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Field Evaluation Of Diclazuril And Narasin For Treatment Of Coccidiosis In Broiler Chickens

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ABSTRACT

The present work was performed to investigate the effect of diclazuril and narasin in treatment of broiler chicks infected with coccidiosis. Oocyst count, weight gain, feed consumption, feed conversion rate as well as some hematological and biochemical changes were the used criteria for evaluation.

One hundred, day old broiler chicks were divided into 5 equal groups,1st group non infected and non-treated chick (-ve control),2nd group infected and non treated (+ve control),3rd group infected and treated with diclazuril (0.5ml / liter drinking water),4thgroup infected and treated with narasin (40 mg/kg ration),5th group infected and treated with diclazuril and narasin in therapeutic doses. Treatment started at age of 25 days and continues for one week.

All chickens were individually weighed at the beginning of the experiment and at weekly intervals. At the end of the experiment (42 days),Five birds from each group were sacrificed and two blood samples from each bird were collected for hematological study and biochemical analysis .

Infected and non treated birds showed a significant decrease in erythrocytes count, hemoglobin content and packed cell volume % and significant increase in serum levels of aminotransferases (ALT&AST), total globulin, uric acid and creatinine but not in calcium and inorganic phosphorus while an insignificant decrease in serum levels of albumin was reported.

Infected chick with coccidiosis and treated with diclazuril or narasin either alone or in combination revealed significant increase in body weight, weight gain, feed consumption and significant decrease in feed conversion rate .

It could be concluded that coccidia infections in chickens induce several side effects on body weight, weight gain, feed consumption, feed conversion rate as well as some blood picture and some biochemical parameters but diclazuril and narasin in therapeutic doses either alone or in combination induced improvement in these parameters and also decreased oocyst count.

INTRODUCTION

Coccidiosis is an important disease affecting poultry. Intensive rearing of chickens on floor pens creates favorable conditions to rapid spread of the disease resulting in severe economic losses. Coccidiosis is not clinically recognizable until the tissue damage associated with the second or third generation schizogony occur *(1)*.

The ideal anticoccidial should limit the number of coccidian that complete their life cycles and allow at the same time the bird to develop natural immune response, thus encouraging a balance of nature between chicken and parasite without affecting bird performance *(2)* .

Diclazuril is a chemical substance synthesized from the benzene acetonitrile derivative, developed as anticoccidial agent for poultry with high efficacy against important Eimeria species of infecting poultry *(3)* , feed additive as drinking medication *(4)* . Success of diclazuril to interrupt completely the life cycle of the parasite is due to its action against different species of Eimeria (micro and macro gametocytes),In macrogamonts, dilatation of the rough endoplasmic reticulum around type II wall forming bodies which don't develop or

disappear completely whereas in microgamonts, the normal evagination of the microgamete was prevented *(5)*.

Narasin, the polyether ionophores antibiotic, is effective against different Eimeria species *(6)*. In addition to its anticoccidial effect, it is used as growth promoter in poultry, cattle and lambs *(7)*.

Narasin elicited reduction in oocyst count of coccidia oocyst count in naturally infected chickens by rapidly transporting sodium ions across the coccidian cell membrane in the opposite direction to the sodium pump, resulting in osmotic imbalance and cell rupture *(8, 9)*.

Used criteria for evaluation of anticoccidial agents in chickens are gain of weight and coccidian lesion scores *(3)*.The goal of the present study was the efficacy of diclazuril and narasin as anticoccidial drugs as well as the bird performance .

MATERIALS AND METHODS

Drugs

A-Diclazuril (Diclosol^{R}1%) is a water soluble formulation obtained from Pharma- Sweed Company .It was administered at a concentration of 5 ppm in drinking water (0.5 ml/L) for one week .

B-Narasin is ionophores compound manufactured by Elanco Comp. for Pharmaceutical Preparation. It was administered at a concentration of 40 mg/kg ration for one week.

Experimental Chickens

A total of 100, one-day old Hubbard broiler chicks and their ration were obtained from Cairo Poultry Company and used in this study.

Avian Eimeria spp.

Recent field of mixed species of sporulated Eimeria oocysts were isolated from fecal matter of diseased cases(7).

Vaccines

a-Hitchner,Lasota live vaccine was obtained from Intervet Boxmeer Company Holland.

b-Gumboro vaccine was obtained from Rhone-Merieau Company ,France.

Experimental design

Experimental broiler chicks used in this trial were kept under hygienic measures and given feed and water free from antimicrobial or anticoccidial drugs until used in the experiment. All chicks were vaccinated with Newcastle vaccines (Hitchner B1 at 7days & lasota at 18 days) and Gumboro vaccine at 14 days. On the 19th day of age all chickens were randomly divided into 5 equal groups(1-5),20 each. Group 1 non-infected and non treated (-ve control), group 2 infected chick with coccidia and non treated(+ve control),group 3 infected and treated with diclazuril (0.5ml /liter drinking water), group 4 infected and treated with narasin (40mg/kg ration),group 5 infected and treated with diclazuril and narasin in their therapeutic doses (Table 1).

Table 1. Experimental design

Group	Total number	Treatment	Dose
1	20	Non-infected and non-treated.(-ve control)	----
2	20	Infected non treated(+ve control)	----
3	20	Infected and treated with diclazuril	0.5ml /liter D.W.
4	20	Infected and treated with narasin	40mg/kg ration
5	20	Infected and treated with diclazuril and narasin	40mg/kg ration 0.5ml /liter d.w.

Coccidial infection

On day 19 of age chickens were given mixed inoculums of recent field isolates of sporulated Eimeria oocysts{5x(10)5} per chicken via gavages *(10).*

Treatment trail

Diclazuril (0.5ml/liter)was administered to birds in drinking water and narasin (40mg/kg ration) for one week at the age of 25 days to 31 days after the first appearance of blood in feces of infected birds. All chickens in each group were individually weighed at the beginning of the experiment and at weekly intervals. Body weight, feed consumption, feed conversion rate were also recorded.

Sampling and haematobiochemical study

At the end of the experiment (42days),5 birds from each group were sacrificed and two blood samples from each bird were collected .

a-First sample was taken on heparinized tube for determination of erythrocytes count, hemoglobin and packed cell volume percent *(11).*

b-Second blood sample was collected in centrifuge tubes and serum was separated and kept at-20℃ until used for measuring the serum transaminases (AST -ALT)*(12),* total proteins *(13),* Serum albumin *(14).* Serum globulin was determined by subtraction of obtained serum albumin from total protein *(15)* uric acid *(16)* creatinine *(17),* calcium *(18)* and inorganic phosphorus *(19).*

Oocysts count

At 24^{th},31^{th} and 41st day-old oocyst outputs were counted by Mc Master technique as described previously *(20).*

Statistical analysis

The obtained data were statistically analyzed using student ś (t) test *(21).*

RESULTS

Our results revealed that most common clinical symptoms in the chicken infected with coccidiosis and non treated were loss of appetite, depression, ruffled feathers, debility, dropped head and bloody diarrhea.

Infection of chickens with coccidia induced significant increase in mortality rate, lesion scores (Table 2),significant increase in oocyst count (Table 3) and significant decrease in body weight, weight gain and feed conversion rate (Table 4).Chickens experimentally infected with coccidiosis and non treated showed a significant decrease in RBCs count,Hb and PCV% and significant increase in serum levels of ALT,AST,total protein,globulin,uric acid and creatinine. Calcium and inorganic phosphorous were insignificantly increased as well as insignificant decrease in albumin were reported (Tables 5-7).

Administration of diclazuril and narasin either alone or in combination evoked a significant reduction in oocyst count (Table 3) and improved body weight gain and feed conversion rate, blood picture and biochemical parameters compared with untreated control birds (Tables 4 -7).

Table 2. Effect of diclazuril and narasin on mortality rate and lesions score of infected broiler with coccidiosis (n= 20).

Groups	Mortality		Lesion scores % (degree of enteritis)			
	N.	%	1	2	3	4
(1) non infected (control)	00	00	00	00	00	00
(2) infected non- treated	5	25	00	00	00	+
(3) infected treated with diclazuril	1	5	00	+	00	00
(4) Infected treated with narasin	1	5	00	+	00	00
(5) infected treated with diclazuril+ narasin	00	00	+	00	00	00

Table 3. Effect of diclazuril and narasin on oocyst count of infected broiler with coccidiosis (Mean±S.E.) (n=5).

Groups	oocysts count /gm feces(X1000)		
	24 day	31 day	41day
Healthy non infected (control)	6.2±0.98	8.5±2.8	6.6±2.9
infected non- treated	16.8±0.69**	15.6±0.46**	12.1±0.98**
infected treated with diclazuril	10.3±0.94++	6.1±0.93+++	8.6±0.52++
Infected treated with narasin	10.3±0.85+++	5.8±0.99+++	7.5±0.83+
infected treated with diclazuril+ narasin	8.1±0.93++	4.6±0.62+++	6.3±0.63+

** Significant at $P < 0.01$
+ $P< 0.05$ ++ $P< 0.01$ +++ $P< 0.001$
* Compared with non infected non treated group.
+ Compared with infected non treated group.

Table 4. Effect of either diclazuril(5ppm) (DI) and/ or narasin (40mg/kg b.wt.) on the Body weight gain (B.W.G.), Feed consumption (F.C.) and feed conversion rate(F.C.R.) of healthy and infected broiler chickens with coccidia(Mean±S.E.) (n=5)

Parameters / Group	Body weight 21 days	28 days				35 days				42 days			
		B.W. Gm/ chick	B.W.G. Gm/ chick	F.C. Gm/ chick	F.C.R	B.W. /chick	B.W.G. /chick	F.C. Gm chick	F.C.R	B.W. Gm /chick	.W.G. Gm /chick	F.C. Gm/ chick	F.C.R
Healthy non infected (control)	520.52 ± 6.43	790.32 ± 7.13	268.80 ± 3.45	521.32 ± 9.61	1.94	1220.23 ± 10.36	430.01 ± 5.31	914.14 ± 16.84	2.13	1680.95 ± 14.95	460.72 ± 6.28	1174.43 ± 10.65	2.55
Infected non- treated	525.41 ± 6.12	730.62 ± 9.62**	205.21 ± 3.21	473.64 ± 5.75**	2.30	1156.54 ± 6.73*	425.92 ± 6.19**	900.73 ± 6.54*	2.18	1568.62 ± 14.63**	412.08 ± 6.28	1110.8 ± 9.87*	2.70
Infected treated with diclazuril	535.49 ± 6.19	789.06 ± 13.96*	264.44 ± 2.63	515.21 ± 1.96	1.95	1218.14 ± 18.20	428.52 ± 1.96	909.64 ± 21.09*	2.10	1700.73 ± 20.95	482.59 ± 3.96	1170 ± 37.76*	2.42
Infected treated with narasin	530.62 ± 4.23	790.16 ± 10.61*	259.54 ± 4.23	521 ± 7.26**	1.96	1221.25 ± 25.35	431.09 ± 5.26**	908.93 ± 30.75	2.09	1710.16 ± 19.63	488.91 ± 5.07	1125.9 ± 45.73*	2.29
Infected treated with (diclazuril)+ narasin	519.38 ± 5.06	789.23 ± 8.64	269.85 ± 2.63	526.95 ± 9.87	1.95	1225.36 ± 14.17	436.13 ± 4.87	910.43 ± 18.96*	2.08	1720.93 ± 18.95	495.57 ± 6.68	1120.54 ± 46.98*	2.26

* $P < 0.05$ ** $P < 0.01$

Table 5. Effect of either diclazuril(5ppm) (DI) and/ or narasin(40mg/kg b.wt.) on hemogram of 42 days old healthy and infected broiler chickens with coccidia (Mean±S.E.) (n=5)

Parameters / Group	Healthy non infected (control)	infected non-treated	infected treated with diclazuril	Infected treated with Narasin	infected treated with diclazuril+ Narasin
R.B.Cs(106/mm)	2.91±0.09	2.89±0.09	2.76±0.08	2.79±0.09	2.89±0.09
HB gm%	10.11±0.18	9.25±0.28*	9.23±0.32*	9.15±0.28*	10.02±0.21
PCV (%)	29.51±0.51	30.02±0.24	29.73±0.32	29.92±0.24	30.02±0.24

*$P < 0.05$

Table 6. Effect of either diclazuril (5ppm) and/ or narasin (40mg/kg b.wt.) on the liver and kidney function of 42 days old healthy and infected broiler chickens with coccidia (Mean±S.E.) (n=5)

Parameters / Group	Liver enzymes		Kidney function	
	ALT u/ml	AST u/ml	Uric acid mg/dl	Creatinine mg/dl
Healthy non infected (control)	26.43± 1.36	36.7± 1.76	1.68± 0.08	0.95± 0.07
infected non- treated	34.41± 1.87**	41.73± 1.38*	2.73± 0.36*	1.25± 0.09*
infected treated with diclazuril	30.10± 1.03*	40.00± 1.40*	2.93± 0.44*	1.00± 0.04
Infected treated with narasin	32.05± 1.16**	41.0± 3.03*	2.10± 0.12*	1.35± 0.15*
infected treated with diclazuril narasin	34.04± 1.32**	42.00± 1.22*	2.15± 0.14*	1.13± 0.03*

* P < 0.05 ** P < 0.01

Table 7. Effect of either diclazuril(5ppm) and/ or narasin (40mg/kg b.wt.) on protein profile ,calcium and phosphorus of 42 days old healthy and infected broiler chickens with coccidia (Mean±S.E.) (n=5)

Parameters / Group	protein profile				Mineral	
	T.protein gm/dl	Albumin gm/dl	Globulin gm/dl	A/G ratio	Calcium mg/dl	Phosphorus mg/dl
Healthy non infected (control)	5.73± 0.06	3.22 ± 0.19	2.51 ± 0.34	1.28± 0.30	10.99± 1.28	4.89± 0.49
infected non- treated	6.06± 0.15*	3.07 ± 0.23*	2.99 ± 0.11*	1.03± 0.27	12.08± 0.89	5.80± 0.83
infected treated with diclazuril	5.90± 0.10*	3.14± 0.33	2.86 ± 0.24	1.09± 0.25	9.19± 1.06	5.45± 0.68
Infected treated with narasin	5.31± 0.11*	3.18± 0.25	2.13 ± 0.27	1.49± 0.27	10.21± 1.65	5.27± 0.35
infected treated with diclazuril narasin	5.97± 0.13*	3.19± 0.27	2.78 ± 0.26	1.15± 0.24	11.32± 1.45	5.57± 0.67

* P < 0.05 ** P < 0.01

DISCUSSION

Chicken infected with coccidiosis displayed loss of appetite, depression ,ruffled feathers, dropped wings and bloody diarrhea .Similar results were previously reported ***(22).***

In the present study it has been observed that diclazuril and narasin produced a significant decrease in oocyst shedding/ gm droppings in chickens experimentally infected with coccidiosis. This might be attributed to the action of diclazuril and narasin on developmental stages of coccidia, Diclazuril induce significant reduction in the number of oocysts count Narasin acts by rapidly transporting sodium ions across the coccidian cell membrane in the opposite direction to the sodium pump, so that the sodium concentrations inside and outside the cell are eventually equalized ***(4)*** . The cell counteracts this effect by speeding up the sodium pump, but by time, its energy reserves are exhausted and the sodium pump can no longer cope. The influx of sodium ions into the coccidian creates an excess of intracellular ions and water enters the cell to compensate for this

osmotic imbalance. The cell then begins to swell and finally ruptures *(8)* and narasin had antisporozoite activity *(23).* Similar improvement was observed by use of narasin in a dose of 60 or 80 ppm to broiler chickens when given for 45 days *(24).* Narasin was effective in reducing the number of oocysts count *(25,26)*. It has been recorded also that narasin had significant anticoccidial activity against all pathogenic species of chickens coccidian and recorded that the two higher levels of narasin (80 and 100ppm) decreased the total number of oocysts produced in battery raised broiler chickens *(27).*

Treatment of infected chickens with either diclazuril or narasin in therapeutic doses either alone or in combination reduced the clinical symptoms and improved the health status of the chickens infected with coccidiosis as evidenced in this study by the decreased mortalities and lesions scores. Our results coincides with previous study *(4),* where diclazuril induce reduction in clinical symptoms and improved the health status of the chickens infected with coccidiosis as evidenced in this study by the decreased mortalities and lesions scores in broiler chickens. Significant reduction in the severity of cecal and intestinal lesions after narasin application at levels of 60-80 ppm has been recorded *(24,28).*It has been reported that narasin produced a significant reduction in the severity of cecal and intestinal lesions when compared with those in infected non medicated controls. On similar grounds, the administration of narasin had significant anticoccidial activity and decreased lesion scores *(27).*

Results showed that the use of both drugs in treatment of infected chickens results in increased body weight and weight gain allover the experiment when compared with other infected non treated chickens. These improvements in the body weight may be due to the anticoccidial effect of diclazuril and narasin. Anticoccidial drugs causing improvement of the general health condition of the chickens, increasing food intake and absorption of nutrients *(2).* It has been showed that under diclazuril supplementation a great improvement in bird performance represented by marked increase in body weight gain and improvement of feed conversion *(28).* Nicarbazin (50 ppm) alone or in combination with narasin (50)ppm of each induce significant improve in body weight *(29)* and the use of narasin and nicarbazin combination gave an improved performance in treated birds *(23).*

Significant increase in feed consumption and reduction in feed conversion rate was recorded .This could be attributed to the increased integrity of the epithelial lining of the gastrointestinal tract leading to increased absorption of amino acids and other nutrients, in addition to increased feed intake leading to improvement in body performance *(30&31)* .

Our findings were confirmed by the previous finding obtained **(32),**which indicated an improve weight gain in turkey treated with diclazuril at a dose level of 1.0ppm better than at 0.5 ppm. In addition, It has been shown that diclazuril administration to chickens resulted in improved feed efficiency *(4).* Nicarbazin (50ppm) alone or in combination with narasin (50) ppm of each induced significant improvement of feed conversion *(28).*

The present study showed a significant decrease in erythrocytes count, hemoglobin content and packed cell volume percent in chickens infected with coccidia when compared with the non-infected control group. These results supported study *(3)* which showed that previous coccidian infection caused a significant decrease in the total erythrocytes count, hemoglobin content and packed cell volume percent. The decrease in hemogram of chickens infected with coccidia was conceivably attributed to hemorrhage associated with E. tenella infection *(33).*

Our study showed that the serum activities of liver transaminases (AST-ALT),total protein,globulin,uric acid and creatinine were significantly elevated but calcium and inorganic phosphorous were insignificantly increased as well as insignificant decrease in albumin in the

infected non treated broiler chickens with coccdia compared with non infected non treated chick. This might be due to the pathological changes induced by infection as it is well documented that the increase of AST and ALT activity is concomitantly recorded with liver damage leading to alteration in cellular permeability due to change in normal cell membrane. This allows the escape of these enzymes to the serum in abnormal high levels *(34&35).* Decreased the enzyme activities (AST-ALT) after treatment compared with pretreatment values was recorded *(36).* The increased uric acid and creatinine levels in serum of infected birds could be resorted to the degenerative changes in renal tubules preventing their excretion, thereby increasing their serum concentrations *(37).*

Summing up our observations, it could be concluded that coccidia infection in chickens induce several side effects in body weight, weight gain, gain %, feed consumption, feed conversion rate as well as some blood picture and some biochemical parameters but diclazuril and narasin in therapeutic doses either alone or in combination induce improvement in these parameters and decrease in oocyst count.

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الملخص العربى

محاولة حقلية لتقييم علاج الكوكسيديا باستخدام الداى كلازيوريل والنارسين في بداري تسمين الدواجن

شهيرة حنفي محمود حسبن* ، فاطمة السيد جابر* ، فوزي إبراهيم شحاتة**

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لقد تطورت صناعة الدواجن في جمهورية مصر العربية خلال الأعوام الأخيرة لتغطية الاحتياجات المختلفة من لحوم وبيض الدواجن لغذاء الإنسان وذلك لما تمتاز به هذه اللحوم البيض على قيمة غذائية عالية .ومن الأمراض الشائعة التي تهاجم صناعة الدواجن الإصابة بطفيل الكوكسيديا حيث يؤدى إلى فقد شديد في هذه الصناعة بسبب زيادة نسبة النفوق ونقص معدل النمو وإنتاج البيض.للأسباب السابقة فان علاج الإصابة بالكوكسيديا والقضاء عليها ذو أهمية كبيرة وذلك بالاستخدام الوقائي والعلاجي لبعض مضادات الكوكسيديا مثل الداى كلازيوريل والناراسين.

في هذه الدراسة تم استخدام مائة كتكوت هبرد عمر يوم وتم تقسيمهم إلي خمس مجموعات متساوية تحتوى كلا منها على عشرين كتكوت.المجموعة الأولى تركت بدون عدوى أو اى علاجات(مجموعة ضابطة),المجموعة الثانية كتاكيت مصابة بالكوكسيديا ولم يتم علاجها, المجموعة الثالثة كتاكيت مصابة بالكوكسيديا وتم علاجها باستخدام الجرعة العلاجية من الداى كلازوريل(١/٢ ملى لكل لتر من مياه الشرب يوميا لمدة أسبوع) عن طريق مياه الشرب من اليوم ٢٥حتى اليوم٣١, المجموعة الرابعة كتاكيت مصابة بالكوكسيديا وتم علاجها باستخدام الجرعة العلاجية من الناراسين(٤٠ جزء في المليون لكل كيلو جرام من العليقة) في العلف من اليوم ٢٥حتى اليوم٣١, المجموعة الخامسة كتاكيت مصابة بالكوكسيديا وتم علاجها باستخدام الجرعة العلاجية من الداى كلازيوريل و الناراسين معا من اليوم ٢٥حتى اليوم٣١.وفى اليوم ٤٢ من عمر الكتاكيت أخذت عينتين دم من كل كتكوت, العينة الأولى على هيبارين وذلك لدراسة تأثير الكوكسيديا على صورة الدم.أما العينة الثانية لفصل السيرم (المصل) اللازم لقياس بعض الوظائف البيوكيميائية.

تشير النتائج إلى حدوث نقص معنوي في عدد oocyst عند استخدام الداى كلازيوريل والنارسين بالجرعة العلاجية سواء منفردين أو معا في علاج الإصابة بالكوكسيديا عنها في المجموعة التي لم تعالج .

تبين لنا من تلك الدراسة أن الإصابة بالكوكسيديا أحدثت بعض التغيرات في صورة الدم وبعض الوظائف البيوكيميائية ومعدل النمو ,تشير النتائج أن الإصابة بالكوكسيديا أدت إلى حدوث نقص معنوي في وزن الجسم, وزن الجسم المكتسب, عدد كرات الدم الحمراء , تركيز الهيموجلوبين وحجم الخلايا المضغوطة وزيادة معنوية في معدل التحويل الغذائي, مستوى إنزيم الالانين أمينوترانسفيريز وإنزيم الأسبارتيت أمينوترانسفيريز وكذلك في مستوى البروتين الكلى,الجلوبيولين,حمض البوليك والكرياتينين,الكالسيوم والفسفور ونقص غير معنوي في الزلال.

نتج عن إعطاء الدوائين معا تحسن إيجابي في القياسات الدموية, إنزيمات الكبد ووظائف الكلى في مصل الطيور المعالجة.

نستخلص من هذه الدراسة أن الإصابة بالكوكسيديا أحدثت تأثيرات عكسية على وزن الجسم و معدل استهلاك العلف, التحويل الغذائي ,صورة الدم وبعض الوظائف البيوكيميائية في بداري التسمين ولكن استخدام الداى كلازيوريل والناراسين بالجرعة العلاجية سواء منفردين أو معا لعلاج الإصابة بالكوكسيديا في بداري التسمين لهما اثر فعال وجيد ضد طفيل الكوكسيديا حيث أديا إلى تقليل عدد oocyst, زيادة في معدل استهلاك العلف وتقليل معدل التحويل الغذائي وتحسن في صورة الدم وبعض الوظائف البيوكيميائية طوال مدة التجربة.لذلك ينصح باستخدام الداى كلازيوريل والناراسين بالجرعات العلاجية لعلاج الإصابة بالكوكسيديا.