

The efficacy of diclazuril (liquid formulation) in the prevention and control of coccidiosis in broiler chicken.

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This study was carried out to evaluate the efficacy of water soluble formulation of diclazuril (1%) in the prevention and control of mixed *Eimeria* infection in two experimental (semifield) trials and testing efficacy of this medicament in controlling of natural field case of mixed *Eimeria* infection. The experimental (semifield) trials carried out each on 150-day-old broiler chicks, as they were divided into 3 equal separate subgroups. Infected groups were orally received 1ml of an inoculum containing 50.000 sporulated oocysts of *Eimeria tenella*, *E. acervulina* and *E. necatrix*/chick at 14 days of age. Diclazuril (1%) liquid at dose of 1 ml / 4 liter of drinking water for 2 successive days just after appearance of bloody dropping (at the 5th day post infection). Birds were given prophylactic diclazuril dose (1 ml / 8 liter) at the 7th day before the experimental inoculation. In both trials were oocyst count (shedding) in the dropping, dropping score, lesion score, body weight gain % and mortality rate were evaluated. Groups treated with diclazuril either in the prophylactic and curative studies gave significant and satisfactory improvement in the assessment criteria when compared with infected non medicated groups.

A field trial was conducted 2 commercial broiler chicken farm consisted of 2 flocks aged 33 days; 6000 birds each. Flock (1) was kept as control positive infected non medicated group, whereas flock (2) was medicated with diclazuril (1%) at dose of 1 ml / 4 liter of the drinking water for 2 successive days. The results revealed more reduction in total oocyst count, lesion score and the mortality rate in the medicated than the infected non-treated group. The results proved that diclazuril (1%) liquid is effective in prevention and control of both experimental and field infection field circulating *Eimeria* species still sensitive to the drug.

Coccidiosis is a common disease complex in poultry caused by infection by members of the genus *Eimeria*. Economic losses are incurred not only as a result of mortality and poor performance of surviving birds, but also as a result of the cost of routine prophylactic and therapeutic medication (Williams, 1999).

There are several species of *Eimeria* that parasitize chickens (Shirley, 1986) and the pathogenic effects of virulent field strains vary, according to *Eimeria* species, from a mild to severe haemorrhagic enteritis and death (Williams, 1998).

As the world's poultry production continues to grow, so do concerns about the control of coccidiosis, which remains one of the most commonly reported disease of chickens (Biggs, 1982; Xie *et al.*, 2001). Moreover, although prophylactic administration of conventional anticoccidial drugs in the feed, outbreaks of

coccidiosis still occurred in the field (Chapman, 1987).

Evaluation of any anticoccidial drug is based upon estimation of bird's performance criteria such as growth rate and feed conversion, and parasitological criteria including oocyst shedding and the presence of pathognomonic intestinal lesions (Reid *et al.*, 1969; Champan, 1998).

Anticoccidial compounds should be highly effective against all developmental stages of *Eimeria* species, don't affect on the host immune response as well as have no residues in the tissues. In this respect, diclazuril is one of a series of benzenacetone nitrile derivatives. The prophylactic anticoccidial efficacy of diclazuril in feed was extensively studied in chickens (Chapman, 1989; Vanparijs *et al.*, 1989b,e,f; McDougald *et al.*, 1990a,b; Vieira and Clemente, 1995; Chapman, 1998; Conway *et al.*, 2001a,b and 2002a,b ; Kiaei *et al.*, 2001; Awaad

et al., 2003 ; Meireles *et al.*, 2003), turkeys (Vanparijs *et al.*, 1989c; McDougald *et al.*, 1991 ; Chapman *et al.*, 2004), pigeons (Vercruyse, 1990), pheasants (Vanparijs *et al.*, 1990), partridges (Vanparijs *et al.*, 1991) and rabbits (Vanparijs *et al.*, 1989a,d) and the drug proved its efficacy in the prevention of coccidial infection without development of drug resistance.

New formulations of water soluble diclazuril for administration in the drinking water were introduced recently in many parts of the world, and few studies were conducted to study its efficacy in the prevention and control of chicken's coccidiosis (Jiang-ZhongQi, 1999; Mukiibi-Muka *et al.*, 2001; El-Banna *et al.*, 2005 ; El-Dakhly *et al.*, 2006). This investigation was designed to evaluate the efficacy of using water soluble formulation of diclazuril (1%) in the prevention and control of mixed Eimeria species infections in broiler chickens as a experimental (semifield) trial, also testing the efficacy of this medicament in the control of naturally infected broiler chickens with mixed Eimeria species as a field trial.

Materials and methods

Chickens.

Experimental (Semi field) trial. Three hundreds, day-old (Hubbard breed) broiler chicks obtained from commercial poultry company were reared on cleaned and disinfected wire floor cages and given feed and water ad libitum without any additives or coccidiostates. These chicks were given Hitchner B1 and La Sota vaccines at 5 and 19 days of age, respectively for Newcastle disease vaccination and also given IBD 228E vaccine at the 12 days of age for infectious bursal disease vaccination; all vaccines were used via eye drop method.

Field trial. A commercial Hubbard breed broiler chicken farm consisted of two flocks of 6000 birds each, aged 33 days and reared on deep litter system were tested in this trial. These birds were suffered severe intestinal and caecal coccidiosis.

The used anticoccidial drug. Diclazuril water soluble formulation (A synthetic molecule belongs to the benzeneacetone nitrates) as 1% in liquid was obtained from Jordan Vet. and Agr. Med. Ind. Co., Jovet. Batch No. 050508. In experimental (semifield) trial, diclazuril was administered in a prophylactic dose of 1 ml / 8 liter of the drinking water at the 7th day before the experimental infection, while the therapeutic dose of (1 ml / 4 liter) was used for 2 successive

days once the blood was observed in the dropping [at the 5th day post infection (PI)].

In the field trial, diclazuril was administered in a dose of 1 ml / 4 liter for 2 successive days after appearance of signs and mortalities.

Preparation of sporulated oocysts. Mixed Eimeria species non-sporulated oocysts containing E. tenella, E. acervulina and E. necatrix in approximate ratio of 1: 2: 1 was obtained from Prof. Dr. M. Kutkat, National Research Center, Egypt. The non sporulated oocysts were emulsified in 2.5% potassium dichromate solution, then filtrated and the filtrate was left for sedimentation. The sediment was washed several times with distilled water. Lastly, the washed oocysts were kept in 2.5% potassium dichromate solution at room temperature for sporulation. The sporulated oocyst count/ml done using Mc-Master technique according to Maff (1977); Soulsby (1978). Different Sporulated oocysts species used for experimental infection or field diagnosis identified according to their size (measuring 100 oocysts) as described by Soulsby (1978).

Experimental infection. Each bird of the infected and medicated groups in the experimental (semifield) trial was intracropally inoculated with 1 ml solution containing 50,000 mixed species of E. tenella, E. acervulina and E. necatrix sporulated oocysts on the 14th day of age.

Evaluation of the drug efficacy.

Total oocyst count (shedding). The dropping of birds in the semifield (experimental) and field trial were collected daily from the 5th (first appearance of blood) till the 7th day post infection (PI) for testing drug efficacy, while the dropping were collected during 2 days before treatment and 7 days post treatment to test the drug curative efficacy. The mean number of non sporulated oocysts / gram of dropping for each group were counted and calculated using Mc-Master technique as described by Soulsby (1978).

Dropping score. In the (the dropping of each group in the preventive study were collected daily from the 5th to the 7th day (PI), while they were collected during 2 days before and 7 days post treatment in the semi field and field one. The dropping score was graded (1-4) according to the consistency of the dropping and the presence of mucus/ or blood (Johnson and Reid, 1970; Ramadan *et al.*, 1997).

Lesion scoring . Dead and sacrificed birds were

subjected for macroscopical examination of the intestine and caeci. The lesions were scored on a scale of 0 to 5 according to the severity of the lesions in the small intestine and the caeci (Johnson and Reid, 1970, Conway, 1979; Conway and Mackenzie, 1991). A score (0) denoted no lesions whereas (5) denoted severe lesions.

The body weight gain. The mean body weight gain at the end of observation period (7 days PI) in the experimental was calculated.

The mortality rate. The number of dead birds in both semi-field and field trials were recorded daily.

Experimental design.

Semifield (experimental) trial. Out of 300, day old chicks, the first group containing 150 birds was divided into three equal separate subgroups (A1, B1 and C1), each consisted of 50 birds. Group (A1) was kept as control positive infected non medicated group, while group (B1) was kept as control negative non infected non medicated group. Group (C1) was medicated with diclazuril 1% (water soluble) in a prophylactic dose of 1 ml / 8 liter of the drinking water at the 7th day before the experimental infection. Both of group (A1) and (C1) were orally infected with 1 ml solution containing 50.000 mixed species of sporulated *E. tenella*, *E. acervulina* and *E. necatrix* oocysts at 14 days of life. The rest of 150 birds in the second group were equally divided into 3 equal subgroups (A2, B2 and C2), 50 birds each. Group (A2) was kept as control positive infected non medicated group, whereas group (B2) was considered as control negative non infected non medicated group. Groups (A2) and (C2) were experimentally infected per os with 1 ml solution containing 50.000 mixed species of sporulated *E. tenella*, *E. acervulina* and *E. necatrix* oocysts at 14 days of life. Group (C2) was medicated with diclazuril 1% (water soluble) in a curative dose of 1 ml / 4 liter of the drinking water at the 5th day (PI), just after detection of blood in the dropping. The drug was given for 2 successive days.

The dropping of birds in the prophylactic study were daily collected from the 5th till the 7th day (PI), while the dropping were collected during 2 days before and 7 days post treatment for oocyst count (shedding) and mean dropping score. Dead and sacrificed birds from each group were macroscopically examined and the mean gross lesions of the intestine and caeci were recorded. The mean body weight and the mortality rate at the end of observation period were calculated.

Once a field infection was diagnosed in the two flocks (1 and 2), birds of flock (1) were considered as infected non medicated group, whereas birds of flock (2) were medicated with diclazuril 1% water soluble formulation at dose 1 ml / 4 liter of the drinking water for 2 successive days.

Freshly voided dropping from each group were collected for 2 days before treatment and for 7 days after treatment from 5 floor areas in X manner / meter square for oocyst count (shedding). Dead and sacrificed birds before and after medication were subjected to post mortem examination for detection of the mean lesion score in the intestine and caeci. The number of dead birds was also recorded for determination of mortality rate in each flock.

Statistical analysis. The obtained data were statistically analyzed by using Analysis of Variance (ANOVA) according to Snedecor and Cochran (1980).

Results and Discussion

Table (1) showed the prophylactic efficacy of water soluble diclazuril (1%) at dose of 1 ml / 8 liter of the drinking water against experimental infection with mixed *Eimeria* species at the 7th day before the in the semifield (experimental) trial. Water soluble diclazuril induced significant ($P \leq 0.05$) reduction in the mean oocyst shedding, dropping score, lesion score and mortality rate when compared with infected non medicated control positive group. Moreover, the drug induced significant ($P \leq 0.05$) improvement in the mean body weight gain at the end of observation period in comparison with infected non medicated group. Efficacy of diclazuril as a feed additive in prevention and control of coccidiosis had been studied and proved to be highly effective (Chapman, 1989; Awaad *et al.*, 2003; Meireles *et al.*, 2003).

Efficacy of Diclazuril administration in drinking water was also proved by El-Banna *et al.*, (2005) who investigated that diclazuril in water or feed were of similar efficacy in elimination of experimental infection with *E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima* and *E. Brunetti* as shown by increasing body weight gain and survival rate and reduction in faecal shedding, dropping score and lesion score. Furthermore, the results of using diclazuril (1%) water soluble solution as 1ml/ 4 liter of the drinking water for 2 successive days just after appearance of blood in the dropping (5th day PI)

Table (1): The prophylactic efficacy of diclazuril (water soluble formulation) in experimentally infected broiler chickens with mixed Eimeria species (semifield trial).

| Group No. | Treatment | Infection | Total No. of oocysts/gm dropping x 10 ³ | | Mean dropping score | Mean lesion score | Mortality rate | Body weight gain % |
|-----------|-----------|-----------|--|--|-----------------------|------------------------|----------------|-------------------------|
| | | | | | | | | |
| A1 | - | + | 46.9±2.1 ^a | | 4.8±0.91 ^a | 3.1±0.79 ^a | (35/50) 70 | 33.25±4.52 ^c |
| B1 | - | - | 0.0±0.0 ^c | | 1.0±0.0 ^b | 0.0±0.0 ^b | (0/50) 0 | 48.9±1.04 ^a |
| C1 | + | + | 3.45±1.02 ^b | | 1.21±0.1 ^b | 1.02±0.03 ^b | (2/50) 4 | 44.01±1.06 ^b |

Values within a column with no common superscript are significantly different ($P \leq 0.05$).

Table (2): The curative efficacy of diclazuril (water formulation) in experimentally infected broiler chickens with mixed Eimeria species (semifield trial).

| Group No. | Treatment | Infection | Total No. of oocysts/gm dropping x 10 ³ | | Mean dropping score | Mean Lesion score | Mortality rate | Body weight gain % |
|-----------|-----------|-----------|--|-------------------------|----------------------|------------------------|----------------|-------------------------|
| | | | 2 DPT* | 7 DPT * | | | | |
| A2 | - | + | 8.9±0.69 ^a | 55.03±4.02 ^a | 4.6±0.3 ^a | 3.7±0.89 ^a | (38/50) 76 | 31.3±2.13 ^b |
| B2 | - | - | 0.0±0.0 ^b | 0.0±0.0 ^c | 1.0±0.0 ^b | 0.0±0.0 ^c | (0/50) 0 | 42.04±1.05 ^a |
| C2 | + | + | 7.81±0.87 ^a | 1.95±0.11 ^b | 1.5±0.2 ^b | 1.79±0.95 ^b | (6/50) 12 | 40.99±1.08 ^a |

* DBT: Days before treatment

Values within a column with no common superscript are significantly different ($P \leq 0.05$).

in experimentally infected broiler chickens with mixed Eimeria species were obtained in table (2). The drug was very effective as shown by significant ($P \leq 0.05$) decrease in the number of oocyst shedding, dropping and lesion scores as compared with infected non-medicated group. Also, diclazuril had the ability to reduce the mortality rate and prevent the reduction of body weight gain caused by coccidial infection. These results agree with those reported by El-Banna *et al.*, (2005); El-Dakhly *et al.*, (2006) who reported that diclazuril in the drinking water was appropriate for use in prevention and treatment of Eimeria infection in chickens as indicated by decreased oocyst number and lesion score in the treated groups. In addition, Jiang-ZhongQi (1999) demonstrated that usage of diclazuril in concentrations 0.25, 0.5 or 1 mg / liter of drinking water of E. tenella infected chickens, body weight increased, losses due to infection were decreased and index of resistance to the infection increased with increasing rate of diclazuril.. The mode of action of diclazuril was studied by Varheven *et al.*, (1989) and concluded that diclazuril treatment primarily affect particular stages in the sexual development of E. maxima and E. brunetti resulting in complete eradication of infection. Brander *et al.*, (1991) stated that diclazuril

breaks down all intracellular developmental stages of asexual and sexual cycles of E. tenella, asexual later shizonts of E. acervulina and works against sexual and zygote for E. maxima and gametocytes for E. brunetti. Furthermore, Kwazone and Fabio (1994); El-Banna *et al.*, (2005) ; El-Dakhly *et al.*, (2006) confirmed that diclazuril solution induced marked activity in stopping the cycle of coccidial development inside the medicated birds especially when applied on the day of first blood appearance of bird's drooping.

Results of field study (table 3) proved that two days water medication of diclazuril improved the total oocyst count, the lesion score and the mortality rate than non medicated infected group natural infection with Eimeria species. In this respect, Mukiibi-Muka *et al.*, (2001) found both diclazuril and amprolium water treatment were effective in controlling of natural coccidial infection, but diclazuril was the superior in reduction of oocyst shedding.

Due to diclazuril water solubility, short duration of treatment (2 days), small dosage, rapid anticoccidial action against all intracellular developmental stages of the coccidial infections in all poultry species make diclazuril to be more effective in prevention and control of coccidiosis.

Table (3): The curative efficacy of diclazuril (water soluble formulation) in naturally infected broiler chickens with mixed Eimeria species (field trial).

| Flock No. | Infection | Treatment | Total No. of oocysts /gm dropping $\times 10^3$ | | Mean lesion score | Mortality rate |
|-----------|-----------|-----------|---|-------------------------|------------------------|----------------|
| | | | 2 DPT* | 7 DPT* | | |
| 1 | + | - | 9.47±0.46 | 60.12±4.11 ^a | 3.2±0.33 ^a | (90/6000) 1.5 |
| 2 | + | - | 8.99±0.05 | 0.37±0.16 ^b | 1.11±0.74 ^b | (20/6000) 0.3 |

* DPT: Days before treatment.

Values within a column with no common superscript are significantly different ($P \leq 0.05$).

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أجريت هذه الدراسة للتعرف على كفاءة عقار "الدايكلزوريل" في وقاية وعلاج الإصابة بالكوكسيديا في دجاج التسمين

أجريت الدراسة للتعرف على كفاءة عقار الـدايكلزوريل السائل بتركيز ١% في وقاية وعلاج عدوى الدجاج بطفيل الكوكسيديا متعدد الأنواع في محاولتين معمليتين (شبه حقلية) أحدهما للوقاية والأخرى للعلاج والأخرى محاولة علاج حالة إصابة حقلية بالطفيل. أجريت المحاولات المعملية كل على ١٥٠ كتكوت عمر يوم قسمت إلى ٣ مجموعات متساوية. أجريت العدوى بواحد مللي يحتوي على ٥٠٠٠٠ من الحويصلات المتجرثمة sporulated oocysts من أنواع التينلا والاسيرفيولينا والنيكاتركس عند عمر ١٤ يوم. واستخدم عقار الـدايكلزوريل السائل (١%) في الوقاية بجرعة ١ملل/٨ لبر من ماء الشرب ٧ أيام قبل العدوى، أما الجرعة العلاجية فكانت ١ مللي/٤ لتر ماء شرب ليومين متتابعين بدءاً من التعرف على الإصابة بظهور الأعراض على الطيور في صورة زرق مد دم أو الوفيات. قيمت آثار الدواء بعد الحويصلات في الزرق، تدريج الزرق، ودرجة الآفات المرضية المعوية، و معدل اكتساب الوزن النسبي ومعدلات الإصابة والوفيات. أدى إعطاء الدواء سوءاً في العلاج أو الوقاية إلى تحسن معنوي وملحوظ في المعايير المقاسة عند المقارنة بالمجموعات الضابطة. أجريت الدراسة الحقلية على عنبري مزرعة بكل منهما ٦٠٠٠ دجاجة في عمر ٣٣ يوم مع أول ظهور لأعراض الإصابة الطبيعية وتشخيصها تم إعطاء الدواء لأجد العنابر بالجرعة العلاجية ١مللي/٤ لتر ماء شرب ليومين متصلين وترك الآخر كضابط للعلاج. أدى عد حوصلات الطفيل في الزرق وملاحظة الآفات المرضية في الأمعاء وكذا التحسن في معدلات النفوق إلى التأكد من كفاءة الاستخدام الحقلية للدواء. اوضحت نتائج هذه الدراسة المعملية والحقلية إلى التأكيد على كفاءة الـدايكلزوريل ١% السائل في ماء شرب الدجاج في الوقاية من العدوى المتعددة بطفيل الكوكسيديا وإن عترات الطفيل الحقلية والمسببة للحالات

