

COMPARATIVE STUDY ON THE EFFICACY OF A PROBIOTIC AND DIFFERENT ANTICOCCIDIAL DRUGS AGAINST *EIMERIA TENELLA* INFECTION IN BROILER CHICKENS

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SUMMARY

The aim of this study was to determine the proposed prophylactic anticoccidial effects of a probiotic compound containing *Pediococcus acidilactici*, natural microflora producing lactic acid, (as a natural immunopotentiator) compared with the treatment effects of anticoccidial drugs (amprolium, sulphaquinoxaline and toltrazuril) against experimental infection with *Eimeria tenella* (*E. tenella*) in broiler chickens. One hundred and fifty, day old chicks were reared till 15 days of age, where fifty birds of them were divided into two equal groups (25 chicks each), group (1) was kept as control negative non infected and non treated, while the other group (3) was

supplemented with a probiotic containing *Pediococcus acidilactici* in the feed (100 gm / ton) for 3 days before infection; as a prophylactic treatment. At 18th day of age, the group supplemented with the probiotic and the rest of 100 birds were inoculated with 100.000 sporulated *E. tenella* oocysts orally in the crop then (100) birds were divided into 4 equal separate groups 2, 4, 5 and 6 (25 birds, each). Group (2) was kept as control positive infected non treated one. At 24th day of age (the 6th day after infection), group (4) and group (5) were treated with amprolium (1 ml / liter of the drinking water) and sulphaquinoxaline (1 ml / liter of the drinking water) for 5 successive

days, respectively, while birds of group (6) were given toltrazuril in the drinking water (1 ml / liter) for 2 successive days. Body weight (BW), body weight gain (BWG), relative growth rate (RGR), the oocysts count in the caecal contents and the lesion score as well as the histopathological findings in the sacrificed birds were used to evaluate the antagonistic activity of the probiotic and the other anticoccidial drugs against *E. tenella* infection. The results revealed that group treated with toltrazuril showed great and significant improvement in (BW), (BWG) and (RGR) and also revealed the highest reduction in mortalities, the number of oocysts and the lesion score when compared with sulphaquinoxaline and amprolium indicating that toltrazuril is the most effective anticoccidial drug in treatment of *E. tenella* infection in chickens. Comparing with infected and non treated group, the dietary supplementation of the probiotic resulted in improving the performance, reduction of the clinical signs, lesion score and oocysts count. Further investigation is needed to spotlight on the antagonistic activity of natural microflora

as *Pediococcus acidilactici* against infection with *E. tenella* in chickens.

Key words:

Coccidiosis control, *E. tenella*, Probiotics, Anticoccidial drugs, Chickens.

INTRODUCTION

Coccidiosis, a disease caused by obligatory intracellular protozoan parasites of the genus *Eimeria*, constitutes a significant economic impact under intensive poultry production. Lower weight gain, inefficient feed utilization, mortality (Edgar, 1955), and prophylactic medication are the important cost factors (Danforth and Augustine, 1985).

The protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract of poultry and produces tissue damage, resulting in reduced growth and increased susceptibility to pathogens (MacDougald, 2003) such as *Clostridium perfringens*, leading to necrotic enteritis (Stephens and Vestal, 1966; Helmboldt and Brynat, 1971; Maxy and Page, 1977; Al-Sheikhly and Al-Saieg, 1980 and

Shane et al., 1985). In bacteria-free chickens infected with *E. tenella* oocysts, clinical signs did not develop unlike in chickens with one or more indigenous species of bacteria (Radhhakrishnan, 1971; Johnson and Reid, 1972 and Visco and Burns, 1972 a and b). Apparently, indigenous bacteria are required for the occurrence of typical caecal coccidiosis in chickens. In the course of development of caecal coccidiosis, the growth of *Clostridium perfringens* and coilforms, especially *Eschericia coli*, is stimulated whereas the growth of *Lactobacillus* species is suppressed (Johansson and Sarles, 1948 and Radhhakrishnan, 1971). *Lactobacillus* species have been shown to inhibit *E. tenella* invasion in vitro (Tierney et al., 2004).

Despite the development of better anticoccidial drugs in the past fifty years, the coccidial problem remains unsolved (Logan et al., 1993). Keeping of the efficacy of the existing anticoccidial drugs is an urgent demand as discovery of new drugs to cover emerging resistance against the drugs is becoming ever more difficult (Chapman, 1999).

More efficient control techniques for coccidiosis, such as the use of the water-

soluble anticoccidials, are thus very important to the future of the poultry industry.

Toltrazuril is a symmetrical triazinetrione compound and has no chemical relationship with other anticoccidial agents. Toltrazuril is a highly efficacious anticoccidial drug due to its water solubility, anticoccidial action against all intracellular developmental stages of the parasite (Haberkorn and Stoltefuss, 1987; Vertommen et al., 1990 and Mathis et al., 1997, 2003 and 2004), and its effectiveness against all coccidial species of chickens (McDougald, 1982; Mehlhorn et al., 1984 and Laczay et al., 1995), ducks (Reynaud et al., 1999) and pigeons (Schumacher, 1983).

Cocciostats have been popularly used in the poultry farms for prevention of avian coccidiosis. These drugs have adverse effects like health risk to both birds and human. Moreover, residual cocciostats drugs cause environmental contamination via bird's waste

(Chapman, 1999). Therefore, an alternative method for the prevention of coccidiosis should be employed to reduce the use of these drugs, at the same time ensure breeding of poultry free from coccidiosis and without residual

coccidiostats in consumer meat, and also reduce environmental contamination due to bird's waste. It's expected that in the near future the coccidiostatic drugs currently used in animal feeds will be banned. Perhaps probiotic containing *Pediococcus acidilactici* preparation can be useful (McDougald, 2003 and Tierney *et al.*, 2004).

The protective role of gut microflora is thought to be due to non-specific barrier effects, competition for intestinal surface sites, production of antipathogen products, and enhancement of the immune response or a combination of all (Tierney *et al.*, 2004). Among this natural microflora that exhibits this probiotic property is species that produces lactic acid like *Pediococcus acidilactici*. This species exhibits properties which include the ability to adhere to specific intestinal sites leading to exclusion or reduction of pathogenic adherence and then persists, multiplies and produces acids, hydrogen peroxides and bacteriocins (Reid, 1999 and Vaughan *et al.*, 1999).

Lactobacillus species producing lactic acid had been shown to inhibit parasitic infestation in vivo. Certain studies demonstrated *Lactobacillus* influence on *Eimeria acervulina* (Dalloul *et al.*, 2003), *Cryptosporidium parvum*

(Alak *et al.*, 1999 and Waters *et al.*, 1999) and *Giardia lamblia* (Singer and Narsh, 2000).

Pediococcus acidilactici is non pathogenic member of normal chickens gut flora (Barnes *et al.*, 1980) that colonize the caecum producing antagonistic substances like acids which found to be active against different emerging chicken's enteric bacterial pathogens.

The objective of this trial was to investigate the proposed prophylactic anticoccidial effects of a probiotic compound containing *Pediococcus acidilactici* on *E. tenella* compared with the treatment effects of anticoccidial drugs (amprolium, sulphaquinoxaline and toltrazuril) in *E. tenella* experimentally infected broiler chickens.

MATERIAL AND METHODS

1. Chickens:

One hundred and fifty, day-old Hubbard broiler chicks were obtained from a commercial hatchery as hatched. The chicks were reared on wire floor cages, where the cages were thoroughly cleaned with boiled water and soap, disinfected with 5% formalin solution and finally fumigated with 10% ammonium hydroxide. The birds were given broiler starter ration ad libitum without any growth promoters or coccidiostatics, also water was given ad libitum. The used chicks were vaccinated

against Newcastle disease using Hitchner B1 and La Sota vaccines at 8 and 21 days of age, respectively and against infectious bursal disease at 14 days of age. All the vaccines were given through eye drop method.

2. The probiotic and the anticoccidial drugs used:

A. A probiotic containing stabilized strain *Pediococcus acidilactici* MA18/5M produced by Lallmand, France, was supplemented as a dried fed microbial in the birds ration in a dose of 100 gm / ton for 3 days from 15th day until 18th day of age (the day of experimental inoculation). The dose level was as recommended by the manufacturer.

B. *Amprolium* (20%) soluble powder produced by SIDICO, batch number 04j04, was given as 1 gm / liter of the drinking water at 21 days of age (the 6th day after infection) for 5 successive days as recommended by the manufacturer.

C. *Sulphaquinoxaline sodium* (25%) soluble powder produced by Marcyrl Pharmaceutical Industries with a batch number 51158, was given as 1 gm / liter of the drinking water at 21 days of age (the 6th after infection) for 5 successive days as recommended by the manufacturer.

D. *Toltrazuril* (2.5%) in liquid form produced by Arab Company for Medical products, batch

number 0236/05, was given at concentration of 7 mg/kg body weight as 1 gm / liter of the drinking water at 21 days of age (the 6th after infection) for 2 successive days as recommended by the manufacturer.

3. Preparation of *E. tenella* sporulated oocysts:

Oocysts of *E. tenella* were obtained from the parasitological laboratory of poultry diseases department, Faculty of Veterinary Medicine, Cairo University. The caeci of naturally infected chickens were separated by sieving and sedimentation techniques (Soulsby, 1978). The two caeci were emulsified in 2.5% potassium dichromate solution (in a ratio of one part of faecal sample to two parts of the solution), then filtrated and the filtrate was left for sedimentation. The sediment was taken and washed with distilled water several times. Finally, the washed oocysts were kept in 2.5% potassium dichromate solution at room temperature for sporulation.

4. Experimental infection:

Each chick in the infected groups was orally inoculated at 18th day of age with 1 ml solution containing about 100.000 sporulated *E. tenella* oocysts in the crop using a wide mouthed 1 ml pipette (Dalloul *et al.*, 2003).

5. Parameters of evaluation:

A. Relative growth rate:

Relative growth Rate (RGR) was calculated according to the following equation (Samar, 1991):

$$\text{Relative growth Rate (RGR)} = 100 \times \frac{W_2 - W_1 \text{ (weight gain)}}{W_2 + W_1 / 2}$$

Where W_1 = Mean initial weight of birds in each group just before treatment

(24th day of age).

W_2 = Mean final weight at the end of the experiment (29th day of age).

B. Counting of oocysts:

Sporulated *E. tenella* oocysts used for experimental infection and non sporulated ones present in the caecal contents of experimentally infected and infected treated birds were counted (Hodgson, 1970 and Echert *et al.*, 1995) as 0.3 ml of oocysts suspension was thoroughly mixed with 2.7 ml of saturated sodium chloride solution. The McMaster slide chambers were filled using micropipette and left for few minutes till floating of oocysts. The oocysts were counted in each chamber and the average values of the number of oocysts / ml of the suspension were calculated.

C. Gross lesion score:

The gross lesion score in the caeci of infected birds is an important criterion to detect the

severity of lesions of infected non treated control positive group and comparing them with those of all infected and treated ones according to Johnson and Reid (1970) and Conway (1979). Based on the severity of the lesions, the score was classified into four grades as follow:

Grade (0): indicated no lesions.

Grade (1): indicated very mild changes (There were very few scattered petechiae on the caecal wall, with no visible thickening. Also normal caecal contents were present).

Grade (2): indicated mild changes (The lesions were more numerous with noticeable blood in the caecal contents).

Grade (3): indicated moderate changes (Large amounts of blood and caecal core were present. The caecal wall was greatly thickened).

Grade (4): indicated severe changes (Caecal pouches either contain blood or large caseous cores. The caeca were greatly distended).

D. Histopathological examination:

The two caeci from each group were collected daily during the treatment, 1 cm of the caecal tissue specimens were fixed in 10% neutral buffered formaline, then these specimens were thoroughly washed, dehydrated in ascending concentrations of ethyl alcohol, followed by clearing in xylol and finally embedded in paraffin according to Bancroft and Steven (1996). Paraffin sections were stained with

Hematoxylin and Eosin (H & E), and then examined microscopically.

8. Experimental design:

The used 150, day-old Hubbard broiler chicks were kept on wire floor cages with daily examination of their dropping till the 15th day of life, where 50 birds were randomly collected and divided into two equal separate groups (1 and 3); 25 chicks each. Birds of group (1) were kept as non infected and non treated control negative group, while chicks of group (3) were supplemented with a probiotic containing *Pediococcus acidilactici* in the ration in a dose of 100 gm / ton for 3 days from 15th day until 18th day of age (the day of oocysts inoculation).

At 18th day of age, the group supplemented with the probiotic (group 3) and the rest of 100 birds were inoculated orally in the crop with 1 ml suspension/chick containing about 100,000 sporulated *E. tenella* oocysts, then divided into 4 equal separate groups 2, 4, 5 and 6 (25 birds, each). Group (2) was kept as control positive infected non treated birds. At 24th day of age (the 6th day after infection), group (4) and group (5) were treated with amprolium (1 ml /

liter of the drinking water) and sulphaquinoxaline (1 ml / liter of the drinking water) for 5 successive days, respectively, while birds of group (6) were given toltrazuril in the drinking water (1 ml / liter) for 2 successive days. Birds of all groups were observed daily and mortalities were recorded as it occurred.

Severe clinical signs (bloody dropping) were appeared at the 6th day post infection (24th day of age). Just before starting of treatment, ten random birds each from groups (1), (3) and from the rest of the infected 100 birds were weighted to obtain initial mean body weight (W_1), then these 100 birds were divided into four equal separate groups (2, 4, 5 and 6), 25 chicks each. Birds of group (2) were left as infected and non treated control positive group. Birds of groups (4) and (5) were medicated with amprolium and sulphaquinoxaline, respectively as 1ml / liter of the drinking water for 5 successive days, while chickens of group (6) was given toltrazuril as 1ml / liter of the drinking water for 2 successive days.

Body weight of 10 random birds / group was taken just after the cessation of the treatment at

the end of the experiment (29th day of age) to calculate the final mean body weight (W_2). Body weight gain (BWG) was calculated for each group as ($W_2 - W_1$).

Three birds / group were sacrificed daily from the 1st to the 5th day of the treatment course for detection of macroscopic caecal lesions score and the caecal contents was collected for counting of *E. tenella* oocysts / gm caecal contents. The two caeci also were collected for histopathological examination.

9. Statistical analysis:

Body weight data was statistically analyzed by using Analysis of Variance (ANOVA) according to Snedecor and Cochran (1980).

RESULTS

The signs of coccidial infection were started in appearance in some of the infected birds at the 3rd day post infection in the form of lower feed intake, ruffled feathers and loose dropping. At the 4th and 5th days post infection, all the infected chickens showed anorexia, ruffled feathers, humped back and chalky mucoid diarrhea, while at the 6th day post infection, the birds showed bloody diarrhea. Mild signs were observed in the group supplemented with the probiotic before infection.

Results of table (1) showed the effect of different treatments on the performance parameters including body weight (BW), body weight gain (BWG) and the relative growth rate (RGR) and also mortalities in infected and treated chickens. Significant reduction in (BW), (BWG) and (RGR) was recorded in the infected non treated control positive chickens as compared with non infected non treated control negative birds. Treated groups showed significant increase in (BW), improvement in (BWG) and (RGR) and reduced mortalities than infected non treated group. Birds of group treated with toltrazuril had the highest performance parameters and lowest mortalities, followed by those of groups treated with sulphaquinoxaline and amprolium, respectively. Chickens supplemented with the probiotic had the lowest performance parameters when compared with treated groups but it was higher than those of infected non treated control group.

The data of table (2) showed the number of *E. tenella* oocysts / gm of the caecal contents of the sacrificed infected and treated birds during the course of the treatment. No oocysts could be detected in the caecal contents of control negative non infected and non treated group along the experimental duration, but the highest count was observed in the infected non treated

control positive birds till the end of the treatment. The lowest count was seen in the group treated with toltrazuril followed by those treated with sulphaquinoxaline and amprolium, respectively. When compared with the treated groups, the oocysts count was higher in the bird's dietary supplemented with the probiotic before infection but the count was lower than infected non treated group.

The effect of different treatments on the caecal lesion score in the infected and treated groups was seen in table (3a). The results revealed that non infected non treated control negative group had no lesions, while infected non treated control positive group showed the highest score along the whole treatment period. The birds treated with toltrazuril showed the lowest caecal lesion score when compared with those treated with sulphaquinoxaline and amprolium, respectively. Among the treated groups, the one prophylactically supplemented with the probiotic showed the highest lesion score but it was still lower than control positive group.

Chickens in all groups were subjected to post-mortem examination as well as lesion score was observed (Table 3b) and (Figure A and B).

The lesions were classified into four grades according to the severity of lesions as follows: Grade (0) no change, grade (1) very mild changes, grade (2) mild changes, grade (3) moderate changes while grade (4) severe changes. From this table it could be observed that infected non treated birds showed the highest grade (4) in comparison with non infected non treated birds which showed the lowest one (0). Treated groups showed variable grades (1, 2 and 3), but the number of chickens showed grade (3) were lower in the group treated with toltrazuril, followed by those treated with sulphaquinoxaline, amprolium and the probiotic, respectively.

Histopathological changes:

Control negative (non infected and non treated) group showed apparently normal caecal mucosa, submucosa, muscosa, and serosa all over the experimental period (Fig.C,1).

Control positive (*E. tenella* infected) group at the 1st and the 2nd day of treatment, revealed presence of undifferentiated gamonts in the mucosal epithelium and glands with mononuclear cells infiltration in both mucosa and submucosa (Fig.C,2). These lesion progressed to massive mucosal necrosis and

submucosal infiltration with undifferentiated gamonts surrounded with mononuclear reaction at the 3rd day of treatment (Fig.C,3). and to shizonts formation with basophilic banana shaped merozoites at the 4th day of treatment (Fig.C,4). While at the 5th day of treatment, there were necrosis in most caecal glands with cystic dilatation of others and severe mucosal infiltration with heterophils (Fig.C,5).

In the probiotic treated group, at the 1st day of treatment, denoted infiltrated mucosa with both undifferentiated and differentiated gamonts together with mononuclear cells infiltration that extended to the submucosa and musculosa (Fig.D,1) was seen. Two days of treatment, the mucosa became necrosed together with submucosal congestion (Fig.D,2). The mucosal epithelium regenerated and invaded with differentiated macrogametocytes and microgametocytes at the 3rd day of treatment (Fig.D,3). At the 4th day of treatment, the parasitic stages decreased to fewly scattered gamonts in the lamina propria with mild

inflammatory reaction (Fig.D,4). This picture improved at the 5th day of treatment and revealed apparently normal mucosal villi and glands (Fig.D,5).

The caecal histology of amprolium treated group at the 1st day of treatment showed destructed villi and undifferentiated gamonts that infiltrated both mucosa and submucosa associated with inflammatory reaction (Fig.E,1). The destruction of the villi progressed to massive necrosis at the 2nd day of treatment, also the necrosis involved most of the glands so that the remained glands compensated and became cystically dilated (Fig.E,2). By the 3rd day of treatment, the gamonts differentiated to macrogametocytes and microgametocytes together with heterophylic infiltration, (Fig.E,3 & E,4). There were diffuse hemorrhagic areas in between the caecal glands at the 4th day of treatment (Fig.E,5). While at the 5th day of treatment, there were severe shortening and fusion of the villi with persisting submucosal infiltration with undifferentiated gamonts and heterophils (Fig.E,6).

Sulphaquinoxaline treated group at the 1st day of treatment, revealed villous destruction that infiltrated with both undifferentiated and differentiated gamonts and heterophils (Fig.F,1). Shizonts formation with basophilic banana shaped merozoites were detected at the 2nd day of treatment surrounded with heterophils (Fig.F,2). Three days of treatment, the villous destruction became completed including most of the caecal glands with cystic dilatation of others. Also there were both mucosal and submucosal infiltration with undifferentiated gamonts and mononuclear cells (Fig.F,3). These gamonts disappeared and the villi regenerated at the 4th day of treatment (Fig.F,4). At the 5th day of treatment, The caecal tissue appeared normal (Fig.F,5).

The microscopical alterations of toltrazuril treated group at 1st day of treatment showed massive mucosal invasion with undifferentiated gamonts associated with mononuclear cells infiltration (Fig.G,1). Then at the 2nd day of treatment, the gamonts became differentiated to macrogametocytes and microgametocytes that infiltrated the mucosa and surrounded with inflammatory reaction (Fig.G,2). While at the 3rd day of treatment, appeared apparently normal mucosa with fewly scattered differentiated gamonts, (Fig.G,3). Then these stages became completely disappeared and the caecal tissue appeared normal at both the 4th day of treatment (Fig.G,4), and the 5th day of treatment (Fig.G,5).

Table (3): The number of *A. baileyi* oocysts per caecal contents X 10³

Group No.	Treatment	Days of treatment				
		1	2	3	4	5
1	-	0	0	0	0	0
2	-	102	137	93	84	173
3	B	89	75.4	82.3	74	83
4	A	104	82.1	92.9	81	73
5	S	82	73	72	72	66
6	T	22	32.2	62	22	1

Table (1): The effect of different treatments on body weight (BW), body weight gain (BWG), relative growth rate (RGR) and mortalities in infected and treated groups.

Item	Groups treatments					
	Non infected non treated	Infected and treated	P	A	S	T
Mean W_1 (gm)	240±15.15	212.5±13.78	220±12.33	212.5±13.78	212.5±13.78	212.5±13.78
Mean W_2 (gm)	450±31.62	330±15.81	351±11.25	372±16.08	388±17.06	396±16.84
$W_2 - W_1$ (weight gain)	210	117.5	131	159.5	175.5	183.5
$\frac{W_2 - W_1}{2}$	345	271.25	285.5	292.25	300.25	304.25
RGR	60.86	43.31	45.88	54.57	58.45	60.31
No. of dead birds/group	0/25	20/25	9/25	6/25	5/25	3/25

P: Probiotic A: Amprolium S: Sulphaquinoxaline T: Toltrazuril

W_1 : Initial mean body weight of each group at 24th day of age (just before treatment) ± SEM

(Standard Error of Mean).

W_2 : Final mean body weight of each group at 29th day of age (just before treatment) ± SEM (Standard Error of Mean).

Table (2): The number of *E. tenella* oocysts /gm caecal contents X 10³.

Group No.	Infection	Treatment	Days of treatment				
			1	2	3	4	5
1	-	-	0	0	0	0	0
2	+	-	105	137	95	84	175
3	+	P	89	75.4	85.2	74	83
4	+	A	104	85.1	92.5	8.1	7.5
5	+	S	85	73	7.5	5.5	6.6
6	+	T	52	33.5	0.5	2.2	1

P: Probiotic A: Amprolium S: Sulphaquinoxaline T: Toltrazuril

Table (3a): The effect of different treatments on the caecal lesions score in the infected and treated groups.

Group No.	Infection	Treatment	Days of treatment				
			1	2	3	4	5
1	-	-	0	0	0	0	0
2	+	-	4	4	3.5	4	3.5
3	+	P	3	2.5	2.5	1.5	3
4	+	A	4	1.5	2	1	1.75
5	+	S	3.5	3.5	1.5	2.5	1.5
6	+	T	2.5	1.5	1.5	1.5	1

P: Probiotic A: Amprolium S: Sulphaquinoxaline T: Toltrazuril

Table (3b): Differences in the caecal lesions score in the infected and treated groups (n=25).

Group No.	Infection	Treatment	No. of birds showed lesions				
			Grade (0)	Grade (1)	Grade (2)	Grade (3)	Grade (4)
1	-	-	25/25	-	-	-	-
2	+	-	-	-	-	-	25/25
3	+	P	-	1/25	15/25	9/25	-
4	+	A	-	5/25	14/25	6/25	-
5	+	S	-	8/25	12/25	5/25	-
6	+	T	-	12/25	10/25	3/25	-

P: Probiotic A: Amprolium S: Sulphaquinoxaline T: Toltrazuril

Grade (0): No changes

Grade (1): Very mild changes

Grade (2): Mild changes

Grade (3): Moderate changes

Grade (4): Severe changes



Fig. (A): Caeci of *E. tenella* infected chicks (left) and non infected ones (right).

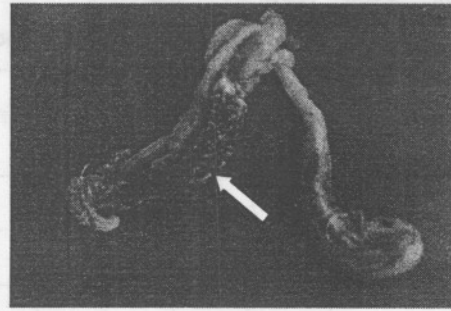


Fig. (B): Opened caecum showed (left) and non haemorrhagic contents and core.

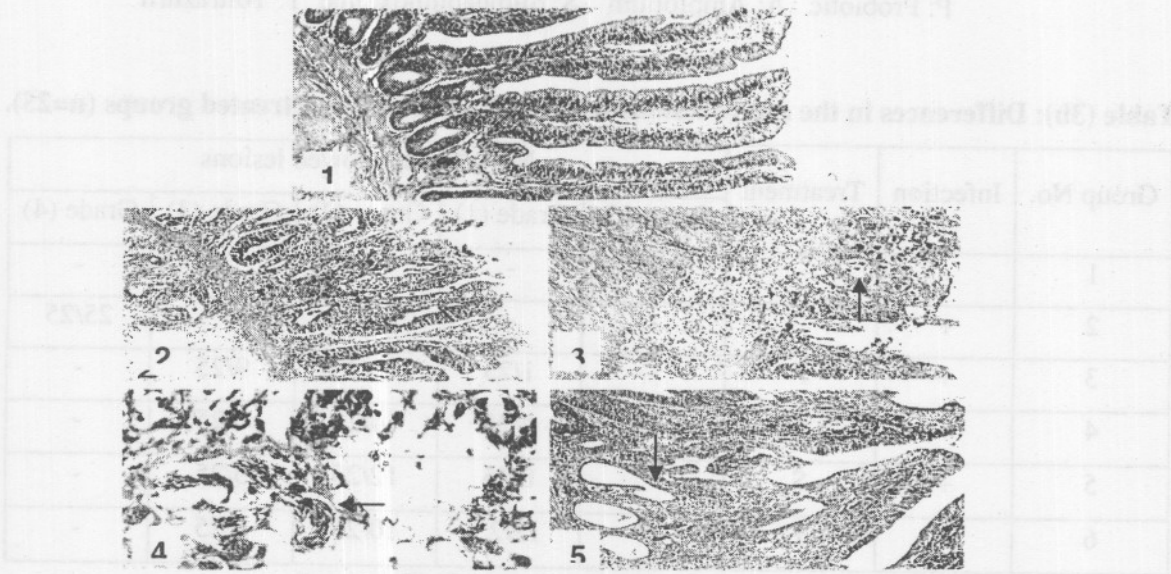


Fig. (C). Sections of chicken's caecum of non infected non treated (1) and of infected non treated (2, 3, 4 and 5) stained by H&E.

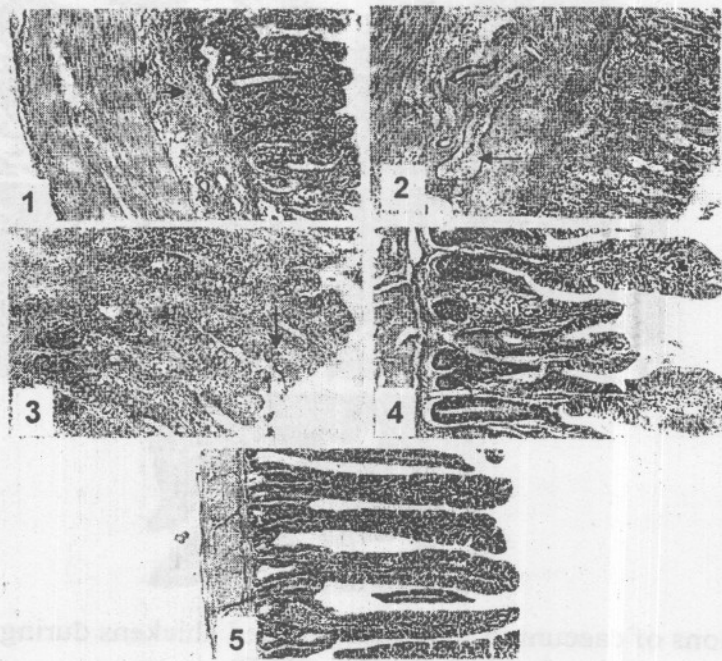


Fig. (D): Sections of caecum in *E. tenella* infected chickens supplemented with probiotic (H&E).

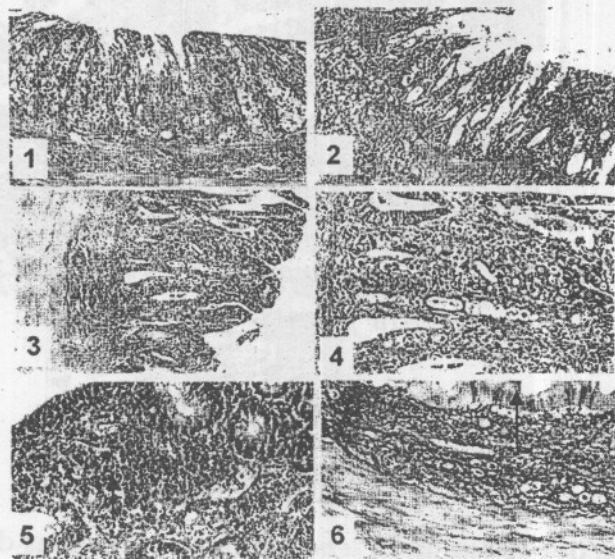


Fig. (E): Sections of caecum in *E. tenella* infected chickens during the treatment with amprolium (H&E).

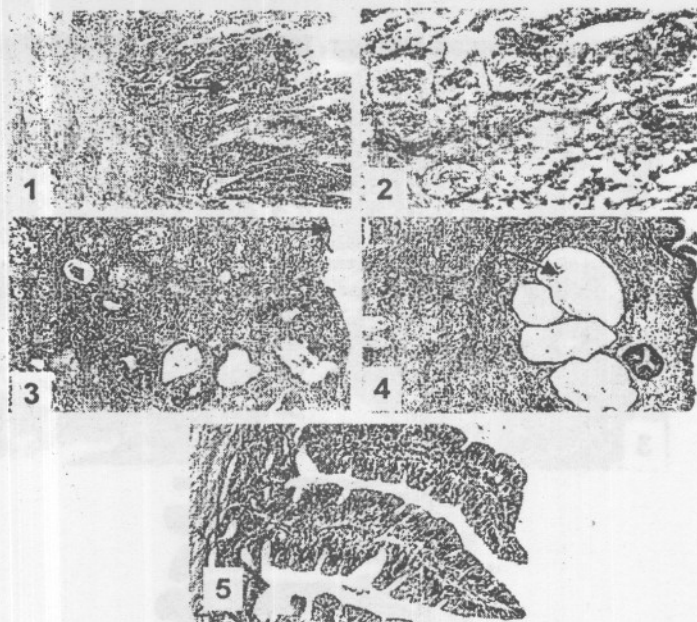


Fig. (F): Sections of caecum in *E. tenella* infected chickens during the treatment with sulphaquinoxaline (H&E).

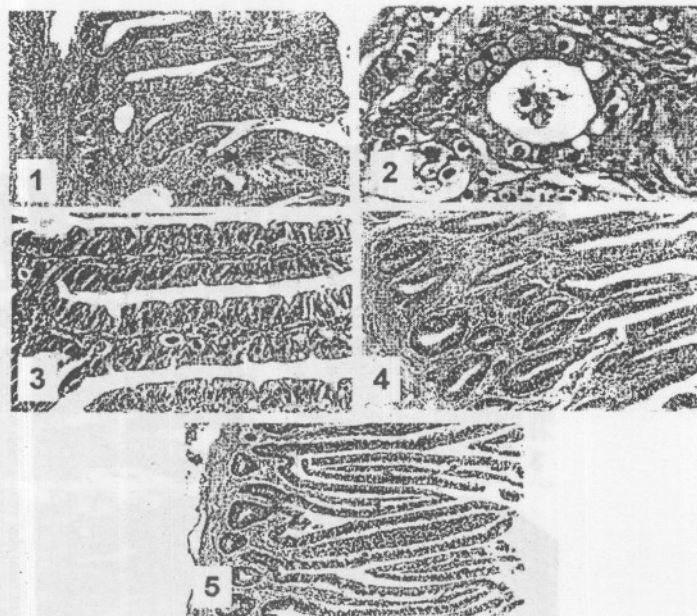


Fig. (G): Sections of caecum in *E. tenella* infected chickens during the treatment with toltrazuril (H&E).

LEGANDS OF FIGURES:

Fig.(C,1): Caecum of non infected non treated chickens (control negative group), showing apparently normal caecal tissue. (H&E X100).

Fig. (C,2): Caecum of non treated chickens infected with *E. tenella* (1st & 2nd day of treatment), showing undifferentiated gamonts in the mucosal epithelium and glands, with mononuclear cells infiltration in both mucosa and submucosa. (H&E X100).

Fig. (C,3): Caecum of non treated chickens infected with *E. tenella* (3rd day of treatment), showing massive mucosal necrosis with undifferentiated gamonts (arrow) that extending to the submucosa and surrounding with cellular reaction. (H&E X100).

Fig. (C,4): Caecum of non treated chickens infected with *E. tenella* (4th day of treatment), showing schizonts formation with basophilic banana shaped merozoites (arrow). (H&E X400).

Fig (C,5): Caecum of non treated chickens infected with *E. tenella* (5th day of treatment), showing necrosis of caecal glands with cystic dilatation (arrow) of others and scattering of undifferentiated gamonts among the mucosa. (H&E X100).

Fig. (D,1): Caecum of *E. tenella* infected chickens treated with (Probiotic), (1st day of treatment), showing undifferentiated and differentiated gamonts infiltrating the mucosa with mononuclear cells infiltration that extending to the submucosa (arrow) and tunica muscularis.(H&E X100).

Fig. (D,2): Caecum of *E. tenella* infected chickens treated with (Probiotic), (2nd day of treatment), showing necrosed mucosa that infiltrated with undifferentiated gamonts and mononuclear cells. The submucosa showing congested blood vessel and leucocytic infiltration (arrow). (H&E X100).

Fig. (D,3): Caecum of *E. tenella* infected chickens treated with (Probiotic), (3th day of treatment), showing large number of differentiated macrogametocytes and microgametocytes in the mucosal epithelium (arrow) and glands, as well as heavy infiltration of the lamina propria and submucosa with mononuclear cells and heterophils. (H&E X100).

Fig. (D,4): Caecum of *E. tenella* infected chickens treated with (Probiotic), (4th day of treatment), showing fewly scattered undifferentiated gamonts in the lamina propria with massive mononuclear cells infiltration. (H&E X100).

Fig.(D,5): Caecum of *E. tenella* infected chickens treated with (Probiotic), (5th day of treatment), showing apparently normal mucosal villi and glands.(H&E X40).

Fig.(E,1): Caecum of *E. tenella* infected chickens treated with (Amprolium) (1st day of treatment), showing destruction of villi as well as massive intraepithelial and

submucosal presence of undifferentiated gamonts together with mononuclear cells infiltration.(H&E X100).

Fig.(E,2): Caecum of *E. tenella* infected chickens treated with (Amprolium), (2nd day of treatment), showing marked necrosis of villous epithelium, cystically dilated caecal glands, and infiltrated undifferentiated gamonts in both lamina propria and submucosa.(H&E X100).

Fig.(E,3): Caecum of *E. tenella* infected chickens treated with (Amprolium) (3rd day of treatment), showing marked infiltration of the mucosa with differentiated macrogametocytes and microgametocytes associated with heterophils infiltration that extending to the submucosal layer. (H&E X100).

Fig.(E,4): Higher power of figure (E,3) (H&E X200).

Fig.(E,5): Caecum of *E. tenella* infected chickens treated with (Amprolium), (4th day of treatment), showing diffuse hemorrhagic areas (arrow) in between the caecal glands associated with heterophils infiltration. (H&E X200).

Fig.(E,6): Caecum of *E. tenella* infected chickens treated with (Amprolium), (5th day of treatment), showing sever shortening and fusion of the villi (arrow) as well as submucosal infiltration with undifferentiated gamonts and mononuclear cells.(H&E X200).

Fig.(F,1): Caecum of *E. tenella* infected chickens treated with (Sulphaquinoxaline), (1st day of treatment), showing destruction of the villous epithelium that heavily infiltrated with both undifferentiated and differentiated gamonts together with inflammatory reaction (arrow) that extends to the submucosa.(H&E X100).

Fig.(F,2): Caecum of *E. tenella* infected chickens treated with (Sulphaquinoxaline), (2nd day of treatment), showing shizonts formation with basophilic banana shaped merozoites surrounded with inflammatory reaction.(H&E X200).

Fig.(F,3): Caecum of *E. tenella* infected chickens treated with (Sulphaquinoxaline), (3rd day of treatment), showing complete villous destruction (arrow), cystic dilatation of caecal glands, and mucosal infiltration of undifferentiated gamonts associated with inflammatory reaction that extends to the submucosa.(H&E X100).

Fig.(F,4): Caecum of *E. tenella* infected chickens treated with (Sulphaquinoxaline), (4th day of treatment), showing shortening and fusion of the villi, marked dilatation of the caecal glands (arrow) associated with mononuclear cells infiltration.(H&E X100).

Fig.(F,5): Caecum of *E. tenella* infected chickens treated with (Sulphaquinoxaline), (5th day of treatment), showing apparently normal caecal tissue.(H&E X 100).
(1st day of treatment), showing massive invasion of the lamina epithelialis and glandular epithelium with undifferentiated gamonts with diffuse mononuclear cells infiltration (arrow).(H&E X100).

Fig.(G,2): Caecum of *E. tenella* infected chickens treated with (Toltrazuril) (2nd day of treatment), showing differentiated macrogametocytes and microgametocytes infiltrating the mucosa (arrow) and surrounding with inflammatory reaction. (H&E X400).

Fig.(G,3): Caecum of *E. tenella* infected chickens treated with (Toltrazuril) (3rd day of treatment), showing fewly scattered differentiated macrogametocytes and microgametocytes among the apparently normal mucosa. (H&E X200).

Fig.(G,4): Caecum of *E. tenella* infected chickens treated with (Toltrazuril) (4th day of treatment), showing apparently normal caecal tissue. (H&E X100).

Fig.(G,5): Caecum of *E. tenella* infected chickens treated with (Toltrazuril) (5th day of treatment), showing normal caecal tissue. (H&E X100).

DISCUSSION

In our study, we tried to spotlight on the inhibition of *E. tenella* infection mainly by using of a probiotic containing *Pediococcus acidilactici* and comparing that effect with those of three anticoccidial medicaments (amprolium, sulphaquinoxaline and toltrazuril) to evaluate their effectiveness as anticoccidials.

Mean body weight (BW), body weight gain (BWG) and relative growth rate (RGR), mortalities, oocysts count in caecal contents and lesions score were used as criteria to assess the efficacy of any anticoccidial drug (Long, 1970).

Infected non treated birds showed severe haemorrhages in their dropping at the 6th day post challenge, the highest mortalities and also showed the lowest weight gain and relative growth rate. The reduction in the birds production associated with coccidial infection might be explained by the inflammatory reactions that divert energy from the growth which affects the weight gain (Klasing *et al.*, 1987). Also the oocysts count in these birds was the highest among the other groups and this was associated with the highest lesion score.

Group treated with toltrazuril showed great improvement in its performance parameters including mean body weight, body weight gain and relative growth rate and reduction in oocysts count as well as macroscopic and microscopic lesion score. Our results are in a great accordance with Ramadan *et al.*, (1993a and b) who showed that the addition of toltrazuril to the drinking water of chickens improved body weight gains and feed utilization. Also, Dhillon *et al.*, (2004) found that treatment of birds by toltrazuril in the drinking water for 2 successive days at 9 hours after infection induced high performance indices and complete elimination of signs at lower dose of infection, also treated groups showed lower mortalities and reduction of oocyst production.

The efficiency of toltrazuril against coccidian of chickens and turkeys had been confirmed by many field trials (Greuel and Mundt, 1984; Kutzer *et al.*, 1985; Greuel, 1986; Johnson *et al.*, 1986; Muangyai *et al.*, 1991; Deghidy and El-Askalany, 1993; Voeten, 1993 and Laczay *et al.*, 1995). Coccidiosis of geese, including the renal form (Friedhoff *et al.*, 1983 and Greuel, 1984) and of pigeons (Schumacher, 1983) can also treated effectively with toltrazuril.

The mode of action of toltrazuril was studied by Harder and Heberkorn, (1989) who proved that toltrazuril primarily affects the respiratory chain and secondarily, two enzymes involved in pyrimidine synthesis of *Eimeria* species, as toltrazuril reduced activities of some enzymes of the respiratory chain, such as succinate-cytochrome C reductase, NADH oxidase and succinate oxidase. Moreover, Mehlhorn *et al.*, (1984) and Haberkorn and Stoltefuss (1987) reported that toltrazuril affects all the intracellular developmental stages (schizogony and gametogony), and is also effective against all *Eimeria* species of poultry and mammals. Greif and Haberkorn (1997) and Greif (2000) recorded that despite the high efficacy of toltrazuril; it doesn't interfere with the development of natural immunity but can even enhance it. They suggested that this efficacy may be due to the destruction of the intracellular stages that stay in the host cells and act as antigens; also the non affected free stages may also enhance immune reactions by invading to host cells.

The solubility of toltrazuril for administration in the drinking water and its good efficacy after two days of treatment (short application period and rapid mode of action), significant reduction of oocyst shedding and good compatibility shows that it is highly

appropriate and superior to conventional chemotherapy for the prophylaxis, therapy and intermittent treatment of *E. tenella* infected chickens (Haberkorn, 1984 and 1986; Peeters and Geeroms, 1986; Haberkorn and Stoltefuss, 1987; Schmid *et al.*, 1991 and Voeten, 1993).

Comparing with the group treated with toltrazuril, the birds of groups treated with sulphaquinoxaline and amprolium had lower body weight gain and relative growth rate, higher mortalities, oocysts count and higher lesion score. These results are in agreement with the results obtained by Chapman, (1989) who studied the efficacy of toltrazuril, sulphaquinoxaline/pyrimethamine and amprolium/ethopabate, given in drinking water, against field isolates of *E. tenella* and found that oocysts of *E. tenella* were found in high count in birds medicated with sulphaquinoxaline/ pyrimethamine or amprolium/ethopabate and non in those medicated with toltrazuril.

Also, the therapeutic efficacy of sulphachlorpyrazine and toltrazuril against experimentally induced *E. tenella* infection was compared in battery and floor pen raised broiler chickens. In the battery studies, both

drugs prevented coccidiosis-related mortality and decrease the weight gain to a similar degree, but toltrazuril was more effective in reducing intestinal lesions and faecal score (Laczay *et al.*, 1995).

The mode of action of sodium sulphapyrazine on *Eimeria* infection was studied by Penev and Lozanov (1983), when the preparation of sulphonamide compound was applied at the 72nd hour after infection of chickens with *E. tenella*, it led to the degeneration of most of the second generation of schizonts and inhibited their further development. As a result no oocysts were found in the feces of birds up to the 10th -11th day after infection. When applied at the 92nd and the 120th hour sulphapyrazine interfered with the development of the already found forms of gametogony without concurrent degenerative effects.

In this study, addition of a probiotic compound containing *Pediococcus acidilactici* in the ration of the birds before experimental infection with *E. tenella* resulted in mild improvement in the performance parameters, slight reduction in lesion score and in the oocysts count when compared with the birds treated with anticoccidial drugs, but that

picture was better than infected non treated group.

The addition of compounds containing natural microflora (especially those producing lactic acid) to the poultry feed or water to overcome coccidial infection especially *E. tenella* was studied by many authors. Tortuero, (1973) found that *Lactobacillus* species reduced the severity of clinical disease associated with *E. tenella* infection. Also, chickens fed on *Lactobacillus*-based ration showed reduced oocysts output compared to controls after challenge with *E. acervulina* (Dalloul *et al.*, 2003).

In explanation of the probiotic effect, Tierney *et al.*, (2004) found that *Lactobacillus* species isolates from chicken's gastrointestinal tract significantly inhibited *E. tenella* invasion in vitro. They referred that inhibition due to the extracellular metabolic factors secreted by *Lactobacillus* species into the surrounding media which inhibit the parasite invasion. *Eimeria* species are highly specific to intestinal locations as are *Lactobacillus* species (Jin *et al.*, 1996). It was initially postulated that a *Lactobacillus* species, which colonizes the lower gastrointestinal tract, might demonstrate optimal parasite inhibition

as both organisms share similar site location (Tierney *et al.*, 2004).

The protective effect of both a prebiotic such as mannanoligosaccharide (MOS) derived from the wall of yeast (*Saccharomyces cerevisiae*) and the bacteria producing acids like *Pediococcus acidilactici* against *Eimeria* species might be related to their role in improvement of intestinal function (Loddi *et al.*, 2002), immunity modulation (Ferket *et al.*, 2002) or reduce the number of schizonts (Elmushara *et al.*, 2006).

Additionally, the effect of MOS addition to the broiler ration to prevent experimental infection with *E. tenella* was evaluated by Elmushara *et al.*, (2006); the results indicated that MOS preparation had enhanced immunity and accordingly decreased the number of schizonts in the intestinal lamina propria of the infected birds. Moreover, Fernandez and Van Gils, (2002) suggested that perhaps dietary MOS supplementation increased the level of *Bifidobacterium* and *Lactobacillus* species in the intestinal tract and depressed the number of *Enterobacteriaceae*. *Lactobacillus* species are known to compete with *Clostridium* species (Shane *et al.*, 1985).

In the light of the above-mentioned findings, an increase in bacteria species producing lactic

acid and a decrease in *Clostridium* species might reduce caecal coccidiosis in broiler chickens. These hypotheses explain the results of this study about the partial protective effect of *Pediococcus acidilactici* probiotic preparation against *E. tenella* infection.

The findings of the histopathological examination in this study were confirmative and accord with the macroscopic lesion score. No microscopic lesions were seen in non infected non treated group, while the severest lesions were observed in infected non treated birds. Chickens treated with toltrazuril showed the mildest lesions followed by those treated with sulphaquinoxaline and amprolium, respectively. Our results were in accordance with Lakkundi *et al.*, (2002) who found that toltrazuril prevented the establishment of *E. tenella* by degeneration and disintegration of the first generation of schizonts. However, in amprolium treated birds, few intact first and second generation of schizonts and also micro and macrogametocytes were noticed, occasionally, oocysts were also found. The probiotic preparation containing *Pediococcus acidilactici* reduced the number of schizonts in the intestinal lamina propria.

Similar observations were recorded by Jeurissen *et al.*, (1996) who found that treated chickens with MOS showed significantly fewer sporozoites reached the crypt epithelium and so the formation of shizonts was inhibited.

Sporozoites that had failed to reach the crypt epithelium were detected within the macrophages or surrounded by them, pointing at control of the intensity of a primary infection. A reduction of schizonts in infected birds fed MOS should be associated with lower caecal lesions score mediated by *E. tenella* infection (McDougald, 2003). It's not known why feeding on MOS reduced the number of schizonts, which mature after 4 days of the production of hundreds of lamina propria, so that the schizonts mature and the merozoites are released. Perhaps MOS and so *Pediococcus acidilactici* enhanced the

immunity of the infected birds and consequently reduce the number of schizonts (Elmushara *et al.*, 2006).

Our study pointed out that; 1) toltrazuril which is the more recent anticoccidial drug is still more effective than sulphaquinoxaline or amprolium in elimination of *E. tenella* infection in chickens and can be used successfully in control of caecal coccidiosis in chickens. 2) Probiotic has a partial indirect protective effect against *E. tenella* infection which may be through improvement of general health condition or through increase the body defense (immunopotential). Using of the probiotic in the future can be extended to include alternative strategy which may contribute to the prevention of chicken coccidiosis. So, further studies are required to investigate the antagonistic activity of natural microflora as *Pediococcus acidilactici* against *Eimeria* species infections in chicken

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ضد عدوى الأيمريا تينيلاً في دجاج التسمين

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أجريت هذه الدراسة لمقارنة التأثير الوقائى المقترح لإحدى مركبات البروبيوتيك التى تحتوى على بكتريا (البيديوكوكس أسيدى لاكتيسى) بالتأثير العلاجى لكل من مضادات الكوكسيديا الآتية (الأمبروليوم، و السلفاكوينوكساليين و التولترازوريل) ضد العدوى الإصطناعية لطفيل الأيمريا تينيلاً فى دجاج التسمين.

تمت هذه الدراسة باستخدام 150 كتكوت تسمين عمر يوم حيث تم تربيتها حتى عمر 15 يوم ثم تم تقسيم عدد 50 كتكوت منهم إلى مجموعتين متساويتين، المجموعة (1) مجموعة ضابطة سلبية (لم يتم إحداث العدوى فيها ولم يتم علاجها)، بينما الأخرى (مجموعة 3) تم تغذيتها على عليقة تحتوى على مركب البروبيوتيك بمعدل 100 جرام/ طن علف لمدة ثلاثة أيام فقط قبل إحداث العدوى الإصطناعية بطفيل الأيمريا تينيلاً.

عند عمر 18 يوم تم إحداث العدوى الإصطناعية عن طريق التجريع بالفم بمعدل 100000 من حويصلات الكوكسيديا لكل طائر. ثم تم تقسيم المائة طائر بعد العدوى إلى أربع مجموعات متساوية العدد (2: 4، 5، 6). المجموعة (2) بقيت كمجموعة ضابطة إيجابية (معدة و غير معالجة).

وعند اليوم 24 من عمر الطيور (اليوم السادس من العدوى) تم علاج المجموعة (4) بعقار الأمبروليم 1 جم/لتر ماء شرب والمجموعة (5) بعقار السلفاكوينوكساليين بمعدل 1 جم/ لتر ماء لمدة 5 أيام متتالية لكلا المجموعتين على النوالى، بينما عولجت المجموعة (6) بعقار التولترازوريل بمعدل 1 سم/ لتر ماء لمدة يومين متتاليين.

أظهرت النتائج أن المجموعة التى تم علاجها بعقار التولترازوريل كان بها تحسن معنوى و ملحوظ فى معدل الأوزان، و معدل إكتساب الوزن وكذلك المعدل النسبى للنمو، بينما أظهرت إنخفاض فى النافق و عدد الحويصلات و كذلك إنخفاض فى حدة الآفات التشريحية وذلك مقارنة ببقية المجموعات العلاجية الأخرى. ومقارنة بالمجموعة الضابطة الإيجابية فإن الطيور التى تم تغذيتها على البروبيوتيك أظهرت تحسن نسبى فى القياسات السابقة ولكن أقل من التحسن الذى حدث فى المجموعات العلاجية. وعلى ذلك فإن النتائج خلصت إلى أن التولترازوريل كان الأكثر تأثيراً على عدوى الأيمريا تينيلاً فى الدجاج، وأن هناك حاجة إلى دراسات تكميلية عن تأثير البروبيوتيك على العدوى بطفيل الأيمريا تينيلاً.