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EVALUATION OF THE PROTECTIVE EFFICACY OF ANTICOCCIDIAL DRUGS AND VACCINE IN PREVENTION OF COCCIDIOSIS IN BATTEZ0041SWRY REARED CHICKENS

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SUMMARY

Efficacy of feed additive anticoccidial drugs (Salinomycin 60 ppm or diclazuril 200 g/ton) and live vaccine "Coccivac®)" in prevention of experimental coccidiosis in battery reared reared chickens was conducted. Clinical signs and/or mortalities, weekly average body weight and feed intake, feed conversion rate (FCR), challenge test, oocyst count, and lesion score were taken as criteria for evaluation. Salinomycin group showed FCR similar to diclazuril, while Vaccinated group showed lower rates than vaccinated medicated groups at the 3 weeks of life. Total FCR of Salinomycin and diclazuril were lower (2.4) than control negative (2.1) and diclazuril + vaccine (2.2) while vaccinated group and vaccinated Salinomycin medicated group are moderate in between. Vaccinated group and vaccinated medicated with Salinomycin showed lesions at all parts of the intestine at 29 and 33 days postvaccination, while in vaccinated medicated with diclazuril, lesions only in upper and middle part. Vaccinated non-medicated group 4 showed higher oocyst count/gm of drooping from the 4th day post vaccination (dpv) than vaccinated medicated groups 5 and 6. Birds received diclazuril and vaccine showed lower count than vaccine + Salinomycin group at all intervals. Following challenge, vaccinated groups showed no marked signs or mortalities while examined droppings revealed presence of oocyst in concentration of 180/gram of faces at 2 dpc, 7680/g. at 8 dpc and 1200/g. at 11 dpc. Oocyst count/g. in both vaccinated and/or medicated challenged chickens was lower than negative control groups. Birds received only vaccine induced higher and earlier oocyst shedding than those were vaccinated and medicated. Our study pointed out that the use of coccidiostate in the ration of "Coccivac®) vaccinated floor reared chickens was of value in lowering of the vaccinal reaction, oocyst shedding and improve FCR; where re-infection with vaccinal oocyst is possible.

Key words: Coccidiosis in chickens, Coccivac, control, Prevention, anticoccidial drugs.

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INTRODUCTION

As coccidial oocysts are ubiquitous and easily disseminated in the poultry house environment and have such a large reproduction potential, Inspite of the improvement in management and hygienic conditions in poultry production in recent years, outbreaks of coccidiosis still occur.

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It is very difficult to keep chickens coccidian free, especially under current intensive rearing conditions (Allen, 1986; Bhopal et al., 1992 and Saif et al.,2003). The use of anticoccidial feed additives over the past 50 years has played a major role in the growth of poultry industry. These anticoccidials could be classified as chemicals having specific mode of action against parasite metabolism. and of polyether ionophore which act through general mechanisms of altering ion transport and disrupting osmotic balance (Jeffers, 1997). However the hazardous use of anticoccidials and coccidiostates in poultry farms had been resulted in development of drug resistant Eimeria that threatened the economic stability of the poultry industry (Ruff and Danforth, 1966: Chapman, 1984, 1989, 1994 and 1998; Li et,al., 2004and William, 2006)

The work of Edgar had lead to the introduction of the first commercial vaccine, "Coccivac" in the 1950's, (Williams 2002) Live vaccines were used in coccidiosis control to a limited degree by the poultry industry for about 50 years primarily to protect the breeder and layer flocks. Their effectiveness depends on the recycling of initial doses of oocyst and gradual build up of solid immunity (Shirley et al. 1995). In broilers the live vaccine required careful determination of the dose to avoid depressing effect on the growth performance (Abu-El Ezz et al. 2002). In comparison to the usage of anticoccidials, and Coccivac® still used for control of disease in broilers, however a new generation of attenuated precocious live vaccines were introduced.

Many workers recommended the usage of coccidiostates in combination with vaccine in prevention of coccidiosis(Edgar, 1958, and Williams, 2002). Disease control strategies rely heavily on chemoprophylaxis and to a certain extent, live vaccines. Combined these factors inflict tremendous economic losses to poultry industry.beside Increasing regulations on the use of anticoccidial drugs coupled with costs for developing new drugs and live vaccines (Dalloul and Lillehoj, 2006).

Our study was planed to investigate the ability of Diclazuril and Salinomycin as anticoccidial drugs in control of vaccinal reaction "Coccivac®" in vaccinated floor reared chickens. Ability of vaccine and /or drugs to overcome challenge with field isolates.

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MATERIALS and METHODS

CHICKS:

Two hundred and sixteen; 1-day old chicks (avian-43) from commercial hatchery were used. The used chickens were reared on straw deep litter in clean, disinfected and isolated battery.

RATION:

The chicks were fed on prepared ration according to the National Research Council (NRC, 1984). Ration without feed additives was given to the chicks adlibitum.

EIMERIAL OOCYSTS:

Sporulated oocysts from field cases were purified. The collected oocysts were sporulated and passed in susceptible chicks 3 times. Virulence of the 3rd passage sporulated oocyst was tested according to Walelzky (1970) and FDA (1992).Sporulatedoocysts were kept in 2.5% potassium dichromate in screw capped bottles at 4-8 C° till used for challenge test.

COCCIDIAL VACCINE:

Commercial coccidiosis vaccine (Coccivac®) Sheering plough, Animal Health Corporation, USA. Batch No.66/03 was used. The vaccine components are as follows E. tenella, E.acervulina, E.brunetti, E.necatrix, E.praecox, E.maxima and E.mitis species.Coccivac is given to chicks at the 5th day of age by intra-ocular dropping after dilution in 30 ml saline.

ANTICOCCIDIAL DRUGS:

The used drugs were ZOX® (diclazuril) where each gram contains 5 mg diclazuril manufactured by Marcyrl Pharmaceutical Industries B.NO 51213. The drug was used in a dosage of 200 gm/ton. Coccifree® 12% (salinomycin granular 12%) manufactured by Almasria for industrials and trading Reg. no. 2089/2003 and used as instructed by the producer in dosage of 0.5 kg / ton to be finally 60 ppm.

OOCYST COUNT:

The collected fecal samples from experimental birds and intestinal contents were subjected to concentration flotation method and oocysts were counted by McMaster.

CHALLENGE TEST:

From each group 10 birds were challenged with 50 000 sporulated oocyst from filed isolates intracroup. Challenged birds were kept under daily observation for clinical signs and mortality.

SAMPLES FOR OOCYST COUNT:

Freshly voided droppings from living and intestinal and cecal contents from sacrificed or dead chickens were collected and subjected for presence of oocyst and oocyst count. Collection was done 3 times at 9th, 16th, and 23 day old, and 5 times at 2, 5, 7, 11 and 15 after challenge.

LESION SCORE:

Lesion scores were recorded according to the procedure described by Johnson and Reid (1970) was used for determining efficacy of vaccine or drug. From each group 3 chicks/ group were randomly taken and slaughtered at 5, 12, and 19 days after vaccination and 3, 6, 9, 12 days after challenge.

CHICKEN PERFORMANCE:

Weekly mean body weights gain, weekly feed intake as well as total feed intake were calculated on weekly basis according to Sainsbury (1984).

EXPERIMENTAL DESIGN:

At the 1st day of life the used chicks (216) were randomly divided into 6 equal groups (1-6); 36 chicks each. Chicks of group (1) were kept as negative control group (non-vaccinated non treated) while those of groups 2 and 5 as well as groups 3 and 6 were given medicated ration containing Salinomycin (600 ppm) as well as diclazuril (200 g/ton); respectively.

At the 5th day of life 3 chicks from each group were sacrificed and there intestine were examined to be still free from Eimerial infection.The other groups 4-6 (33 chicks/group) were vaccinated via eye drops with live attenuated coccidial vaccine. All chicken groups were daily observed for clinical signs and/or mortalities with weekly recording of average body weight and feed intake to calculate FCR (Table 1). Fresh droppings and 3 scarified chicks from each group were collected at 4, 11, 18, 20, 25, 29, and 33 days post vaccination to be examined for Eimerial oocysts (Table 2) and intestinal lesion score (Table 3).

At 17 dpv (22 days of age), 10 chicks from each group were randomly collected and separately kept on floor pens. Each chicken was orally challenged with 0.2 ml containing 5×10^5 mixed sporulatedoocysts. The challenged birds were subjected to daily observation for clinical signs and/or mortalities. Three chicks were randomly collected from each challenged groups and their intestine were examined for lesion score(Table 4) and oocyst count(Table 5).

RESULTS

Average weight of 1-day old chicks was 41.67 gm. All chicken groups showed no detectable signs or mortalities during the 1st three weeks of age. Examined dropping samples of negative control and medicated groups revealed no detectable oocysts during the first 3 weeks of life.

Control negative FCR was 1.7,1.9,2.1 and 2.4 in the 1st, 2nd, 3rd and 4th week of age, respectively (Table1). Salinomycin group 2 is 1.8, 2.0, 2.3 and 3.0 as well as diclazuril group 3 was 1.7, 2.0, 2.3 and 2.9 at the 4 weeks of life, Vaccinated group 3 was 1.7, 2.1,2.2 and 2.8. While vaccinated

medicated groups 5 and 6 are 1.8 and 1.9 at 1st week, 2.0 and 1.95 at 2nd week as well as 2.1 and 2.0 at the 3rd week.Total FCR of Salinomycin gr. 2 and diclazuril gr. 3 were the lowest (2.4) than control negative (2.1) and diclazuril + vaccine gr. 6 (2.2) while vaccine gr 4and vaccine+ Salinomycin gr.5 are moderate in between. Oocyst output/gm of drooping (table2) reveal that all non vaccinated groups 1-3 showed undetected Eimerialoocysts in examined drooping samples, vaccinated non medicated group 4 showed higher oocyst count/gm of drooping from the 4th days post vaccination (dpv) than vaccinated medicated groups 5 and 6. Birds received diclazuril and vaccine showed lower count than vaccine + Salinomycin group at all intervals.

At the 2nd week, vaccinated group show slight inflammation in upper and middle part of intestine, while vaccinated medicated with Salinomycin and diclazuril showed slight inflammation in middle and lower part respectively. At 25 dpv,vaccinated group showed inflammation extend to lower part, while vaccinated medicated with diclazuril showed no intestinal lesion. At both 29 and 33 dpv, vaccinated group and vaccinated medicated with Salinomycin showed inflammation in all parts of intestine, while in vaccinated medicated with diclazuril, inflammation found in upper and middle part only (Table 3).

Examined intestines showed no detectable lesion score in all negative control and medicated groups while vaccinated and vaccinated medicated showed lesion in upper part of. intestine in 1st, 2nd and 3rd week post-vaccination (table 3) .

Vaccinated groups showed no marked signs or mortalities while examined droppings revealed the presence of oocysts in concentration of 180/gram of faces at 2 days post challenge (dpc), 7680/gram at 8 dpc and 1200/gram at 11dpc. Oocyst count/gm (table 4) of challenged chickens showed that both vaccinated and/or medicated groups have lower count than control negative groups(1). Birds that received only vaccine induced oocyst shedding have higher count than those vaccinated and medicated but vaccinated gr 4 showed early elimination of oocyst sheding at 11thdpc than all groups.

Challenged control groups, 2 birds were died at 5 dpc with sever hemorrhagic cecum with white foci. General signs started to appear at 3 dpc such as ruffling feathers, huddling to each other, off food and dropping tinged with blood was seen in the 5thdpc (Table 5).at 2-dpc, upper intestinal mucosa showed slight inflammation. Medicated non-vaccinated groups showed no mortality, while at the 2-dpc inflammation found in upper and

middle part of intestine and general signs start to appear. At the 8th dpc in all parts of intestine.

DISCUSSION

From the 1st incident of coccidiosis as an enteric protozoal infection of young chickens (Tyzzer, 1929) until now, this affection causes major economic losses in intensive poultry farms (Long et al,) 1979 and Saif et al 2003). Many lines and strategies were planned to minimize the economic losses of such affection including hygienic measures, anticoccidial or coccidiostates drugs, and/or immunization with live attenuated vaccines. Salinomycin in feed (60 ppm) and diclazuril (200g/ton) as recommended by Abu El Ezz et al. (2002), Li et al (2004) and Suo et al., (2006).Coccivac vaccine was given through eye drop at the 5th day of age as previously used by Rose and Long (1980).

negative Control gr. 1, medicated diclazuril gr. 2 and Salinomycin gr. 3 and vaccinated groups (4-6) showed no detectable signs or mortalities during the 1st 3 weeks of age. These findings were also recorded in 9 trials of vaccinated birds by Bushel et.al. (1992)and Williams et.al. (1999) who stated that no coccidiosis was observed in vaccinated flocks and no lesions were recorded at p.m. While clinical signs of cecalcoccidiosis appeared about 2 weeks after vaccination (Lee 1987) and coccidial lesions in chicks between 5-23 days after vaccination (Williams and Andrews 2001).

Salinomycin in ration induced higherTotal FCR (2.3) than all groups(table 1), while diclazuril FCR (2.1) in group 3 is similar to these of groups 5 and 6 and these results agreed with Abu El Ezz et.al (2002) who stated that FCR improved in groups that received Salinomycin.

Vaccinated group 4 showed total conversion rate at the 4th week of age similar to that of control negative (2.2) and this agreed with Ruiz and Tamasaukas (1995) who proved no body weight difference was observed between vaccinated and non-vaccinated groups. Youn et. al., (1998) found that body weigh gains and groups immunized with coccidial vaccine and treated with anticoccidial drugs were moderately higher than groups just treated with anticoccidial drugs.

Similar results were reported by Chapman and Johnson (1992) who recorded the presence of oocysts in the litter before and after withdrawal of Salinomycin from the broiler feed and Amer et. al., (2007) found that diclazuril was more effective in controlling of coccidiosis in experimentally infected chickens.

Examined intestine showed no detectable lesion score in all control negative and medicated groups; while vaccinated and

vaccinated medicated showed lesion in upper part of intestine in 1st, 2nd and 3rd week post-vaccination (table 3), and this agreed with Youn et. al., (1998) who stated that the lesion score of all groups immunized with coccidial vaccine and/or treated with anticoccidial drugs were milder than those of the infected control groups. Vaccinated group showed slight inflammation in upper part of the intestine, while vaccinated medicated with Salinomycin and diclazuril showed slight inflammation in upper and middle parts; respectively. This result was proved by Williams and Andrews (2001) as coccidial lesions found in chickens between 5 and 23 dpv, where lesions observed up to 5 dpv were identified as primary a host response to the 1st vaccinal life cycle and those observed from 6 days onwards were designated as primary or secondary host response to the second and subsequent vaccinal life cycle. Williams (1994) reported that vaccinated birds had mild coccidial lesions when sampled at 26, 33 or 40 days after vaccination. Williams (2003) reported the presence of gross lesions in commercially vaccinated chickens does not indicate vaccine failure unless performance is also adversely affected.

All non vaccinated groups 1-3 showed undetected oocysts in examined drooping samples (table5) and this indicate complete hygienic measures. Vaccinated non medicated group 4 showed higher oocyst count/gm of drooping from the 4thdpv than vaccinated medicated groups 5 and 6. Birds received diclazuril and vaccine showed lower count than Salinomycin group at all intervals. Vaccinated group showed total FCR nearly similar to that of negative control and higher than vaccinated medicated ones. The result was similar to those of Bednik et. al., (1990) who stated despite of presence of some coccidial oocysts in dropping after vaccination the weight gain and feed conversion of vaccinated chicks were about the same as those given coccidiostats.

Vaccinated non-medicated group showed higher oocyst count/gm of drooping from the 4thdpv than vaccinated medicated groups. Birds that received only vaccine induced oocyst shedding higher than those were vaccinated and medicated. The result indicated at reduction of oocyst output in the vaccinated groups (Ruiz and tamasaukas 1995). The detection of no signs, reduced lesion and mortalities in vaccinated challenged group was previously reported by Norton et. al., (1989). The use of Anticoccidial drugs with vaccine may affect the level of immunity by lowering the effect of vaccine on intestinal lesions.

The detected signs and lesions in non vaccinated groups post challenge indicates pathogenicity of the used field isolates. The signs and lesions were indicative for establishment of infection (FDA 1992 and Saif et. al.,(2003). Our study pointed out that the use of coccidiostate in the ration of vaccinate birds with coccivac is of value in lowering the post-vaccinal reaction, oocyst shedding as well as improving feed conversion rate especially in battery reared broiler chickens where re-infection with vaccinal oocyst is possible.

Table (1): Average weekly body weight, feed intake and FCR of medicated and/or vaccinated chicks.

Gr. No.	Treatment	1st week			2nd week			3rd week			4th week			Total FCR
		Feed intake/g.	Weight gain/g.	FCR	Feed intake/g.	Weight gain /gm	FCR	Feed intake/g.	Weight gain/g.	FCR	Feed intake/g.	Weight gain/g.	FCR	
1	-ve	71.2	41.15	1.7	120.0	61.66	1.9	185.0	88.88	2.1	250.0	103.6	2.4	2.1
2	Sal.	69.7	37.57	1.8	123.3	60.0	2.0	185.0	77.77	2.3	250.0	82.14	3.0	2.4
3	Dicl.	60.6	34.28	1.7	125.0	61.83	2.0	181.5	77.77	2.3	235.7	79.52	2.9	2.4
4	Vacc.	63.6	36.93	1.7	126.7	60.0	2.1	1850	84.66	2.2	228.5	81.42	2.8	2.3
5	Sal.+Vacc.	60.6	33.33	1.8	130.0	63.33	2.0	185.2	87.84	2.1	242.0	88.57	2.7	2.3
6	Dicl.+Vacc.	60.6	30.88	1.9	130.0	66.66	1.9	181.5	90.74	2.0	245.7	98.5	2.5	2.2

Table (2): oocyst count / gm of medicated and/or Eimeria vaccinated chickens.

Gr. no.	Treatment	4 dpv	11 dpv	18 dpv	20 dpv	23 dpv	25 dpv	29 dpv	33 dpv
1	-ve	-	-	-	-	-	-	-	-
2	Sal.	-	-	-	-	-	-	-	-
3	Dicl.	-	-	-	-	-	-	-	-
4	Vacc.	120	340	1340	1460	1410	143	400	890
5	Sal.+Vacc.	110	210	1250	1410	1370	140	350	730
6	Dicl.+Vacc.	70	200	1170	1380	1380	139	355	680

Dpv = day post vaccination

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Table (3): lesion score of medicated and/or Eimeria vaccinated chickens.

Gr. no.	Trea.	11 dpv				18 dpv				20 dpv				23 dpv				25 dpv				29 dpv				33 dpv			
		u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c
1	-ve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Sal.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Dicl.	-	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+
4	Vacc.	+	-	-	-	+	+	+	-	+	+	+	-	-	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-
5	Sal.+Vacc.	+	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-
6	Dicl.+Vacc.	+	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-

dpv: day post vaccination. U : upper intestine. M : middle intestine L : lower intestine. C : Cecum.

Table (4): lesion score of Eimeria challenged chickens groups.

Gr. no.	Treat.	2days post challenge				5days post challenge				8days post challenge				11days post challenge				15days post challenge			
		u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c	u	m	l	c
1	-ve	+	-	-	-	+	+	+	+++	+	+	+	++++	+	+	++	+++	++	+	+	+
2	Sal.	+	+	-	-	+	+	-	+	+	+	+	++	+	-	++	++	++	+	+	-
3	Dicl.	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	++
4	Vacc.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-
5	Sal.+Vacc.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+
6	Dicl.+Vacc.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-

Table (5): Average Oocyst count / gm of Eimeria challenged chickens.

Gr. no.	Treatment	2days post challenge	5days post challenge	8days post challenge	11days post challenge	15days post challenge
1	-ve	180	1630	7680	1200	2440
2	Sal.	50	850	1120	1320	560
3	Dicl.	30	380	1100	1240	430
4	Vacc.	120	340	410	360	620
5	Sal.+Vacc.	50	112	1230	1560	1330
6	Dicl.+Vacc.	30	110	1430	1550	1160

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الملخص

فى محاولة للتحكم فى مرض الكوكسيديا فى الدجاج عن طريق لقاح الكوكسى فاك والحد من رد الفعل بمضادات الكوكسيديا التى تستخدم لنفس الغرض فى العدوى الطبيعية مثل عقار السالينومايسين (Salinomycin 60 ppm) وعقار الداكلازوريل (diclazuril 200 g/ton).

تم دراسة مدى كفاءة لقاح الكوكسى فاك فى التحكم فى مرض الكوكسيديا خلال تجربة اجريت على كتاكيت عمر واحد يوم تم تربيتهم على الأرض (فرشة) حيث تم اعطاء التحصين عن طريق التنقيط فى العين على عمر ٥ ايام.

تم ايضا دراسة مدى كفاءة عقار السالينومايسين وعقار الداكلازوريل فى التحكم فى مرض الكوكسيديا. تم مقارنة المجموعات المختلفة من ناحية معدل الصفة التشريحية المرضية (lesion score) بعد التحصين باللقاح وكذلك بعد احداث العدوى وكذلك عدد الحويصلات الناتجة بعد التحصين وكذلك بعد التحدى.

عند اضافة السالينومايسين الى العليقة يكون معدل التحويل اقل من باقى المجموعات بينما عند استخدام الداكلازوريل يكون معدل التحويل الغذائى مماثل للمجاميع التى تم تحصينها مع وجود كوكسيدوستات فى العليقة كذلك وجد ان معامل التحويل الغذائى للمجموعة التى تم تحصينها تقريبا مثل المجموعة السالبة .معدل الصفة التشريحية المرضية (lesion score) فى المجموعات المحصنة والمغذاة على علف يحتوى على كوكسيدوستات وكذلك المجموعة المحصنة فقط كان يوجد فى الجزء العلوى من الامعاء فى الاسابيع الثلاثة الاولى بعد التحصين.

معدل انتاج الحويصلات فى الزرق فى المجموعة المحصنة فقط كان اعلى من المجاميع المحصنة وتتغذى على علف بة كوكسيدوستات وكذلك المجموعة المحصنة مع وجود الداكلازوريل فى العليقة تنتج حويصلات اقل من التى تتغذى على عليقة بها سالينومايسين.

فى المجاميع التى تلقت علاج فقط دون تحصين، لا يوجد نافق بها ووجد انة بعد ظهرت التهابات فى الجزء العلوى والاوسط من الامعاء وبدأت الاعراض فى الظهور يومين من التحدى.

اوضحت الدراسة ان استخدام العقارات مع التحصين بالكوكسيفاك فى الدجاج المربى على الارض كان له تاثيات ايجابية من حيث الحد من الافات التشريحية وتحسين الكفائة التحويلية وتقليل اخراج الحويصلات فى الزرق. كان عقار الدايكلازوريل اكثر تاثيرا من السالينومايسين.

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