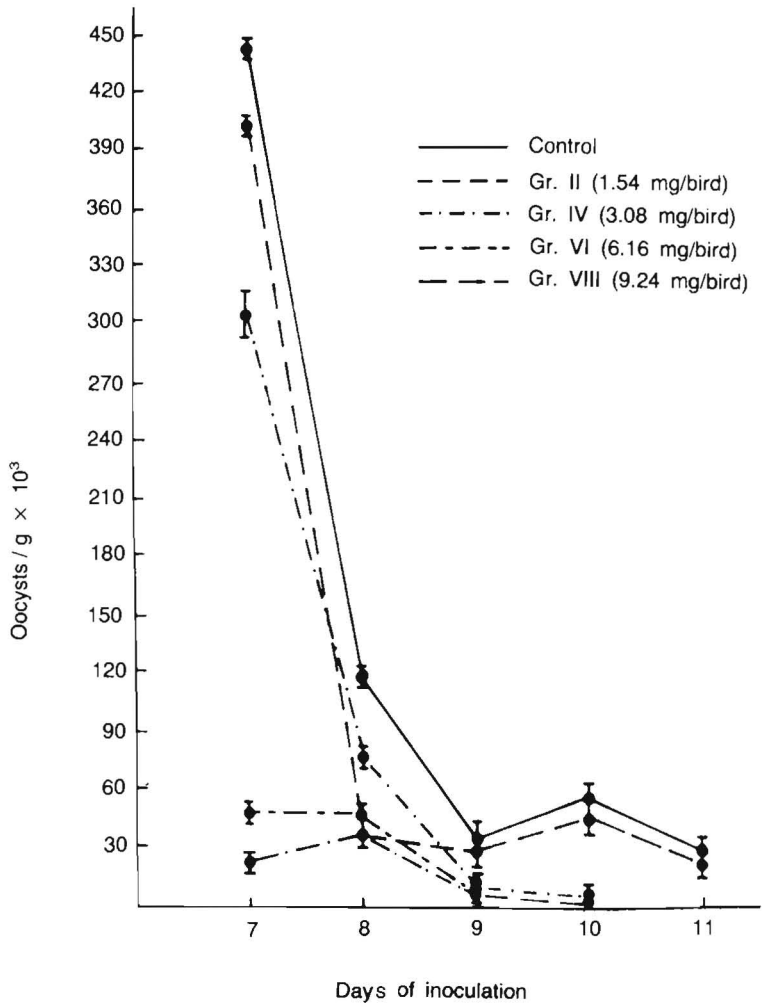

Fig. 1. Mean daily oocyst output of *E. tenella* per 1.0 g feces from chickens treated with thiotepa during patent period starting 7 days after inoculation. Each point is the mean ± SEM of six replicants.

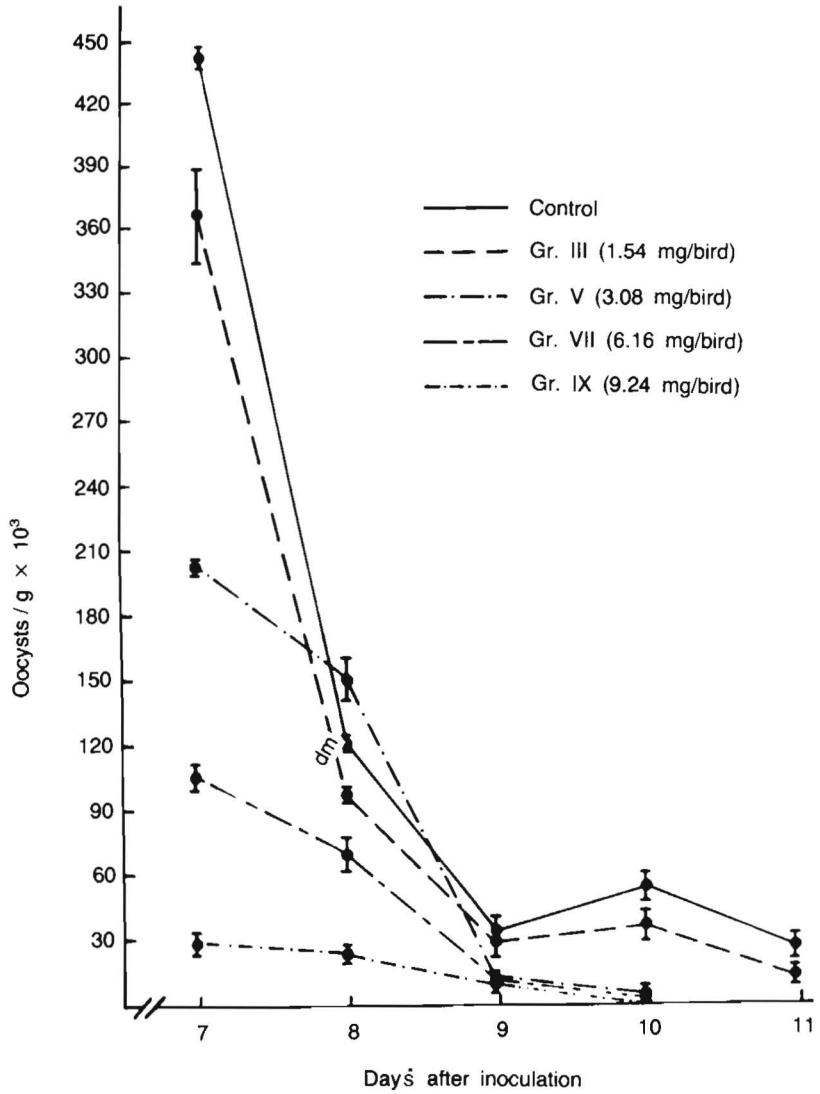

Fig. 2. Mean daily oocyst output of *E. tenella* per 1.0 g feces from chickens treated with 2,5 dichloro 2,4-dinitro benzenesulfonanilide during patent period starting 7 days after inoculation. Each point is the mean ± SEM of six replicants.

Table 1. Duration and number of discharged oocysts from chickens inoculated with 15,000 sporulated oocysts of *E. tenella* and treated with different levels of thiotepa

Groups treated with thiotepa	Drug level	Oocyst Discharge	
		Total number of collected oocysts per 1.0 g feces	Duration (days)
Group 2	1.54 mg/bird	544,100	5
Group 4	3.08 mg/bird	397,300	4
Group 6	6.16 mg/bird	107,35	4
Group 8	9.24 mg/bird	74,25	4
Untreated control		679,300	5

Table 2. Duration and number of discharged oocysts from chickens inoculated with 15,000 sporulated oocysts of *E. tenella* and treated with different levels of 2,5 dichloro-2,4 dinitro benzensulfonanilide

Groups treated with 2,5 dichloro-2,4 dinitro	Drug level	Oocyst Discharge	
		Total number of collected oocysts per 1.0 g feces	Duration (days)
Group 3	1.54 mg/bird	549,000	5
Group 5	3.08 mg/bird	374,300	4
Group 7	6.16 mg/bird	189,700	4
Group 9	9.24 mg/bird	65,650	4
Untreated control		679,300	5

Discussion

Much of the early work on coccidial infections in poultry was carried out with *Eimeria tenella* (Railliet et Lucet). Not only is this species a major cause of loss but it proved to be a convenient parasite for experimentation. For these reasons, most of the fundamental principles of the chemotheraphy of coccidiosis have been worked out with *E. tenella* (Dickinson and Schofield 1939, Goff and Upp 1940, Horton-Smith and Boyland 1946, Waletzky and Hughes 1946, and Joyner and Kendall 1956).

From the present study, it is apparent that both thiotepa and 2,5 dichloro-2,4 dinitro benzenesulfonanilide reduced production of oocysts in chickens infected with *E. tenella*. Peak numers of oocysts discharged were reduced, as was the duration of patent period. There is a possibility that these drugs may act on both the first and second-generation schizonts of this parasite. This view is supported by the absence of hemorrhage on the fifth day after infection. This, in turn, may be due to the action of the drug on the second-generation schizonts which normally mature 4 to 5 days after inoculation with oocysts.

Kendall and McCullough (1952) found that high level doses of sulfamezathine had an inhibitory effect on the first-generation schizonts of *E. tenella* in chickens. As only high level doses of thiotepa and 2,5 dichlor-2,4 dinitro benzenesulfonanilide reduced oocyst production in the present study, it is possible that these compounds also have some effect on the first-generation schizonts of this species.

In an early study, after a careful analysis of the effect of 1.0% sulfadimidine on *E. tenella*, Farr and Wehr (1947) concluded that there was some effect on early first-generation schizonts, no effect on the first generation merozoites, and destruction of second-generation schizonts. These authors also noted that when a high dose of drug was given during the early part of the development cycle, hemorrhage occurred after the medicated mash had been removed. This was several days after it normally would be expected to occur. These observations were confirmed and extended by Kendall and McCullough (1952), who concluded that at ordinary therapeutic levels, sulfadimidine affects the second-generation schizonts, while at higher levels, first-generation schizonts were affected. Mature second-generation schizonts of *E. tenella* are found 96 hr after inoculation.

In practice using therapeutic doses of sulfamezathine, there is a wide margin (*e.g.*, four to six-fold increase) between the dose required to inhibit the second-generation schizonts and that which will affect the first-generation schizonts Kendall and McCullough (1952).

Conclusion

Eimeria tenella (Railliet et Lucet, 1891) was used to determine the effectiveness of thiotepa and 2,5 dichloro-2,4-dinitro-benzenesulfonanilide (insect chemosterilants) in controlling coccidiosis in chickens. A total of 33 chickens were used in nine groups, each chicken was inoculated with approximately 12,000 to 15,000 sporulated oocysts. Four groups were treated with thiotepa and four groups were treated with 2,5 dichloro-2,4-dinitro-benzenesulfonanilide while the remaining group served as a control. The results showed that administration of either thiotepa or 2,5 dichloro-2,4 dinitro benezenesulfonanilide (6 consecutive days at

1.54 mg/bird) reduced oocyst production in the treated groups as compared with controls. However, the treated groups as well as the controls exhibited clinical signs of coccidiosis (diarrhea and bloody feces). By increasing the dose two-fold (*i.e.* 3.08 mg/bird) oocyst production was further decreased, but chickens in these groups showed no clinical symptoms. A further increase of the dose-level (*i.e.* 6.16 mg/bird) resulted in greater reduction in oocyst production. None of the birds showed symptoms of the disease. In birds which received a sixfold dose (*i.e.* 9.24 mg/bird) oocyst production was lowest. These results indicated that both thiotepa and 2,5 dichloro-2,4-dinitro-benzenesulfonanilide reduce oocyst production proportional to dose concentration in chickens under these test conditions.

References

Altman, R.M. (1963) The effects of tepa on *Plasmodium gallinaceum* in *Aedes-aegypti*, *Am. J. Hyg.* **77:** 221-227.

Amoudi, M.A. (1985) Evaluation of two insect chemosterilants (hempa and thiotepa) for their effect on *Eimeria vermiformis* infection in mice, *Arab Gulf J. Scinet. Res.* **3:** 691-703.

Bertram, D.S. (1964) Entomological and parasitological aspects of vector chemosterilization, *Trans. Royal Soc. Trop. Med. Hyg.* **58:** 296-317.

Bertram, D.S., Srivastava, S.C. and **Msangi, A.S.** (1964) Transmission of *Plasmodium gallinaceum* Brumpt to chicks by *Aedes aegypt* (L). Sterilized by an alkylating agent, thiotepa, *J. Trop. Med. Hyg.* **67:** 51-57.

Dame, D.A., Dean, C.J.W., and **Ford, J.** (1964) Investigations on the sterile male technique with *Glossina morsitans. Proc. 10th Meet. Intern. Sci. Comm. Trypanosomiasis Res.* Kampala, Uganda, p. 39.

Dickinson, E.M. and **Schofield, R.H.** (1939) The effect of Sulphur against Artificial Infection with *Eimera acervulina* and *Eimeria tenella, Poult. Sci.* **18:** 419-431.

Farr, M.M. and **Wehr, E.E.** (1947) Development stages in the life cycle of *Eimeria tenella* affected by sulphamethazine treatment, *Proc. Helm. Soc. Wash.* **14:** p. 12.

Goff, O.E. and **Upp, C.W.** (1940) Effect of four grades of sulfur on artificially produced coccidiosis, *Poult. Sci.* **19:** 180-186.

Horoton-Smith, C. (1949) The acquisition of resistance to coccidiosis by chickens during treatment with sulfonamides, *Vet. Rec.* **61:** 237-238.

Horton-Smith, C. and **Boyland, E.** (1946) Sulphonamides in the treatment of caecal coccidiosis of chickens, *Brit. J. Pharmacol.* **1:** 139-145.

Joyner, L.P. and **Kendall, S.B.** (1956) The mode of action of a mixture of pyrimethamine and sulphadimidine on *Eimeria tenella, Brit. J. Pharmacol.* **11:** 454-459.

Kendall, S.B. and **McCulloguh, F.S.** (1952) Relationships between sulphamethazine therapy and the acquisition of immunity to *Eimeria tenella, J. Com. Pathol. Therap.* **62:** 116-124.

LeBrecque, G.C. and **Smith, C.N.** eds. (1968) *Principles of Insect Chemo-Sterilization,* New York, Appletion-Centure-Crofts.

Levine, N.D. (1973) *Protozoan Parasites of Domestic Animals and of Man.* 2nd ed. Minneapolis, Minnesota; Burgess Publishing Co., pp. 381-381.

Lindquist, A.W. (1961) Chemical to sterilized insects, *J. Washington Acad. Sci.* **106:** 1.

Waletzky, E. and **Hughes, C.O.** (1946) The relative activity of sulfanilamides and other compounds in avian coccidiosis (*Eimeria tenella*), *Amer. J. Vet. Res.* **7:** 365-377.

(Received 23/11/1986;
in revised form 01/04/1987)

دراسات تقويمية لنوعين من المركبات الكيميائية التعقيمية (ثيوتيبا) ٢ ، ٥ دايكلورو ٢ ، ٤ داينترو بنزين سلفانيد الينيد وفعاليتهما كعوامل لمكافحة مرض الكوكسيديا في الدجاج

مكي عبدالله العمودي*

قسم علم الاحياء ـ جامعة ولاية يوتا ـ لوقان ـ يوتا ٨٤٣٢١ ـ الولايات المتحدة الأمريكية

بلغ عدد الحيوانات المستعملة في هذه الدراسة ٣٣ دجاجة، مقسمة إلى تسعة مجاميع، أعطيت كل دجاجة منها جرعة من المرض تقدر بـ ١٢,٠٠٠ إلى ١٥,٠٠٠ كيس من البيض الناضج من الطفيلي ايميريا تينيلا *Eimeria tenella* ، وقد عوملت الأربع مجموعات الأولى، وهي الثانية والرابعة والسادسة والثامنة بجرعات من الدواء ثيوتيبا Thiotepa عند تراكيز ١,٥٤ ملجم/ دجاجة، ٣,٠٨ ملجم/ دجاجة، ٦,١٦ ملجم/ دجاجة و ٩,٢٤ ملجم/ دجاجة. كما عوملت الأربع مجموعات الأخرى وهي الثالثة والخامسة والسابعة والتاسعة بجرعات من الدواء ٢,٥ دايكلورو ٢,٤ ـ داينترو بنزين سلفانيد الينيد عند تراكيز ١,٥٤ ملجم/ دجاجة، ٣,٠٨ ملجم/ دجاجة و ٦,١٦ ملجم/ دجاجة و ٩,٢٤ ملجم/ دجاجة، بينما استعملت المجموعة المتبقية كمجموعة ضابطة. وقد دلت النتائج على أن جميع المجاميع المعاملة بالدوائين سابقين الذكر تحت تراكيز مختلفة قد أعطت انخفاضاً واضحاً ومضطرداً في إنتاج البيض، وذلك كلما إزداد تركيز الدواء مما يدل على فعالية هذين المركبين في مكافحة مرض الكوكسيديا الذي يسببه طفيلي الايميريا تينيلا *Eimeria tenella* في الدجاج.

* العنوان الحالي: قسم علم الحيوان، كلية الهندسة، جامعة الملك سعود، ص.ب ٢٤٥٥ ـ الرياض ١١٤٥١ المملكة العربية السعودية.