Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (2) April-June, pp. 355-360/Shojaei
Research Article

EVALUATION OF EXPERIMENTAL COCCIDIOSIS IN TWO COMMERCIAL STRAINS OF BROILER CHICKEN BY LESION SCORING AND OPG QUANTITATION

***S.S.R. Shojaei**
Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran
**Author for Correspondence*

ABSTRACT
The study was initiated to compare of two internationally reputed broiler strains in experimental coccidiosis by quantitation of defecated eimerial oocysts and intestinal lesions scoring under the local environmental and management conditions. 40 one-day old male chickens of Arbor Acres strain and Ross308 strain (20 chicks from each strain) used in this study and were divided randomly into four control and challenge groups. Feed and water were provided for ad libitum consumption. At 15 d of age, chickens of challenge groups (from each strain) were individually inoculated with a mixture of 50000 of sporulated oocysts of 4 species including of E. acervulina (20%), E. maxima (40%), E. tenella (25%) and E. necatrix (15%) and from the fourth day after Eimerial challenge, faecal droppings (litter samples) were collected 10 days consecutively for counting oocyst per gram (OPG). Also at day 7 after Eimerial challenge, 5 chicks from each challenge groups were euthanized and intestinal lesions were scored. The results indicated that in both challenge groups, there was an increasing process of OPG in days of 4-7 post challenging and the pick level of OPG was seen at seventh day after inoculation. The average of OPG in the Arbor Acres group was lower than the group Ross in days post inoculation and this difference was significant ($P<0.05$). Mean lesion scoring of intestine of Arbor acres and Ross challenge groups was 2.0 and 2.6 respectively. Lesions scoring difference was significant ($p<0.05$). According to the obtained results in this study and since oocyst index and lesions scoring almost always are considered as the most important indicators for coccidiosis evaluation, it can be realized that in the same surveillance condition the, regarding the severity evaluation of coccidiosis, Arbor Acres strain broilers shed less oocysts and had lower lesion scores than Ross308 strain broilers.

Keywords: *Arbor Acres, Ross308, Broiler, Coccidiosis, OPG, Lesions score*

INTRODUCTION
Coccidiosis of chickens, one of the most costly diseases affecting the poultry industry worldwide, is caused by infection with one or more of 7 species of the intracellular protozoan parasite Eimeria (Lee *et al.,* 2010; Sharman *et al.,* 2010). Eimeria infections occur when susceptible chickens ingest viable sporulated oocysts from contaminated litter. The ingested oocysts invade the intestinal epithelium in a region-specific manner, causing variant pathogenicity in poultry ranging from reduced feed conversion, weight loss,dysentery, enteritis, emaciation, drooping wings, poor growth to, at times, high rate of mortality and morbidity (Shirzad *et al.,* 2011; Lee *et al.,* 2010; Hadipour *et al.,* 2011).
After at least 2 generations of asexual reproduction, several hundred thousand Eimeria oocysts can be produced from a single oocyst, and are excreted in feces over several days or weeks (Morris *et al.,* 2006). The oocysts have cyst walls that are highly refractory to environmental extremes and disinfectants. Therefore, the oocysts can be transported mechanically by animals, insects, dust, and contaminated feed, water, and other fomites (Stotish *et al.,* 1987; Morris *et al.,* 2006)
Eimeria spp. is omnipresent and can survive in infected birds and the environment for long times (McDougald, 2003). It causes high mortality in young chicks because most of the Eimeria spp affects birds between the age of 3 and 18 weeks (Nematollahi *et al.,* 2009).
The tremendous growth of poultry industry in Iran is hampered by various factors and prevalence of various diseases in poultry which is of main concern. Among the various diseases, protozoan parasite of

the genus Eimeria causing coccidiosis is considered as one of the most common parasites (Hadipour *et al.*, 2011; Shirzad *et al.*, 2011). Due to higher stocking densities and intensive husbandry practices, its incidence is being increased in poultry (Nnadi and George, 2010).

The genetic selection of poultry for superior growth rate has arguably been the primary method for increasing productivity. However, many studies have been shown that such selection may be coincidentally accompanied by decreased resistance to diseases or changes in immunological response (Makram *et al.*, 2010; Li *et al.*, 2001; Fathi *et al.*, 2003; Huff *et al.*, 2005).

Vaccination programs alone cannot cope adequately with infectious diseases. A combination of vaccination and genetic resistance is essential to maximize the protection from diseases. The endeavor of this work was to compare of response of two modern broiler strains to coccidiosis by OPG counting and lesion scoring during an experimental infection

MATERIALS AND METHODS

This experiment was carried out at poultry research farm, faculty of Veterinary Medecine, Islamic Azad University, Karaj branch.

40 one-day old male chickens of Arbor Acres strain and Ross strain (20 chicks from each strain) were chosen and divided randomly into four control and challenge groups (10 chicks per group). After leg labeling, the chicks were reared under similar managerial, housed in cages covered plastic floor and according to their strains, fed ad libitum and kept in breeding room with appropriate distance.

They were fed a corn-soybean meal commercial diet containing18%-20% crude protein and 2800 kcal ME/kg diet. No antibiotic growth promoter or additives was included in the feed. The temperature was maintained at 30-32 °C for the first week and was reduced by 2°C on weekly basis. Lighting was provided 23 hours light and one hour dark. No vaccine was used during the test period. Ambient ventilation and other environmental conditions fully met the requirements laid down in the technical instructions of each strain.

In order to purification and standardization of doses of the inoculum for experimental infection, four selected species including *E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix* were propagated in 8-wk-old Eimeria-free chickens by oral inoculation and the oocysts were recovered from feces which were collected for up to 8 days post inoculation, sporulated and stored in 2% potassium dichromate at 4°C before inoculation (Conway, 2007). The appropriate dose had been estimated in a preceding test inoculation from the results of these initial tests (Shirley, 1995).

Then, at 15 d of age, chickens of challenge groups (from each strain) were inoculated orally with a dosage of 50000 of the mixed sporulated oocysts of 4 species including of *E. acervulina* (20%), *E. maxima* (40%), *E. tenella* (25%) and *E. necatrix* (15%)

From the fourth day after Eimerial challenge, samples of the chickens feces were taken from their cages for 10 consecutive days. With regard to control of probable infection, OPG counting was performed on days 5 and 14 before inoculation. The number of oocysts was quantitated by Mac master method (Conway, 2007).

On the other hand, since another current valuable diagnostic method is lesion scoring, an interpretation of subclinical coccidiosis based on macroscopic visible lesions caused by Eimeria (Johnson and Reid, 1970), at day 7 after inoculation, we selected randomly 5 chicks from each challenge group and intestinal lesions were scored after euthanizing.

For statistical analysis, data were subjected to a one-way analysis of ANOVA test. When significant differences among means were found, data were analyzed by Post-hoc and compared by Tukey test.

RESULTS

A comparative study was undertaken on two reputed broiler strains namely, Arbor Acres and Ross308 regarding production and defecation of Eimerial oocysts and intestine lesion scoring, as well.

Table 1: OPG comparison between Ross308 and Arbor Acres strains in different days post infection

Groups	**Daily oocyst per gram of feces during a 10-day period after Eimerial inoculation**									
	4	5	6	7	8	9	10	11	12	13
Uninoculated group	0	0	0	0	0	0	0	0	0	0
	55200	65000	175000	181000	122000	87000	47500	15000	9800	3500
	54000	67000	183000	179000	126000	88000	26500	16000	9600	2500
Group	52500	68000	180000	179000	123000	85000	28000	17000	9750	4000
includes	53500	64000	178000	180500	125000	84000	26000	14000	9650	2000
Ross308	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
strain	53000	66000	179000	180000	124000	86000	27000	15500	9700	3000
	50000	50000	145000	151000	98000	72000	25000	13500	7900	1800
	51000	52000	113000	150500	100000	70000	24000	125000	7700	1600
Group	47000	51500	146000	153000	97000	71500	26000	14000	7500	1650
includes	48000	50500	142000	149000	101000	70500	23000	12000	8100	1750
Arbor Acres	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
strain	49000	51000	144000	151000	99000	71000	24500	13000	7800	1700

Table 2: Gross Lesion Score for 5 randomly selected chicks from each strain 7 days post inoculation

	GLS at Day 7 after challenge	
Selected chickens	**Arbor Acres strain**	**Ross Strain**
Chick 1	2	3
Chick 2	2	3
Chick 3	2	3
Chick 4	1	2
Chick 5	3	2
Mean GLS	2	2.6

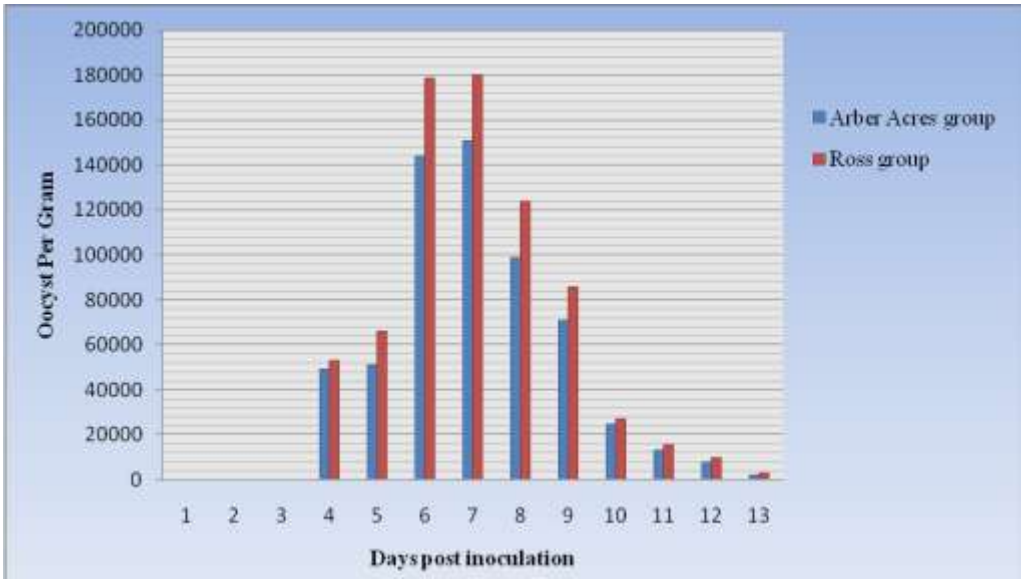

Figure 1: Oocysts per gram feces in 10 days post experimental infection by Eimeria oocysts in Arbor Acres and Ross308 strains. OPG on vertical axis has been plotted against days post Eimerial inoculation on horizontal axis

The results of microscopic examination of 90 fecal samples are depicted in the Table 1. The OPG of pre-infection period of all groups and control group was zero at all days of sampling such as we expected, which is indicative of the appropriate qualitative control of the experiment process. In the challenge groups, there was an increasing process of OPG in days of 4-7 post challenging and the pick level of OPG was seen at seventh day after challenging. From day8 to 9, decreasing of OPG was happened, this decreasing continues with mild, fast and mild process to day of 13, respectively.

In addition, the Mean Gross lesion scoring(GLS) of intestinal damages in Arbor Acres and Ross challenge groups was 2 and 2.6 respectively which were evaluated by Johnson and Reid method (1970) (Table 2).

Discussion

According to the Table 1, OPG of uninfected control was zero in all experiment days which were representative of proper control process during the study. In both strains on days 4 to 13 post infections, a raising trend, one peak and then a decreasing trend was seen, respectively. Also in both strains the highest OPG was measured in three days 6, 7 and 8 after the challenge and between these days, day 7th dedicated the peak of mean OPG to itself by 180000 in the Ross group and 151000 in the Arbor Acres group.

Moreover, the average of OPG in the Arbor Acres group was lower than the group Ross while this difference was significant according to t-test. This significant difference was 95% ($p<0.05$) in days 4, 9 and 10 post challenge and in the other days except day 13after infection was 99% ($p<0.01$). On day 13, the difference of counted oocysts declined ($p=0.064$) ($p>0.05$), so this difference with the confidence level of 95% is not significant whereas the Arbor acres group had lesser OPG in compare to Ross group.

Besides OPG counting, lesion scoring is considered as another common diagnostic method in evaluation of coccidiosis, therefore this index was used in evaluation of two mentioned strains in this study, as well. Total lesion scores of 5 selected chicks from each challenged group indicated that Arbor Acres inoculated group showed mean GLS of 2.6 whereas the average GLS of Ross308 infected group was calculated 2 and this difference was significant among mentioned groups.

Regarding both groups were reared under the complete same condition of growing and with consideration of obtained results from both oocysts output and lesion scoring, it could be realized that the Arbor Acres has more partly resistance to coccidiosis than the Ross strain since the Arbor group shed lesser oocysts and had less intestinal lesions than the Ross group in all days post infection.

Actually there is no evidence about comparison of OPG and lesion scoring between mentioned strains yet, but in the aforementioned studies, these strains have been compared of other indexes which showed that totally Arbor Acres performance is better than Ross in poultry industry

Hussain *et al.,* (1993) conducted a study based on comparison of two stains Ross 308 and Arbor Acres in feed conversion ratio (FCR) and consequently the best FCR was observed for strain Arbor Acres.

In another study, performance of these two commercial broiler strains which were reared under local environmental conditions, were examined and compared which each other (Zahid and Hussain, 2002). The observations were recorded on body-weight gain, feed consumption, general health and mortality. Also FRC was computed from the data and dressing percentage was determined for all broilers separately. The average weekly live weight of Arbor Acres was significantly higher than chicks of Ross strain while the statistical analysis revealed no substantial difference in feed consumption and FCR among the mentioned broiler strains. Although statistically there were no significant differences in mortality rate for these strains, apparent basis mortality was substantially higher in Ross as compared to Arbor Acres. Similar findings were reported by Chew (1987) who compared Ross and Arbor Acres strains and found no significant difference in mortality percent. The dressing percentage of Ross strain was considerably higher ($P<0.05$) as compared to Arbor Acres. Similar results were obtained by Singh *et al.,* (1981) who reported the studies on phenotype correlations among live weight and dressed weight in poultry.

Iqbal *et al.,* (2012) compared the performance of these two economic strains and economic traits measured were body weight, feed intake, feed conversion ratio (FCR), mortality, antibody titres, dressing percentage and economic evaluation as cost of broiler per kilogram live weight produced. Although in

some cases no significant difference was shown between these two strains, overall Arbor Acres indicated better performance as compared to Ross.

Evaluation of OPG variation during different days post inoculation was another aim of this study. As can be seen in figure 1, an ascendant and descendant trend occurred in both experimented groups respectively during the study, thus from this aspect no difference was among them. To state the matter differently, the OPG peak in both groups was in days 6 and 7 after challenge (with a negligible difference) since the life cycle accomplishment of the most Eimeria species is occurred at these days (days 6 and 7 post infection) (Velker, 2011) and oocysts are produced and consequently shed by destruction of epithelial cells through poultry feces. In addition, the reduction pattern after this peak is related to natural decrease of oocysts production since the number of merozoites which enter to gametogony phase will decline. Because gametogony starts after the second or third schizogony and after that if no re-infection occurs the number of oocysts will decrease gradually as the intrinsic potential of merozoites declines naturally. As in the present study chicks were reared in chicken cage systems in order to keep the operation clean, efficient and profitable, they did not have the chance of re-infection by feed-pecking of scattered grains on the ground. As a result, the presented survey could be an authentic pattern for future complementary studies.

According to the obtained results of this study and since oocyst index and lesion scoring almost always are considered as the most important indicators for coccidiosis evaluation, it can be realized that in the same surveillance condition the severity of coccidiosis in Arbor Acres is less than in Ross. In other words, the Eimerial oocyst shedding potential and the intestine lesions induced by coccidiosis which are substantial factors in continuance procedure of coccidiosis, occurance of this disease and economic losses due to poultry coccidiosis, are lower in Arbor Acres as compared to Ross.

Finally, in order to access a more accurate assessment and comprehensive comparison of vital reactions of these two strains against coccidiosis, other important indexes like Carotenoid level and absorption coefficient should be taken in to account in the future complementary studies.

ACKNWLEDGEMENT

I am grateful to Islamic Azad University, Karaj Branch authorities, for their useful collaboration.

REFERENCES

Akhtar M, Anwar MI, Iqbal Z, Muhammad F, Awais MM, Haq AU and Sawicka EH (2012). Immunological evaluation of two local isolates of Eimeria tenella gametocytes against coccidiosis in poultry. *Pakistan Veterinary Journal* **32** 77-80.

Chew PC (1987). A trial on compare the performance of two broiler strains. *Poultry Abstracts* 4(8) 1914.

Conway DP, Dayton AD, Mckenzie ME (1999). Comparative Testing of Anticoccidials in Broiler Chickens:The Role of Coccidial Lesion Scores. *Poultry Science* **78** 529–535.

Conway DP, McKenzie ME (2007). *Poultry Coccidiosis Diagnosis and Testing Procedures* (Iowa: Blackwell publishing) **3**rd edition.

Fathi MM, Ali RA, Qureshi MA (2003). Comparison of immune responses of inducible nitric oxide synthase (iNOS) hyper-and hypo responsive genotypes of chickens. *International Journal of Poultry Science* **2** 280-286.

Hadipour MM, Olyaie A, Naderi M, Azad F, Nekouie O (2011). Prevalence of Eimeria species in scavenging native chickens of Shiraz, Iran. *African Journal of Microbiology Research* **5** 3296-3299.

Hansen J, Perry B (1994). *The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants:* A Handbook (International Laboratory for Research on Animal Diseases, Nairobi) 171.

Huff GR, Huff WE, Balog JM, Rath NC, Anthony NB and Nestor KE (2005). Stress response differences and disease susceptibility reflected by heterophil tolymphocyte ratio in turkeys selected for increased body weight. *Poultry Science* **84** 709-717.

Iqbal J, Mian AA, Ahmad T, Hassan S and Hassan Khan S (2012). Comparative performance of different economic traits of four imported broiler strains under local conditions of Pakistan. *Pakistan Journal of Agricultural Research* **25**(1).

Jeong Mi Y, Sung H, Kang J, Woo H, Kim S, Hyun S, Lillehoj HS and Min W (2011). Effects of Simple and Disposable Chicken Cages for Experimental Eimeria Infections. *The Korean Journal of Parasitology* **49**(3) 299-302.

Johnson J, Reid WM (1970). Anticoccidial drugs: Lesionscoring techniques in battery and floor-pen experiments with chickens. *Experimental Parasitology* **28** 30–36.

Lee BH, Kim WH, Jeong J, Yoo J, Kwon YK, Jung BY, K Won JH, Lillehoj HS and Min W (2010) Prevalence and cross-immunity of Eimeria species on Korean chicken farms. *Journal of Veterinary Medical Science* **72** 985- 989.

Li Z, Nestor KE, Saif YM, Anderson JW and Patterson RA (2001). Effect of selection for increased body weight in Turkey on lymphoid organ weights. Phagocytosis and antibody responses to fowl cholera and Newcastle disease-localization of a protein antigen in the chicken spleen. Effect of various manipulative procedures on the morphogenesis of the germinal center. *Immunology* **28** 1-21.

Mahmood A, Mumtaz A, Khan M and Nisarkhan A (2001). Application of Lesion Scoring Technique for the Assessment of Pathology and Treatment of Coccidiosis in Broiler Chicks. *International journal of Agriculture and Biology* **4** 464–468.

Makram A, Galal M, Fathi M, El-Attar AH (2010). Carcass Characteristics and immunocompetence Parameters of Four Commercial Broiler Strain Chickens under Summer Season of Egypt. *International Journal of Poultry Science* **9**(2) 171-176.

McDougald LR (2003). Coccidiosis. In: *Diseases of Poultry* (Iowa State Press, Blackwell Publishing Company, USA) **11**th Edition 974-991.

Morris GM, Gasser RB (2006). Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in Eimeria. *Biotechnology Advances* **24** 590-603.

Nandi PA and George SO (2010). A Cross-Sectional Survey on Parasites of Chickens in Selected Villages in the Subhumid Zones of South-Eastern Nigeria. *Journal of Parasitology Research* **141** 1-6.

Nematollahi A, Moghaddam GH and Pourabad RF (2009). Prevalence of Eimeria species among broiler chicks in Tabriz (Northwest of Iran). *Munis Entomology & Zoology Journal* **4** 53-58.

Sharman PA, Smith NC, Wallach MG and Katrib M (2010). Chasing the golden egg Vaccination against poultry coccidiosis. *Parasite Immunology* **32** 590-598.

Shirzad MR, Seifi S, Gheisari HR, Hachesoo BA, Habibi H and Mehrani H (2011). Prevalence and risk factors for subclinical coccidiosis in broiler chicken farms in Mazandaran province, Iran. *Tropical Animal Health and Production* **43**(8) 1601-4.

Singh CSP, Singh SK, Mishra HR and Jaruhar SB (1981). Studies on phenotype correlations among live weight and dressed weight in poultry. *Poultry Abstracts* 7(4) 795.

Stotish RL, Wang CC and Meyenhofer M (1978). Structure and composition of the oocyst wall of Eimeria tenella. *Journal of Parasitology* **64** 1074- 1081.

Vadlejch J, Miloslav P, Lukešov D, Čadkov Z, KudrnčovIvana M and Langrov JI (2013). The Concentration McMaster Technique is Suitable for Quantification of Coccidia Oocysts in Bird Droppings *Pakistan Veterinary Journal* **33**(3) 291.

Velker F (2011). Transmission dynamics of Eimeria acervulina in broilers. PhD Thesis, Wageningen University, Wageningen, the Netherlands.

Williams RB (1999). A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International Journal for Parasitology* **29** 1209-1229.

Zahid S and Hussain SA (2002). performance of six commercial broilers strains reared under local environmental conditions. *Pakistan Journal of Agricultural Research* **17**(2).