

Exposure of Broiler Chickens to a Weak Electromagnetic Field Reduces the Impact of a Simulated, Commercial *Eimeria* Infection

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Abstract: Problem statement: In a previous study, the exposure of broiler chickens to a weak Electromagnetic Field (EMF) reduced the severity of a coccidiosis infection. The birds were infected by gavage into the crop which was not representative for the field situation. **Approach:** The possible anticoccidial activity of EMF was investigated in broiler chickens with a simulated, commercial coccidiosis infection. There was an uninfected and infected group not receiving further treatment. Another uninfected and infected group were subjected to EMF treatment. The infection was induced by adding to the litter a mixture of *E. acervulina*, *E. maxima* and *E. tenella*. EMF treatment lasted for 30 min day⁻¹; the field strength within the cages was set to 5 µTesla rms. **Results:** Infection with *Eimeria* resulted in a transient reduction of growth performance in the control chickens. Exposure to EMF counteracted the effect of infection on growth performance. EMF treatment had no effect on oocyst shedding. In the infected birds exposed to EMF, the lesion scores related to the three *Eimeria* species were generally lower than in the infected controls. Due to cross-contamination, the uninfected birds also showed intestinal lesions, the severity being less than in the infected chickens. In the uninfected birds, EMF treatment also had reduced the severity of the lesions. **Conclusion:** In this study EMF exposure protected against coccidiosis in broiler chickens. Consequently, EMF was considered as a possible alternative to anticoccidial drugs.

Key words: Electromagnetic fields, natural infection, coccidia, broilers

INTRODUCTION

Coccidiosis is a common infectious disease in poultry, causing major economic losses. 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 such as *Clostridium perfringens*, leading to necrotic enteritis (Maxey and Page, 1977; Shane *et al.*, 1985). The *Eimeria* species have a complex life cycle that occurs outside the host (exogenous stages) and inside the host (indigenous stages). During the indigenous stage, there are both intracellular and extracellular stages and both asexual and sexual reproduction. The complex life cycle of the *Eimeria* is associated with complex host immune responses to the parasite (Lillehoj, 1994; Lillehoj and Trout, 1996).

The invasion of the *Eimeria* sporozoites into the intestinal epithelium results in massive infiltration of macrophages, granulocytes and lymphocytes into the lamina propria. The macrophages modulate the severity of the infection and the lymphocytes, in particular CD4⁺ T cells, act as inducer of an effective immune response. Both macrophages and lymphocytes are the source of cytokine production in the intestine during *Eimeria* infection and thereby modulate the immune response (Breed *et al.*, 1997; Lillehoj, 1994; Lillehoj and Trout, 1996). It has been reported that an electromagnetic field (EMF) causes stress at the cellular level, leading to the production of cytokines followed by an enhanced immune response (Simko and Mattsson, 2004). We hypothesized that exposure of broiler chickens to EMF may antagonize the effects of coccidiosis infection. Indeed, in broiler

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chickens infected with an *Eimeria* mixture containing *E. acervulina*, *E. maxima* and *E. tenella*, the exposure to EMF was shown to counteract the decrease in weight gain and feed intake as well as the development of intestinal lesions (Elmusharaf *et al.*, 2007).

In our previous study (Elmusharaf *et al.*, 2007) the chickens had been infected with a mixture of *Eimeria* by a single dose administered through gavage into the crop. Infection through gavage with a single high dose of inoculum has no similarity with the field situation and might result in results with no practical relevance. Standardized infection through the litter mimics the field situation, but can be done under controlled conditions and allows experimental flexibility and a large number of experimental units within the research facility (Elmusharaf *et al.*, 2010).

In the present study, the possible anticoccidial activity of EMF was further investigated. In broiler chickens, a natural progression of coccidial infection was simulated by adding to the litter a mixture of *E. acervulina*, *E. maxima* and *E. tenella*. The effects of EMF exposure on growth performance, intestinal lesions and oocyst excretion were measured.

MATERIALS AND METHODS

Birds and housing: About 272-one-day-old female broilers (Ross 308) were purchased from a local hatchery. On the day of arrival (day 1), they were wing-banded and randomly housed in wire-floor, suspended cages. Each cage was provided with thick foil and wood shavings as litter. Continuous lighting was provided throughout the experiment. The temperature in the cages on arrival of the birds was 32°C and then gradually decreased to 20°C at the end experiment.

Diets: Starter and grower diets were used. The diets did not contain growth promoters or coccidiostats. The starter diet was offered until day 13, followed by the grower diet. The ingredient composition of the diets was as follows (g kg⁻¹ diet as fed; starter/grower): Wheat (250/500), soybean meal (49% crude protein) (345.5/253.7), corn (27.50/12.29), animal fat (4.00/500), extracted rape seed meal (2.00/3.00), soybean oil (1.87/1.39), corn gluten (1.0522/0.000), premix (0.50/0.50), salt (0.2119/0.1902), sodium bicarbonate (0.2367/0.2295), monocalcium phosphate (1.0995/0.4276), limestone (1.4674/1.0469), DL-methionine (0.2695/0.2146), L-lysine HCl (0.1723/0.2418), L-threonine (0.0638/0.0855), phytase (0.0100/0.010). Throughout the experiment, the birds had free access to feed and tap water.

Experimental design: The experiment had a 2×2 factorial design with 8 replicates, each replicate consisting of 8 birds. On day 13, the broilers were weighed and divided over the 4 experimental groups so that the weight distributions of the groups were similar. There was an uninfected and infected group not receiving further treatment. Another uninfected and infected group was subjected to EMF treatment, which was started on day one. The infection with a mixture of *Eimeria* species was done on day 15 (Table 1). Under each cage of the EMF treated birds, there was a magnetic coil, consisting of 50 loops of 1.5 mm² electricity wire. The coils were connected via a relays bank to an amplifier and signal generator controlled by a microprocessor, which regulated the period of time and the EM field per cage. The EMF treatment period lasted for 30 min and was given to each cage consecutively and once per 24 h. The field strength within the cages was set to 5 µTesla rms, verified by a FW Bell 5180 Tesla meter with a MOS51-3204 Low field probe. In order to avoid any effect of EMF exposure on the other groups, the EMF-free and EMF-infected groups were housed in adjacent rooms within the facility. The cages of the uninfected and infected groups had evenly distributed locations.

Experimental infection procedure: To test the anticoccidial activity of the EMF, a natural simulation of a coccidial infection within the broilers' test facility was developed. This was accomplished by placing both infected groups on day 15 in new litter seeded with sporulated coccidial oocysts. The infected chickens would pick up the oocysts from the seeded litter.

Table 1: Post-infection growth performance for the four experimental groups

Variable and days PI	Control ¹		EMF		Pooled SEM
	Uninfected	Infected	Uninfected	Infected	
Body weight (g)					
Day 6	738 ^a	658 ^b	690 ^b	684 ^b	15.75
Day 13	1183 ^a	1021 ^b	1191 ^a	1216 ^a	25.04
Day 21	1644 ^a	1539 ^b	1701 ^a	1686 ^a	37.31
Weight gain (g day⁻¹)					
Days 1-6	50 ^a	45 ^b	45 ^b	46 ^{ab}	1.54
Days 7-13	65 ^a	56 ^b	66 ^a	67 ^a	2.08
Day 14-21	61	65	67	68	3.93
Feed intake (g day⁻¹)					
Days 1-6	85 ^a	75 ^b	76 ^b	76 ^b	2.20
Days 7-13	114 ^b	106 ^b	116 ^a	115 ^a	2.68
Day 14-21	139	142	141	136	5.01
Feed Conversion Ratio (FCR)					
Days 1-6	1.71	1.69	1.67	1.64	0.037
Days 7-13	1.77 ^a	1.91 ^b	1.76 ^a	1.72 ^a	0.050
Days 14-21	2.36	2.20	2.18	2.02	0.111

^a, ^b, ^c: Mean values within the same row with different superscript letter are significantly different (p<0.05). Data are presented for 8 cages per experimental group

Individual birds would ingest different numbers of oocysts at different times, which mimic a natural, progressive infection. The uninfected groups were placed in a clean litter. Sporulated oocysts of *E. acervulina* (Weybridge strain), *E. maxima* (Weybridge strain) and *E. tenella* (Houghton strain) laboratory strains were used in this study. The numbers of each species mixed into the wood shavings were equivalent to 1.76×10^4 *E. acervulina*, 1.25×10^4 *E. maxima* and 7.5×10^3 *E. tenella* per bird. The infected litter was spread across the back half of the cage. The oocysts were obtained from the Animal Health Service Ltd., Poultry Health Centre (Deventer, the Netherlands).

Performance measurements: Birds were weighed individually on days 15, 21, 28 and 35. Feed intake was measured per cage on a weekly basis. Average feed intake per broiler within a cage was calculated and corrected for dropouts, if any. Mortality was registered on a daily basis.

Infection measurements: The number of Oocysts Per Gram faeces (OPG) was determined for excreta collected on days 6, 13 and 21 Post Infection (PI). Oocyst shedding was assessed on one sample of homogenized fresh excreta collected from each cage. The modified McMaster counting chamber technique of Hodgson (1970) was used. A 10% (w/v) faeces suspension in a salt solution (151 g NaCl mixed into 1 L of water) was prepared. After shaking thoroughly, 1 mL of the suspension was mixed with 9 mL of a salt solution (311 g of NaCl mixed into 1 L of water). Then, the suspension was put into the McMaster chamber using a micropipette and the number of oocysts was counted and expressed per gram of faeces (Peek and Landman, 2003). The severity of coccidial lesions was scored on day 6, 13 and 21 PI while the investigator was blinded to treatment modality. The 0-4 scoring system described by (Johnson and Reid, 1970) was used.

Statistical analysis: The oocyst values were logarithmically transformed [$\log_{10}(X+1)$] to create a normal distribution and lesion scores were transformed using multinomial transformation. Lesion scores for the various experimental groups were compared using the non-parametric Mann-Whitney U test. Performance and oocyst data were subjected to the LSD test. The statistical program SPSS (SPSS Inc, Chicago, USA) was used. The level of statistical significance was preset at $p < 0.05$.

RESULTS

Growth performance: Post-infection growth performance data are presented in Table 1. The

infection caused a significant growth depression during the first 2 weeks in the control birds, but not in those exposed to EMF. During days 1-6 PI, the uninfected EMF-treated chickens displayed lower weight gain than did the uninfected controls, but this difference had disappeared during the subsequent periods. In the period of 7-13 days PI, the infected EMF-treated chickens grew significantly faster than did the infected controls. The infection produced a decrease in feed intake in the control chickens during days 1-6 and 7-13 PI, but feed intake was left unchanged in their counterparts exposed to EMF. In the control birds, the infection caused an increase in the feed conversion ratio during days 7-14 PI. In the EMF-treated chickens, the infection did not influence the feed conversion ratio.

Coccidiosis infection: The uninfected chickens were found to shed oocysts with the faeces, pointing at cross contamination (Table 2). On day 6 PI, the OPG values were significantly higher in the infected than the uninfected birds, irrespective of whether they had been exposed to EMF or not. Among the EMF-treated chickens the infected animals, consistently showed higher OPG values than did their uninfected counterparts. Among the control chickens, the infected and uninfected animals had similar OPG values on days 13 and 21 PI.

Table 2: Number of Oocysts Per Gram faeces (OPG) expressed as $\log_{10}(X+1)$ for the four experimental groups

Variable and days PI	Control ¹		EMF		Pooled SEM
	Uninfected	Infected	Uninfected	Infected	
Day 6	1.719 ^a	4.033 ^b	2.168 ^a	3.988 ^b	0.606
Day 13	3.259 ^{ab}	4.577 ^b	2.051 ^a	4.384 ^b	0.553
Day 21	4.041 ^a	3.815 ^a	2.358 ^b	3.659 ^a	0.387
Day 6	1.719 ^a	4.033 ^b	2.168 ^a	3.988 ^b	0.606

^{a, b}: Mean values within the same row with different superscript letter are significantly different ($p < 0.05$). Data are presented for 8 cages per experimental group

Table 3: Intestinal lesion scores per *Eimeria* spp. for the four experimental groups

Score per <i>Eimeria</i> spp.	Control ¹		EMF		p-value
	Uninfected	Infected	Uninfected	Infected	
Day 6 PI					
<i>E. acervulina</i>	0.44 ^a ±0.16	2.44 ^b ±0.13	0.44 ^a ±0.13	2.00 ^c ±0.13	<0.05
<i>E. maxima</i>	0.13 ^a ±0.09	2.25 ^b ±0.17	0.06 ^a ±0.06	1.69 ^c ±0.12	<0.05
<i>E. tenella</i>	0.13 ^a ±0.09	1.50 ^b ±0.13	0.06 ^a ±0.06	1.56 ^b ±0.18	<0.05
Day 13 PI					
<i>E. acervulina</i>	1.06 ^a ±0.19	1.69 ^{cd} ±0.15	0.33 ^b ±0.13	1.44 ^{ac} ±0.16	<0.05
<i>E. maxima</i>	0.56 ^a ±0.16	1.63 ^{bc} ±0.15	0.00 ^a ±0.00	1.13 ^b ±0.18	<0.05
<i>E. tenella</i>	0.19 ^a ±0.10	1.31 ^b ±0.15	0.07 ^a ±0.07	0.94 ^c ±0.11	<0.05
Day 21 PI					
<i>E. acervulina</i>	1.38 ^{ab} ±0.27	1.75 ^b ±0.17	0.94 ^a ±0.19	1.25 ^a ±0.13	<0.05
<i>E. maxima</i>	1.50 ^{ac} ±0.33	2.13 ^{cd} ±0.15	0.50 ^b ±0.22	1.25 ^a ±0.18	<0.05
<i>E. tenella</i>	0.56 ^a ±0.16	1.38 ^b ±0.15	0.13 ^c ±0.09	1.17 ^b ±0.11	<0.05

^{a, b, c, d}: Mean values ± SE within the same row with different superscript letter are significantly different ($p < 0.05$). Data are presented for 16 birds per experimental group per time point

The uninfected birds showed intestinal lesions as would be expected based on oocyst shedding (Table 3). However, the severity of the lesions in the uninfected birds was consistently lower than in the infected chickens. Lesion scores due to *E. acervulina* and *E. maxima* on days 6 and 21 PI in the infected chickens were significantly lower in EMF-treated animals than in the controls. The lesions caused by *E. tenella* were less severe on days 13 and 21 PI for the infected EMF-treated chickens versus the infected controls. On days 13 and 21 PI, the uninfected chickens exposed to EMF had lower lesion scores than the uninfected controls, but the difference not always reached statistical significance.

DISCUSSION

It was assumed that the use of litter contaminated with oocysts would simulate a natural coccidiosis infection. Oocyst shedding in the infected birds was found to peak on day 13 PI followed by a decline on day 21 PI. This pattern of oocyst shedding agrees with findings in commercial broiler operations (Peek and Landman, 2003). The presence of oocysts in the excreta of the uninfected chickens indicates that cross contamination had occurred. Although we attempted to prevent cross contamination, we feel that it does not affect the purpose of this study, i.e., studying the effect of EMF exposure on the severity of coccidiosis infection. The lesions in the uninfected chickens were less severe than in the infected ones. It could even be suggested that because of the cross contamination, the effect of EMF at two levels of infection can be evaluated.

Infection with *Eimeria* resulted in a significant reduction of growth performance in the EMF chickens, which was no longer apparent at the end of the experiment. An initial depression of technical performance followed by compensatory weight gain has been reported in chickens immunized against coccidiosis by hatchery spray (Williams and Andrews, 2001). It was obvious that exposure to EMF counteracted the effect of infection on growth performance (Table 1). In the EMF-treated chickens, the infection did not reduce body weight, weight gain and feed intake and did not raise the feed conversion ratio. The positive effect of EMF on the infected birds in this experiment may extend to laying hens. Keirs *et al.* (2005) reported that exposure of egg-layer flocks to EMF improved production, which may have important welfare and economic implications.

Contrary to growth performance, the number of oocysts in excreta was not significantly influenced by

EMF exposure. This observation may not be surprising. Oocyst shedding only reflects the intensity of the infection at the time of sampling (Hodgson, 1970). Indeed, various workers have reported that oocyst production correlates only poorly with the performance of broiler chickens (Jeffers, 1975; Williams and Catchpole, 2000; Williams and Andrews, 2001). Moreover, Reid (1975) has criticized the use of oocyst shedding as the only index of the anticoccidial activity of drugs because medicated chickens may even produce more oocysts than do un-medicated chickens. If any, in this study, the anticoccidial activity of EMF was associated with a tendency towards diminished excretion of oocysts.

Examination of the intestine on day 6 PI showed that in the infected birds exposed to EMF the lesion scores related to *E. acervulina* and *E. maxima* infection were significantly lower than in the infected controls, but the lesions due to the *E. tenella* were not affected by EMF. On days 13 and 21 PI, the group mean lesion scores for all three *Eimeria* species were lower in infected chickens exposed to EMF than in the infected controls. In the uninfected birds, there was no effect of EMF on intestinal lesions on day 6 PI, but on days 13 and 21 PI, EMF treatment had significantly reduced the severity of the lesions. Factors that might contribute to the differences in response of *E. tenella* and *E. acervulina* and *E. maxima* to EMF include site of parasite invasion, host immune reaction at the infection site and parasite infective stage (Allen, 1987). Furthermore, *E. acervulina* and *E. maxima* are more immunogenic than *E. tenella* (Rose and Long, 1962). On day 13 PI, the lesion scores due to *E. acervulina*, *E. maxima* and *E. tenella* in both infected groups were on average lower than on day 6 PI. The opposite was seen in the uninfected chickens. The lesions on day 13 PI in the infected chickens are secondary lesions induced by re-infection as a consequence of the initial infection (Williams and Andrews, 2001; Williams, 2003). Oocysts that are excreted initially after a coccidiosis infection are less pathogenic than oocysts shed later because the latter have completed their life cycle in the host (Jeffers, 1975; Ryley *et al.*, 1976).

The molecular basis underlying the observed antagonistic activity of EMF on coccidiosis infection in broiler chickens is unknown, but it could relate to one or more of the various biological effects that have been described. EMF exposure may have an anti-inflammatory effect (Cronstein *et al.*, 1999; Montesinos *et al.*, 2000; Vallbona and Richard, 1999) and increased blood flow and improved wound healing have been documented (Gessi *et al.*, 2000; Goldin *et al.*, 1981). Possibly, EMF treatment of the

broiler chickens had resulted in increased peripheral blood flow and massive infiltration of macrophages into the damaged tissue, leading to the observed reduction of coccidial lesions. Jeurissen *et al.* (1996) reported that in immune chickens, fewer sporozoites will reach the crypt epithelium and the formation of schizonts is inhibited. Sporozoites that had failed to reach the crypt epithelium within 48 h after infection were detected within or were surrounded by macrophages, indicating that the presence of these cells can moderate the intensity of a primary infection.

CONCLUSION

In this study EMF exposure reduced the severity of coccidiosis in broiler chickens with a simulated, commercial *Eimeria* infection. Further study on the effect of EMF on coccidiosis infection is needed. The uninfected birds treated with EMF showed a lower feed intake and weight gain than did the uninfected control birds. Whether or not this observation was caused by a room effect is not known. The control birds and EMF-treated birds had to be housed in different rooms, albeit that they were adjacent. As mentioned above, the mechanism by which EMF exerts its anticoccidial effect is not known. From a practical point of view, it would be relevant to find out whether optimizing the conditions of EMF could increase its anticoccidial activity. Perhaps, EMF exposure could serve as an alternative to the anticoccidial drugs currently used.

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