

## COMPARATIVE EFFICACY OF DIFFERENT TOLTRAZURIL DOSES AGAINST COCCIDIOSIS IN BROILER CHICKENS

**Rashid, M. I.; Akbar, H.; Al-Araby, M.A.\*; Ismael, A.B.\*\*;**

**Shehzad, W.; Ashraf, K. and Hashmi, H. A.**

Dept. of Parasitology, University of veterinary & animal sciences, Lahore, Pakistan.

\* Dept. of Parasitology, Fac. Vet. Med., Mansoura University, Egypt.

\*\* Dept. of Animal Medicine, Fac. Vet. Med., Zagazig University, Egypt.

### ABSTRACT

The present study was designed to evaluate the therapeutic and prophylactic efficacy of Toltrazuril at different doses against coccidiosis. One hundred and twenty, one day old chicks, were divided into 6 groups, each of 20 chicks. Group A was left as non-infected non-medicated control. Chicks of group B were infected and non-medicated while chicks of group C, D, and E were treated with Toltrazuril in drinking water at rate of 7 (25ppm), 3.5 (12.5ppm), and 1.75 (6.25ppm) mg/kg body weight on day 18 and 19, 25 and 26 respectively. In chicks of group F, Toltrazuril was given as prophylactic at rate of 7mg/kg body weight on days 3 and 10. Chicks of groups (B, C, D, E and F) were challenged orally with 20,000 sporulated oocysts of *E. tenella* on the 16<sup>th</sup> and 26<sup>th</sup> day of their age. Evaluation of the Toltrazuril efficacy was based on oocysts counts per gram (OPG) of faeces, weight gain, mortality and postmortem findings. The maximum reduction of OPG counts were detected in group D (98.04%) and group E (85.39%). Concerning the mean body weight gains as compared to healthy control, group D showed best performance, followed by group E, group C and group F. Chicks of group B showed lowest performance.

**Conclusions:** Ultimately, it was concluded that Toltrazuril at rate of 3.5 mg/kg would be the best solution to solve coccidiosis problem.

**Keywords:** Coccidiosis, *Eimeria*, Toltrazuril, Broiler.

### INTRODUCTION

Intestinal coccidiosis, caused by various species of *Eimeria*, is an economically important, (estimated to be 2 billion dollars a year), infectious disease of poultry and reared livestock throughout the world (Zhang and Zeng, 2005). *Eimeria* spp. are belonging to the phylum Apicomplexa causing coccidiosis of farm

animals and birds. *Eimeria tenella* is the most important species, as it causes caecal coccidiosis in chickens (Shirley, 2000). *Eimeria tenella* primarily invades and resides in the linings of caeca of exposed chickens (Vervelde et al., 1995 and Yun et al., 2000).

Anticoccidial drugs will remain important

for a long time, although resistance development could limit their use (Stephen et al., 1997). Anticoccidial therapeutic agents must fulfill some main criteria including a high level of efficacy against all developmental stages of pathogenic *Eimeria* species infecting poultry and at the same time, they shouldn't interfere with the immune response of the host during and after treatment of coccidial infections at therapeutic dosages (El-Banna et al., 2005). Toltrazuril is a symmetrical triazinetrione compound and 2.5% oral solution has been shown to be effective against all species of *Eimeria* infecting chickens (Mehlhorn et al., 1988). It is active against all intracellular developmental stages including those of schizogony and gametogony (Mehlhorn et al., 1984). Toltrazuril has chemoprophylactic (Gjerde and Helle 1991) and therapeutic effects (Chapman, 1987; Mehlhorn et al., 1988; Mathis et al., 2004; Ghanem et al., 2008) against coccidiosis and does not interfere with the development of immunity (Grief, 2000). Chemoprophylaxis with Toltrazuril enhances immunity development (Grief, 2000). It has been proved that therapeutic medication with toltrazuril protects the birds from clinical coccidiosis (Ramadan et al., 1997).

In the present study, the therapeutic effect of Toltrazuril at different doses as well as its prophylactic efficacy against coccidiosis were investigated. The comparative effects on weight gain, oocyst counts (OPG), mortality and postmortem lesions were also studied.

## 2. MATERIAL AND METHODS

**2.1. Experimental sheds and feed:-** Before the arrival of chicks, the experimental sheds of the Parasitology Department, Univer-

sity of Veterinary & Animal Sciences, Lahore were well washed with water and disinfected with potassium permanganate solution. The sheds were then white-washed and finally fumigated with potassium permanganate and formalin (1:10) for 48 hours. Later on, the room was well ventilated to get rid of the remaining fumes of potassium permanganate and formalin reaction. The birds were fed on commercial poultry coccidiostat free starter ration No. 4 and finisher ration No. 5, donated by Kashmir Feed Mill Limited, Lahore.

**2.2. Chickens:-** One hundred and twenty (120), one day old broiler chicks were purchased from the local hatchery. The chicks were reared under standard hygienic conditions in a clean shed of University of Veterinary & Animal Sciences, Lahore on deep litter system. A commercial feed, free of coccidiostat was provided ad-lib. The electric light was also provided round the clock during the experimental period. All the birds were vaccinated against New Castle Disease (N.D) on 1<sup>st</sup> and 21<sup>th</sup> day of age.

**2.3. Drugs:-** Toltrazuril solution (Baycox<sup>®</sup>, 2.5%) was obtained from Bayer (Leverkusen, Germany). It was administered in different concentrations in drinking water.

**2.4. Coccidial infections:-** The challenge oocysts were isolated from the caeca of naturally infected chickens and then sporulated as described by (Soulsby, 1982). The infected caeca of birds were obtained from the Diagnostic Laboratory of Veterinary Research Institute, Lahore and Livestock, Dairy Development Department, Cooper Road, Lahore. The sporulated oocysts were cleared and counted

per 1.0 ml of the solution using the McMaster technique. The counted sporulated oocysts of *E. tenella* field strain (20,000/bird, orally) were used for experimental infection.

#### Collection and sporulation of coccidial oocysts

- The collected caecal material with the oocysts was mixed with about 20 times of its volume with water and strained through the sieve.
- The fluid mixture was left to be sedimented for 15-20 minutes.
- The supernatant fluid was poured off gently to avoid agitation.
- The sediment was rewashed several times, then the supernatant fluid was decanted and sediment was mixed with sugar solution (150 gm/ 100ml water).
- Centrifuged at about 1500 r.p.m. for 5 minutes.
- The supernatant layer was harvested by touching the surface with wire loop or pipette and placed in glass vials containing small quantity of distilled water.
- The oocystic suspension was mixed with 2.5% potassium dichromate solution and placed in a shallow layer of 2 mm depth in Petri-dishes at temperature 26°C, examined microscopically every 4-6 hours a day to determine the sporulation time. Fresh smears were made from Petri dishes to find out the sporulation time of the oocysts. At each time, the percentage of sporulation was reported.

**2.5. Experimental design:-** At the 3<sup>rd</sup> day of age, the birds were randomly divided into 6 groups, each of 20 birds (Table 1). The groups

were named as A, B, C, D, E and F. Group A was reared as non-infected, non-medicated control group. Group B was kept as infected, non-medicated control group while group C, D, E were treated with toltrazuril orally in drinking water at the rate of 7mg/kg (full dose), 3.5mg/kg (half dose) and 1.75 mg/kg (1/3 of dose) respectively on two occasions (18 and 19 days, 25 and 26 days). Group F was administered with 7mg/kg of Toltrazuril on day 3 and 10 to study its prophylactic effect. Birds in groups B, C, D, E and F were challenged orally with 20,000 sporulated oocysts of *E. tenella* on the 16<sup>th</sup> and 26<sup>th</sup> of their age (Table 1).

#### 2.6. Evaluation of the drug efficacy

**2.6.1. Oocysts count from faeces:-** Oocysts per gram (OPG) counts of faeces for 5 groups (B, C, D, E and F) was carried out on day 3, 7 & 10 post each challenge dose. The OPG counts were performed by the McMaster Egg Counting technique as suggested by (Anonymous, 1986). All the oocysts within the ruled area (1cm<sup>2</sup>) of each chamber were counted using the 10X objective and 10X eyepiece, and the mean value (X) was calculated as following: number of oocysts per gms faeces =  $X / 0.15 \times 45 \times 1/3 = X \times 100$ .

**Where :** X = average number of oocysts in the counting chamber of McMaster slide, 0.15 = volume of samples in 1 cm<sup>2</sup>, 45 = total volume of sample, i.e. 3 g faeces + 42 ml water, 1/3 = correction to 1 gm of faeces.

**2.6.2. Weight gain:-** Weight gains were recorded on day 19, 23, 26, 29, 33 and 36 of age.

**2.6.3. Mortality and postmortem findings:-** Mortalities occurring during the whole of experimental period were recorded and postmortem lesions were observed. The smears prepared from these lesions were examined to confirm the presence of developmental stages and / or oocysts.

**2.7. Statistical analysis:-** So collected data was analyzed statistically by applying two ways analysis of variance (ANOVA) for weight gain to compare the performance of different groups and the efficacy of different concentrations of the used drug.

### 3. RESULTS

**3.1. OPG counts:-** The effect of Toltrazuril on OPG counts was shown in Table (2). In Group A, OPG counts remained "0" throughout the experimental period (healthy control). In case of group B, means of OPG ranged between 5000 and 395,000 showing decline from day 20 to 24 of age then a sharp rise depicted peak of 395,000 OPG counts on day 27 followed by sharp decline by day 29 up to 200,000 OPG which further declined to 125,000 and again rose to 175,000 by the end of experiment. Birds of group C showed similar pattern up to day 29 as shown by group B up to day 27. At day 30-32, mortality started. The range of OPG counts remained between 4000 and 345,500. In response to the treatment, a sharp decline in the OPG counts was experienced by day 33. Then again rise in OPG count occurred during the following period up to the end of experiment. In group D, the mean OPG counts were extremely lower than group B & C and remained suppressed up to day 33 (0-10,000 OPG) which showed slight rise up to 10,000 OPG by the end of the

experiment. It was noticed that the OPG counts remained within the range of 8,500-42,000 in group E. While group F showed range of OPG counts between 0 and 57,000. This was far lower than B and C groups. Gradual increase in OPG counts reached up to 57,000 by day 29 which declined up to 3,500 by day 33 which again rose to 52,000 by day 36.

**3.2. Mean body weight record:-** The effect of Toltrazuril on body weight was shown in Table (3). Maximum mean weight gained by the chicks of non-infected healthy chicks of group A was only 1064 gm (75.09 %) and the mean weight of other groups remained under this limit. The mean weight of chicks of group B was only 686.5 (67.80 %) body weight gains while group C acquired 70.29 % mean weight per bird where the mean weight of chicks was 710 gm. Group D showed mean weight gains by the end of the experiment as 648 (70.81%). Group E showed mean body weight gain as 663.5 gm / bird and the increase was calculated as 70.77%. Group F showed mean weight gains by the end of the experiment as 732 gm / bird (70.24%).

**3.3. Mortality recorded with post-mortem finding:-** A total of 22 chicks died during the experimental period. On day 19, there were two mortalities in group B, one in C, one in D, and two in group E. The mean oocyst counts in groups B, C, D and E were 222200, 131400, 119460, and 113240 respectively. On day 25, four mortalities in B, one in C, four in E and one in F occurred showing the OPG counts as 137375, 14350, 149370, and 14650 respectively. On day 26, three mortalities occurred in groups B and

two in C in which mean OPG counts were 74775, 44550. The last mortality occurred in groups D in which 62675 OPG count was observed on day 33. This showed that mortality occurred when OPG counts reached over 14000 mean. The details are given in Table 4.

The postmortem examination of the chicks was performed. Affected intestines and other organs were examined. There were severe haemorrhages in the cacca which were also markedly swollen. The liver and lungs were dehydrated and pale (Table 4).

#### 4. DISCUSSION

The current study was conducted to estimate the prophylactic and curative efficacy of Toltrazural at different doses on *Elmeria tenella* in Parasitology Department, University of Veterinary & Animal Sciences, Lahore.

The picture of OPG counts in group F showed that the birds became immune to some extent after second dose of infection on day 26. However, due to high challenge (infection, the immunity remained fluctuating. Similar findings have been observed by (Lillehoj and Trout 1996) who reported that host immune responses to coccidial infection were complex and claimed that antibody mediated responses played a minor role in protection against coccidiosis whereas cell mediated immunity played a major role in resistance to infection.

The first dose of infection given on day 16 in groups B and C showed similar behavior up to day 24 irrespective to the fact that group C was given medication on day 18 and 19. This showed that there was no persistent

effect of medicine in the test group. Similarly, infection given on day 26 multiplied and was slightly affected by the medication given on days 25 & 26 but again high peak occurred by day 29 but sharp decline has been observed due to medication on day 32 to control mortality. It was observed that even the full dose of Toltrazuril could not suppress the multiplication persistently and resultantly the OPG counts again rose to the level of 64,000 by the end of experiment on day 36. The findings were in agreement with that of (Laczay et al., 1995) who reported that Toltrazuril was more effective when treatments were initiated 24 hours post infection but when medication was delayed up to 72 hours post-infection, the treatment was less effective. In the present findings the treatment was done 48 hours post infection, therefore, the medicine could not show its full efficacy.

Group F which was administered with Toltrazuril (7.0 mg/kg) as a prophylactic medicine on days 3 and 10, showed range of OPG counts between 0 and 57,000. This was far lower than B and C groups. This depicts prophylactic efficacy of the Toltrazuril. No mortality occurred in this group. The present findings substantiate with the findings of (Grief, 2000) who claimed that during evaluation studies, Toltrazuril acted against all intracellular schizonts and being correlated with a higher reduction in oocyst excretion, lesion scoring and increased weight gains.

According to the reduction in OPG counts as compared to infected control group B, the maximum reduction (98.04%) was observed in group D which was given half (3.5mg/kg) of the recommended dose of toltrazuril. In

descending order, group E was placed as 2nd position as the reduction in total OPG counts was noticed as 85.39%. This group was administered 1/4<sup>th</sup> of the recommended dose (1.75mg/kg). Group F secured the 3rd position in reduction of OPG counts by 83.65%. This group was administered full dose (prophylactic) at day 3 and 10 of age. The result could have been quite better as claimed by (Hashmi et al., 1994). However, it was noticed that group C showed the poorest reduction (38.09%) which was given full dose (7mg/kg) as recommended by the manufacturer.

Chapman (1987) reported that Toltrazuril at 50 ppm was very effective in controlling coccidial infection if given for periods of 3 days, on 2 or 3 occasions at weekly intervals. He claimed that following treatment with Toltrazuril, birds inoculated with high dose of oocysts were immune to subsequent challenge. The findings of the present study were partially supported by Chapman (1987) in terms of prophylactic effect of Toltrazuril given at rate of 7mg/kg but this dose (being lesser) could not provide appropriate protection as quoted by him. The action of Toltrazuril has been reported by (Mehlhorn et al., 1986) that intracellular stages (Schizonts and Gamonts) were destroyed as drug could pass through the host cell membrane and the cytoplasm, thereby ensuring its use as therapeutic. This fact was also supported by Harder and Haberkorn (1989), who reported that Toltrazuril killed E. tenella schizonts in chicken kidney-cell cultures. Grief, (2000) supported the idea and claimed that these damaged stages could remain in the host cell for prolonged time, during which they act as antigens which could be recognized by immune systems. Due

to this effect toltrazuril could act as prophylactic drug. Timings for immunization or administration of prophylactic dose was set as day 3 and 10 in the present experiment which has been supported by (Mathis et al., 2004) who studied the appropriate time to administer Toltrazuril for the control of eoccidiosis in broilers. They claimed that treatment at days 2-3 provided good coccidiosis control with accompanying performance.

From the body weight records, it appeared that the chicks were of low quality and maximum mean weight gained by the chicks of non infected healthy chicks of group A was only 1064 gm and the mean weight of other groups remained under this limit. Moreover, it was also noticed that multiplication of the oocysts administered to test groups was also higher than usual. The members of group D gained 70.81% and the group was placed at 2nd position. The findings of the present study are correlated with Gjerde and Helle (1991) who reported that Toltrazuril at 20 mg/kg improved weight gains in coccidiosis infected animals. Similarly, Ramadan et al. (1997) have also claimed that Toltrazuril at 37.5, 75 and 150 ppm improved the body weight gains and survival percentage. Also El-Banna et al. (2005) obtained similar results using Toltrazuril 25 ppm.

In conclusion, it has been observed that lower doses (3.5mg/kg and 1.75 mg/kg) of toltrazuril proved far better than the higher doses in terms of weight gain and reduction in OPG counts. It was confirmed that the Toltrazuril at rate of 3.50 mg/kg would be the best solution to the coccidiosis problem.

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Table 1 : Experimental protocol.

Groups	Days of Age													
	1	3	10	14	16	18	19	21	23	25 & 26	28	29	33	36
<b>A</b> (n=20)	NDV	-	-	Wt	-		Wt	NDV	Wt	Wt	Wt	Wt	Wt	Wt
<b>B</b> (n=20)	NDV	-	-	Wt	INF.		Wt, OPG	NDV	OPG, Wt	OPG, INF INF, Wt	Wt	OPG, Wt	OPG, Wt	OPG, Wt
<b>C</b> (n=20)	NDV	-	-	Wt	INF	TLT	Wt, OPG, TLT	NDV	OPG, Wt	OPG, INF TLT, Wt	Wt	OPG, Wt	OPG, Wt	OPG, Wt
<b>D</b> (n=20)	NDV	-	-	Wt	INF	TLT	Wt, OPG, TLT	NDV	OPG, Wt	OPG, INF TLT, Wt	Wt	OPG, Wt	OPG, Wt	OPG, Wt
<b>E</b> (n=20)	NDV	-	-	Wt	INF	TLT	Wt, OPG TLT	NDV	OPG, Wt	OPG, INF TLT, Wt	Wt	OPG, Wt	OPG, Wt	OPG, Wt
<b>F</b> (n=20)	NDV	TLT*	TLT*	Wt	INF		Wt, OPG	NDV	OPG, Wt	OPG, Wt,INF	Wt	OPG, Wt	OPG, Wt	OPG, Wt

A, Non-infected non-medicated control (Negative control); B, Infected Non-medicated Control (Positive control); C, Medicated with full dose of Toltrazuril; D, Medicated with half dose of Toltrazuril; E, Medicated with quarter dose of Toltrazuril; F, Toltrazuril Prophylactic Group. OPG, Oocysts per gram of faeces; INF, 20,000 virulent sporulated-oocysts of *Eimeria tenella*; NDV, Newcastle disease vaccine; TLT, Toltrazuril medication; TLT\*, Toltrazuril prophylaxis; Wt, Weight.

Table 2 : Effect of toltrazuril at different doses on OPG counts (mean  $\times 10^3$ ) of different groups.

Groups	Days of Age					
	19	23	26	29	33	36
<b>A</b>	0	0	0	0	0	0
<b>B</b>	450	50	3950	2000	1250	1750
<b>C</b>	435	40	1050	3455	230	640
<b>D</b>	40	5	25	15	0	100
<b>E</b>	450	85	215	100	420	110
<b>F</b>	0	170	250	570	35	520

A, Non-infected non-medicated control (Negative control); B, Infected Non-medicated Control (Positive control); C, Medicated with full dose of Toltrazuril; D, Medicated with half dose of Toltrazuril; E, Medicated with quarter dose of Toltrazuril; F, Toltrazuril Prophylactic Group. OPG, Oocysts per gram.



**Table 3 :** Effect of toltrazuril at different doses on weight gain record (mean in gm) of different Groups.

Groups	Days of Age						Total Weight Gain	Percentage %
	19	23	26	29	33	36		
A	265	394	616	658	900	1064	799	75.09 %
B	326	436	593	639	854	1012.5	686.5	67.80 %
C	300	450	600	630.5	818	1010	710	70.29 %
D	267	372	522	562	746	915	648	70.8 %
E	274	406	547	608	875	937.5	663.5	70.7 %
F	310	435	567	677.5	895	1042	732	70.2 %

A, Non-infected non-medicated control (Negative control); B, Infected Non-medicated Control (Positive control); C, Medicated with full dose of Toltrazuril; D, Medicated with half dose of Toltrazuril; E, Medicated with quarter dose of Toltrazuril; F, Toltrazuril Prophylactic Group.

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**Table 4 :** Mortality chart and post-mortem findings along with OPG counts (mean  $\times 10^3$ ) of relative groups.

Age of Birds	Groups												Postmortem findings*
	A		B		C		D		E		F		
	MN	OPG	MN	OPG	MN	OPG	MN	OPG	MN	OPG	MN	OPG	
19	-	-	2	222.2	1	131.4	1	119.5	2	113	-	-	Internal organs were pale & anaemic
25	-	-	4	137.4	1	143.5	-	-	4	149.4	1	146.5	Caeca were rod & swollen, internal organs were dehydrated & pale
26	-	-	3	747.8	2	445.5	-	-	-	-	-	-	Intestinal haemorrhages, liver and lung become dehydrated & pale (caeca were markedly swollen)
33	-	-	-	-	-	-	1	626.8	-	-	-	-	Caeca & markedly & r color

A, Non-infected non-medicated control (Negative control); B, Infected Non-medicated Control (Pr  
C, Medicated with full dose of Toltrazuril; D, Medicated with half dose of Toltrazuril;  
E, Medicated with quarter dose of Toltrazuril; F, Toltrazuril Prophylactic Group.  
MN, Mortality number; OPG, Oocysts per gram.

\*Coccidiosis confirmed by microscopic examination and postmortem.

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## الملخص العربي

## مقارنة التأثير العلاجي للجرعات المختلفة لدواء التولترازويل على مرض الكوكسيديا في بدارى الدجاج

د / محمد عمران رشيد  
 د / مصطفى عبدالسلام العربي  
 د / وسيم شهذاد  
 د / هارون أكبر  
 د / علاء بسيوني إسماعيل\*\*  
 د / خالد أشرف  
 د / حجي أحمد هاشمي

قسم الطفيليات - جامعة الطب البيطري وعلوم الحيوان بلاهور، باكستان

قسم الطفيليات - كلية الطب البيطري - جامعة النصورة\*

قسم طب الحيوان - كلية الطب البيطري - جامعة الزقازيق\*\*

أجريت هذه الدراسة لتقييم التأثير العلاجي والوقائي لدواء التولترازويل "البايكوكس" بجرعاته المختلفة على مرض الكوكسيديا في الدواجن. أجريت هذه التجربة على عدد 120 كتكوت عمر يوم تم تقسيمهم إلى 6 مجموعات متساوية، المجموعة الضابطة (أ) غير معداة وغير معالجة، أما المجموعات ب، ج، د، هـ، و فقد تم عداوهم بالطور المتواصل للأيميراتينيل بمعدل 20.000 حوصلة ناضجة / كتكوت في اليوم 16 من العمر وكذلك تم تكرار العدوى بجرعة ماثلة في اليوم 26

وقد تركت المجموعة (ب) بدون علاج، أما المجموعات (ج، د، هـ) فقد تم علاجها باستخدام دواء التولترازويل بجرعات مختلفة في الأيام 18.17 و 26.25 من العمر، المجموعة (ج) تم علاجها بجرعة كاملة من التولترازويل (7 مجم / كجم وزن حي) بمعدل 25 مجم/لتر/يوم، المجموعة (د) تم علاجها بنصف الجرعة (3.5 مجم/كجم وزن حي) بمعدل 12.5 مجم/لتر/يوم، أما المجموعة (هـ) فقد تم علاجها بربع الجرعة (1.75 مجم / كجم وزن حي) بمعدل 6.5 مجم/لتر/يوم، أما المجموعة (و) فقد تم إعطاؤها دواء التولترازويل وقتياً في الأيام 10&3 (بجرعة 7 مجم/كجم وزن حي)، وقد تم تقييم الكفاءة العلاجية لدواء التولترازويل عن طريق حساب عدد الحوصلات في جرام الزرق للطيور المعدية، حساب الوزن المكتسب للطنان، معدل التفوق وكذلك الصفة التشريحية للطيور الميتة.

وقد أوضحت أن أقل معدل لحوصلات الأيميراتينيل كان في مجموعة (د) وهو 94.04% ثم مجموعة (هـ) حيث كان 85.39%، أما بالنسبة للزيادة المكتسبة في وزن الطائر فقد كانت أفضل ما يمكن في المجموعة الضابطة أ (75.09%) أما بالنسبة للمجموعات المعدية معيلاً فكانت أقل من ذلك وكانت أحسن نسبة في المجموعة د (70.8%)، ثم في مجموعة هـ (70.7%)، ثم في مجموعة ج (70.29%)، ومجموعة و (70.2%)، أما بالنسبة للمجموعة ب والتي لم تتناول أي علاج فقد كان معدل الوزن المكتسب للطائر أقل ما يمكن (67.8%).

وفي الخلاصة فإن استخدام التولترازويل بنصف جرعه يعتبر هو الحل الأمثل لمشكلة الكوكسيديا.

الكلمات الدالة : كوكسيديا، أيميرسا، بدارى الدجاج، التولترازويل.