

**THE EFFICACY OF DICLAZURIL (LIQUID FORMULATION) IN  
THE PREVENTION AND CONTROL OF COCCIDIOSIS IN BROILER  
CHICKENS (SEMIFIELD AND FIELD TRIALS)**

By

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**SUMMARY**

This study was carried out to evaluate the efficacy of water soluble formulation of diclazuril (1%) in the prevention and control of mixed *Eimeria* species infection at the semifield (experimental) trial and also testing the efficacy of this medicament in controlling of natural field mixed *Eimeria* species infection. The semifield trial was carried out on 300, day-old broiler chicks as they were divided into 2 main equal separate groups (150 chicks; each). The first main chicken group (150 birds) was divided into 3 equal separate subgroups (A1, B1 and C1) each consisting of 50 chicks for studying the prophylactic effect of diclazuril, while the remaining 150 birds were divided into another three equal separate subgroups (A2, B2 and C2) of 50 chicks each for studying the curative efficacy of diclazuril. Groups (A1, A2, C1 and C2) 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. Groups (B1 and B2) were kept as control negative non infected and non medicated groups. Birds of group (C1) was given prophylactic diclazuril (1%) at dose of 1 ml / 8 liter of the drinking water at the 7<sup>th</sup> day before the experimental inoculation, while group (C2) was medicated with diclazuril (1%) at dose of 1 ml / 4 liter of the drinking water for 2 successive days just after appearance of bloody dropping (at the 5<sup>th</sup> day post infection). Both studies were assessed by means of oocyst count (shedding) in the dropping, dropping score, lesion score, body weight gain % and mortality rate. Groups provided 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 on 33 day-old commercial broiler chicken farm consisted of 2 flocks of 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.

**Key words:**

Coccidiosis, Diclazuril, Water soluble, Chickens, Prevention, Control.

**INTRODUCTION**

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 and 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 and 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 benzenacetonitrile 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 and Meireles et al., 2003**), turkeys (**Vanparijs et al., 1989c; McDougald et al., 1991 and Chapman et al., 2004**), pigeons (**Vercruysse, 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 and 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 semifield (experimental) trial, also testing the efficacy of this medicament in the control of naturally infected broiler chickens with mixed *Eimeria* species as a field trial.

## **MATERIAL AND METHODS**

### **1. Chickens:**

#### **A. Semifield (experimental) 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 ad libitum without any feed additives or coccidiostates. Water was given ad libitum. 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.

#### **B. 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.

### **2. 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 the semifield (experimental) trial, diclazuril was administered in a prophylactic dose of 1 ml / 8 liter of the drinking water at the 7<sup>th</sup> day before the experimental infection, while it was given as a treatment dose of 1 ml / 4 liter of the drinking water for 2 successive days once the blood was observed in the

dropping [at the 5<sup>th</sup> day post infection (PI)]. In the field trial, diclazuril was administrated in a treatment dose of 1 ml / 4 liter of the drinking water for 2 successive days after appearance of signs and mortalities.

### **3. Preparation of sporulated oocysts:**

Mixed types of *Eimeria* species non-sporulated oocysts containing *Eimeria tenella* (*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 oocysts were counted per 1.0 ml of the solution according to **Maff (1977)** and **Soulsby (1978)** using Mc-Master technique. Different sporulated oocysts of *Eimeria* species used for experimental infection of birds in the semifield (experimental) trial were identified according to their size differences (measuring 100 oocysts) as previously described by **Soulsby (1978)**. In the field trial, mixed natural infections with *Eimeria* species of *E. tenella*, *E. acervulina* and *E. necatrix* were identified according to **Soulsby (1978)**.

### **4. Experimental infection:**

Each bird of the infected and medicated groups in the semifield (experimental) trial was orally inoculated in the crop with a 1 ml solution containing 50.000 mixed species of *E. tenella*, *E. acervulina* and *E. necatrix* sporulated oocysts on the 14<sup>th</sup> day of age.

### **5. Evaluation of the drug efficacy:**

#### **A. Total oocyst count (shedding):**

The dropping of birds in the semifield (experimental) trial were collected daily from the 5<sup>th</sup> (first appearance of blood) till the 7<sup>th</sup> day post infection (PI) for testing diclazuril prophylactic efficacy, while the dropping were collected during 2 days before treatment and 7 days post treatment to test the drug curative efficacy. In the field trial, similar regimen was applied for testing the curative efficacy of the drug. 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)**.

### **B. Dropping score:**

In the semifield (experimental) trial, the dropping of each group in the preventive study were collected daily from the 5<sup>th</sup> till the 7<sup>th</sup> day (PI), while they were collected during 2 days before and 7 days post treatment in the control study of this trial and the field one. The dropping score was graded (1-4) according to the consistency of the dropping and the presence of mucus/ or blood (**Ramadan *et al.*, 1997**). Grade (1) indicated normal dropping while grade (4) indicated severe diarrhea with presence of mucus and /or blood.

### **C. Lesion scoring:**

Dead birds as well as sacrificed birds from each group from the 5<sup>th</sup> to the 7<sup>th</sup> day (PI) were examined, also dead and sacrificed birds during 2 days before and 7 days post treatment in the control study of the semifield trial and the field one 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 and Conway and Mackenzie, 1991**). A score (0) denoted no lesions whereas (5) denoted severe lesions.

### **D. The body weight gain:**

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

### **E. The mortality rate:**

The number of dead birds in both semifield and field trials were recorded daily.

## **6. Experimental design:**

### **A. 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 7<sup>th</sup> 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 5<sup>th</sup> 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 5<sup>th</sup> till the 7<sup>th</sup> day (PI), while the dropping were collected during 2 days before treatment and 7 days post treatment in the curative study for calculating the total oocyst count (shedding) and the mean dropping score. Dead and sacrificed birds from each group at the previous intervals 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) of a commercial farm (120.000 birds, aged 33 days), birds of flock (1) were considered as control positive 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.

## **7. Statistical analysis:**

The obtained data were statistically analyzed by using Analysis of Variance (ANOVA) according to **Snedecor and Corchran (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 7<sup>th</sup> 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. There were several successful trials had been done to study the effect of using diclazuril alone or in comparison with other anticoccidial drugs in the ration of chickens for the prevention of coccidial infection (**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 and Meireles *et al.*, 2003**) and the drug not only proved high efficacy, but also superceded the others in the prevention of the disease.

The effect of administration of diclazuril in the drinking water method as apposed to in the feed (the usual way), was proved to be effective. **El-Banna *et al.*, (2005)** compared between using of diclazuril in the ration and administration of it as a solution as preventive medicament in the drinking water against mixed experimental infection with *E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima* and *E. Brunetti* and the results revealed that the two methods were have the similar effect in elimination of infection as shown by increasing the body weight gain and the survival rate and reduction in the 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 (5<sup>th</sup> day PI) 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 control positive 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 previously reported by **El-Banna *et al.* (2005) and El-Dakhly *et al.*, (2006)** who reported that diclazuril in the drinking water was appropriate for use in the prevention and treatment of *Eimeria* infected chickens indicated by decrease the oocyst number and the lesion score in the treated groups than the control ones. In addition, **Jiang-ZhongQi (1999)** demonstrated that when diclazuril was given in the drinking water at concentrations (0.25, 0.5 or 1 mg / liter) for controlling of *E. tenella* infection in chickens, the body weight increased when compared with control and that improvement increased by increase the drug concentration, moreover, the losses

caused by the infection were greater in the control than the treated group and the 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) and El-Dakhly *et al.*, (2006)** confirmed the result 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 faeces.

In this study, the efficacy of diclazuril solution in the control of mixed natural infection with *Eimeria* species (field study) was shown in table (3). The results 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. In this respect, **Mukiibi-Muka *et al.*, (2001)** compared between the efficacies of water treatment with diclazuril and amprolium in controlling of natural coccidial infection and found that both drugs were effective but diclazuril was the superior in reduction of oocyst shedding.

Due to diclazuril water solubility, short duration of treatment (2 days), rapid anticoccidial action against all intracellular developmental stages of the parasite and its effectiveness against coccidial infections in all poultry species make diclazuril is not only effective, but also more superior to other anticoccidial drugs in the prevention and treatment of coccidial infection.

To conclude, addition of water soluble formulation of diclazuril (1%) in the drinking water is very efficacious for the prevention and control of experimental and natural infections with mixed *Eimeria* species.

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

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

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

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

Group No.	Treatment	Total No. of oocysts/gm dropping x 10 <sup>3</sup>		Mean dropping score	Mean lesion score	Mortality rate	Body weight gain %
		2 days before treatment	7 days post treatment				
A2	Infected non treated	8.9±0.69 <sup>a</sup>	55.03±4.02 <sup>a</sup>	4.6±0.3 <sup>a</sup>	3.7±0.89 <sup>a</sup>	(38/50) 76	31.3±2.13 <sup>b</sup>
B2	Non infected non treated	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>c</sup>	1.0±0.0 <sup>b</sup>	0.0±0.0 <sup>c</sup>	(0/50) 0	42.04±1.05 <sup>a</sup>
C2	Diclazuril	7.81±0.87 <sup>a</sup>	1.95±0.11 <sup>b</sup>	1.5±0.2 <sup>b</sup>	1.79±0.95 <sup>b</sup>	(6/50) 12	40.99±1.08 <sup>a</sup>

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

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

Flock No.	Treatment	Total No. of oocysts/gm dropping x 10 <sup>3</sup>		Mean lesion score	Mortality rate
		2 days before treatment	7 days post treatment		
1	Infected non treated	9.47±0.46	60.12±4.11 <sup>a</sup>	3.2±0.33 <sup>a</sup>	(90/6000) 1.5
2	Diclazuril	8.99±0.05	0.37±0.16 <sup>b</sup>	1.11±0.74 <sup>b</sup>	(20/6000) 0.3

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

## REFERENCES

- Awaad, M. H.; Manal, A. Afify; Sahar, A. Zouelfakar and Hilali, M. A. (2003):** Anticoccidial efficacy of steroidal sapogenins (organic coccidiostate) in broiler chickens (semi-field and field trials). *Egypt. Vet. Med. Soci. Parasitol. J.*, 1 (1): 123-136.
- Biggs, P. M. (1982):** The world of poultry disease. *Avian Pathol.*, 11: 281-300.
- Brander, G. C.; Pugh, D. M; Bywater, R. J. and Jenkins, W. L. (1991):** *Veterinary Applied Pharmacology and Therapeutic*, 5<sup>th</sup> edn. Bailliere Tindall, London, 552 pp.
- Chapman, H. D. (1987):** Control of *E. tenella*, partly resistant to monensin, by including toltrazuril discontinuously in the drinking water of chickens. *J. Comp. Pathol.*, 97: 21-27.
- Chapman, H. D. (1989):** *Eimeria tenella*, *E. acervulina* and *E. maxima*: Studies on the development of resistance to diclazuril and other anticoccidial drugs in chickens. *Parasitol.*, 99 (2):189-192.
- Champan, H. D. (1998):** Evaluation of the efficacy of anticoccidial drugs against *Eimeria* sp. in the fowls. *Int. J. Parasitol.*, 28: 1141-11440.
- Chapman, H. D.; Matsler, P. L. and Chapman, M. E. (2004):** Control of coccidiosis in turkeys with diclazuril and monensin: effects upon performance and development of immunity to *Eimeria* species. *Avian Dis.*, 48(3):631-640.
- Conway, D. P. (1979):** Examination of lesions and lesion scoring: 17-36 in (Poultry coccidiosis Diagnostics and Testing procedures). Pizer, Int. Inc. New York, USA.
- Conway, D. P. and Mackenzie, M. E. (1991):** Poultry coccidiosis. Diagnostic and testing procedures. Second Ed. Pfizer.
- Conway, D. P.; Mathis, G. F. and Lang, M. (2002a):** The use of diclazuril in extended withdrawal anticoccidial programmes: 1. Efficacy against *Eimeria* spp. In broiler chickens in floor pens. *Poultry Sci.*, 81 (3): 349--352.
- Conway, D. P.; Mathis, G. F. and Lang, M. (2002b):** The use of diclazuril in extended withdrawal anticoccidial programmes: 2. Immunity to *Eimeria tenella* challenge after drug withdrawal. *Poultry Sci.*, 81 (3): 353-355.
- Conway, D. P.; Mathis, G. F.; Johnson, J. and Baldwin, C. (2001a):** The use of diclazuril in extended withdrawal anticoccidial programmes: 1. Efficacy against *Eimeria* species in broiler chickens in floor pens. *Poultry Sci.*, 81: 349-352.
- Conway, D. P.; Mathis, G. F.; Johnson, J; Schwartz, M. and Baldwin, C. (2001b):** Efficacy of diclazuril in comparison with chemical ionophorous anticoccidials against *Eimeria* spp. in broiler chickens in floor pens. *Poultry Sci.*, 80 (4): 426-430.
- El-Banna, H. A.; El-Bahy, M. M.; El-Zorba, H. Y. and El-Hady, M. (2005):** Anticoccidial efficacy of drinking water soluble diclazuril on experimental and field coccidiosis in broiler chickens. *J. Vet. Med.(series A)*. 52 (6): 287-291.
- El-Dakhly, Kh. M.; Azza A. El-Sawah; Shalaby, A, A. and El-Nesr, Kh, A. (2006):** The efficacy of *Lactobacillus acidophilus* and/or diclazuril for inhibition and control of *Eimeria tenella* infection in balady chicks. *Kafr El-Sheikh Vet. Med. J.*, 4 (1): 1-18.
- Jiang-ZhongQi (1999):** Study of the efficacy of diclazuril solution in *Eimeria tenella* inoculated chicks. *Zhejiang Nongye Kexue*, 1: 44-46.

- Johnson, J. and Reid, W. M. (1970):** The development of *E. tenella* in germ free chickens. Fourth International Symposium on Germfree Research. New Orleans, La. April: 16-20.
- Kawazone, U. and Fabio, J. D. (1994):** Resistance to diclazuril in field isolates of *Eimeria* sp. obtained from commercial broiler flocks in Brazil. Avian Pathol., 23: 305-311.
- Kiaei, M. M.; Rahbari, S.; Modirsanei, M.; Rahimi, R. and Aref-Pazhouhi, P. (2001):** The effect of Artemisia sieberi and a chemical anticoccidial drug on control of coccidiosis and broiler chicks performance. J. Fac. Vet. Med., Univ. Tehran. 56 (4): 53-57.
- Maff, A. (1977):** Technical Bulletin no. 18: Manual of veterinary parasitological laboratory techniques. Ministry of Agriculture, Fisheries and Food, London. pp. 58-91.
- McDougald, L. R.; Mathis, G. F. and Seibert, B. P. (1990a):** Anticoccidial efficacy of diclazuril against recent field isolates of *Eimeria* from commercial poultry farms. Avian Dis., 34 (4):911-915.
- McDougald, L. R.; Mathis, G. F. and Seibert, B. P. (1991):** Anticoccidial efficacy of diclazuril against recent field isolates of *Eimeria* from turkeys farms in the United States. Avian Dis., 35 (4): 863-868.
- McDougald, L. R.; Seibert, B. P.; Mathis, G. F. and Quarles, C. L. (1990b):** Anticoccidial efficacy of diclazuril in broilers under simulated natural conditions in floor pens. Avian Dis., 34 (4):905-910.
- Meireles, M. V.; Kavavata, G. M.; Almeida, S. M.; Hisano, M. and Santos, R. F. (2003):** Evaluation of resistance to anticoccidial drugs in field isolates of *Eimeria* oocysts from broiler farms. Revista Brasileira de Ciencia Veterinaria. 10 (2): 72-77.
- Mukiibi-Muka, G.; Otim, M. O.; Musisi, G; Lango, J; Galiwango, T. and Olaho-Mukani, W. (2001):** Comparative study on the efficacy of diclazuril and amprolium in naturally infected broilers in Uganda. Revue-d'Eleve-et-de-Medecine-Veterinaire-des-Payes-Tropicaux. 54 (1): 33-35.
- Ramadan, A.; Abo El-Sooud, K. and El-Bahy, M. M. (1997):** Anticoccidial efficacy of toltrazuril and halofuginone against *Eimeria tenella* infection in broiler chickens in Egypt. Res. Vet. Sci., 62: 175-178.
- Reid, W. M.; Brewer, R. N.; Johnson, J.; Taylor, E. M.; Hegde, K. S. and Kowalski, L. M. (1969):** Evaluation of techniques used in studies on the efficacy of anticoccidial drugs in chickens. American J. Vet. Res., 30: 447-459.
- Shirley, M. W. (1986):** New methods for the identification of species and strains of *Eimeria*. In L. R. McDougald, P. L. Long and L. P. Joyner (Eds.), Research in Avian Coccidiosis, Proceedings of the Georgia Coccidiosis Conference 1985 (pp. 13-35). University of Georgia, Athens, GA.
- Snedecor, G. W. and Corchran, W. G. (1980):** Statistical Methods. Iowa State University Press, Ames, IA.
- Soulsby, E. J. L. (1978):** Helminthes, Arthropod and Protozoa of domestic animals. 6<sup>th</sup> ed. Bailliere and Tindall, London.
- Vieira, M. and Clemente. (1995):** Anticoccidial efficacy of diclazuril in broilers. Veterinaria Tecnica., 5 (4): 36-39.
- Vanparijs, O.; Desplenter, L.; Marsboom, R. (1989a):** Efficacy of diclazuril in the control of intestinal coccidiosis in rabbits. Vet Parasitol., 34 (3):185-190.

- Vanparijs, O.; Desplenter, L.; Marsboom, R. (1989b):** Diclazuril, a new broad spectrum anticoccidial drug in chickens: 1. dose titration studies and pilot floor pen trials. *Poultry Sci.*, 68 (4): 489-495.
- Vanparijs, O.; Desplenter, L.; Marsboom, R. (1990):** Anticoccidial efficacy of diclazuril in pheasants. *Vet. Rec.*, 126 (14):332-333.
- Vanparijs, O.; Hermans, L.; Marsboom, R. (1989c):** Efficacy of diclazuril against turkey coccidiosis in a floor pen experiment. *Avian Dis.*, 33 (3): 479-481.
- Vanparijs, O.; Hermans, L.; Marsboom, R. (1991):** Anticoccidial efficacy of diclazuril in partridges. *Vet. Rec.* 129: 339-340.
- Vanparijs, O.; Hermans, L.; van der Flaes, L. and Marsboom, R. (1989d):** Efficacy of diclazuril in the prevention and cure of intestinal and hepatic coccidiosis in rabbits. *Vet. Parasitol.*, 32 (2-3):109-117.
- Vanparijs, O.; Marsboom, R. ; Hermans, L. and van der Flaes, L. (1989e):** Diclazuril, a new broad spectrum anticoccidial drug in chickens: 2. battery trials. *Poultry Sci.*, 68(4):496-500.
- Vanparijs, O.; Marsboom, R. ; Hermans, L. and van der Flaes, L. (1989f):** Diclazuril, a new broad spectrum anticoccidial for chickens: 3. floor pen trials. *Poultry Sci.*, 69(1): 60-64.
- Varheven, A.; Maes, L; Coussement, W.; Vanparijs, O.;Lauwers, F.; Vlamincx, E. and Marsboom, R. (1989):** Ultrastructural evaluation of the effects of diclazuril on the endogenous stages of *Eimeria maxima* and *Eimeria brunetti* in experimentally inoculated chickens. *Parasitol. Res.*, 75 (8): 604-610.
- Vercruysse, J. (1990):** Efficacy of toltrazuril and clazuril against experimental infections with *Eimeria labbeana* and *Eimeria columbarum* in racing pigeons. *Avian Dis.*, 34 (1):73-79.
- Williams, R. P. (1998):** Epidemiological aspects of the use of live anticoccidial vaccines for chickens. *Int. J. Parasitol.*, 28: 1089-1098.
- Williams, R. P. (1999):** A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry. *Int. J. Parasitol.*, 29: 1209-1229.
- Xie, M.; Cai, J.; Li, A. and Peng, X. (2001):** Coccidiosis of domestic fowl in China. *Proceedings of the VIII<sup>th</sup> International Coccidiosis Conference, Palm Cove* (pp. 153-154). Sydney, Australia.