

Antagonistic Effect of Electromagnetic Field Exposure on Coccidiosis Infection in Broiler Chickens

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ABSTRACT The hypothesis tested was that exposure of broiler chickens to an electromagnetic field (EMF) may reduce the signs of coccidiosis infection, based on recent insights into immunology. The experiment had a 2 × 2 factorial design. An uninfected and an infected group did not receive further treatment, whereas the other uninfected and infected groups were subjected to EMF treatment. In the cages of EMF-treated birds, a field strength of 5 μT root mean square was created for a period of 30 min/d. Infected birds were given a single dose of a mixture of *Eimeria* species (1.76 × 10⁴ sporulated oocysts of *Eimeria acervulina*, 1.25 × 10⁴ sporulated oocysts of *Eimeria*

maxima, and 7.5 × 10³ sporulated oocysts of *Eimeria tenella*) through gavage into the crop. Infection with the *Eimeria* mixture induced intestinal lesions, shedding of oocysts, and a reduction in growth performance. Exposure of broiler chickens to the EMF antagonized the effects of infection. In the EMF-treated birds, the infection caused no effect on weight gain and feed intake, whereas the severity of intestinal lesions mediated by *E. acervulina* and *E. maxima* was less than in the infected controls. We suggest that EMF has anticoccidial activities and its application could serve as an alternative to the anticoccidial drugs currently used in poultry production.

Key words: coccidium, electromagnetic field, broiler

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INTRODUCTION

Coccidiosis is a common infectious disease in poultry that is caused by the protozoan parasite of the genus *Eimeria*. The signs of coccidiosis are lesions in the intestine and associated impaired growth performance. In addition, coccidiosis increases the susceptibility to pathogens (McDougald, 2003). The invasion of *Eimeria* sporozoites into the intestinal epithelium initially results in inflammatory reactions, leading to an effective immune response (Jeurissen and Veldman, 2002). We hypothesized that inhibition of the inflammatory reactions and stimulation of the immune response would diminish the signs of coccidiosis infection in poultry. Exposure to an electromagnetic field (EMF) may have an antiinflammatory effect (Vallbona and Richard, 1999; Jasti et al., 2001), and EMF signals have been reported to stimulate the production of cytokines, mediating an enhanced immune response (Blank et al., 1992; Goodman et al., 1994; Mevissen et al., 1998; Simkó and Mattsson 2004).

In light of the above-mentioned literature, we hypothesized that exposure of broiler chickens to EMF may reduce the signs of coccidiosis infection in broiler chickens. In

the present experiment, our hypothesis was tested. Preliminary results were previously reported in abstract form (Cuppen et al., 2006a)

MATERIALS AND METHODS

Birds and Housing

One-day-old female broilers (Ross 308, n = 288) were purchased from a local hatchery. On the day of arrival (d 1), they were wing-banded and randomly housed in wire-floored, suspended cages. Each cage was 60 × 50 × 38 cm and was provided with thick foil and wood shavings as litter. Continuous lighting was provided throughout the experiment. The temperature in the cages on arrival was 32°C and then was gradually decreased to 20°C at the end of the experiment.

Diets

Starter and grower diets (Research Diet Services BV, Wijk bij Duurstede, the Netherlands) were used. The diets did not contain growth promoters or anticoccidial drugs. The starter diet was offered until d 13, followed by the grower diet. The ingredient composition of the diets was as follows (g/kg of diet, as fed; starter, grower): wheat (250, 500), soybean meal (49% CP; 345.5, 253.7), corn (275.00, 122.90), animal fat (40.00, 50.00), rapeseed meal (extracted; 20.00, 3.00), soybean oil (18.70, 1.39), corn glu-

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ten 60 (1.0522, 0.00), premix (0.50, 0.50), salt (0.2119, 1.902), sodium bicarbonate (2.367, 2.295), monocalcium phosphate (10.995, 4.276), limestone (14.674, 10.469), 99% DL-Met (2.695, 0.2146), 98.5% L-Lys HCl (1.723, 2.418), 98% L-Thr (0.638, 0.855), phytase (Natuphos5000G, BASF AG, Ludwigshafen, Germany; 0.10, 0.10). Throughout the experiment, the birds had free access to feed and tap water.

Experimental Design

The experiment had a 2×2 factorial design. On d 13, the broilers were weighed and divided into the 4 experimental groups so that the weight distributions of the groups were similar. An uninfected and an infected group did not receive further treatment. Other uninfected and infected groups were subjected to the EMF treatment, which was started on d 1. Birds were infected with a mixture of *Eimeria* species on d 15. The 2 groups not receiving the EMF treatment consisted of 10 replicates each (2×10), and the other 2 groups had 8 replicates each (2×8). Each replicate was a cage with 8 birds initially. The cages of the uninfected and infected groups were placed in evenly distributed locations.

Under each cage of the EMF-treated birds was a magnetic coil consisting of 50 loops of electrical wire with a cross-section of 1.5 mm^2 . The coils were connected to an experimental exposure system (Immuent B.V., Veldhoven, the Netherlands) containing a signal generator controlled by a Cygnal C8051F126 microprocessor (Silabs, Austin, TX) that regulated the period of time and the EMF per cage. The coil was placed directly under and along the borders of the cage. No attempt was made to achieve uniformity of the magnetic field in the cage, because previous experiments (see Cuppen et al., 2006b) had shown an equal response from 0.3 to $50 \mu\text{T}$. In the cages the field strength varied between 5 and $10 \mu\text{T}$, as shown by measurements at 7 different points. Coil resistance was 0.9 ohm and the current was less than 60 mA rms. Therefore, heat delivery was less than 50 mW for 30 min/cage so that no detectable temperature increase was caused by the exposure. In addition, no detectable sound was produced by the coils at these very low-exposure settings. The birds were observed multiple times during treatment and no behavioral changes were seen. The EMF treatment period lasted for 30 min, and the treatment was given to each cage consecutively, once every 24 h. The field strength within the cages was set to $5 \mu\text{T}$ rms, as verified by an FW Bell 5180 Tesla meter with a MOS51-3204 low-field probe (www.fwbell.com). To avoid any effect of EMF exposure on the other groups, the EMF-free and EMF-positive groups were housed in adjacent rooms within the facility. The distance between the EMF groups and the control groups was 3 m, and the thickness of the wall between the 2 rooms was 20 cm.

On d 15 of the experiment, the birds in 18 cages (144 birds) were individually challenged with the mixture of *Eimeria* containing 1.76×10^4 sporulated oocysts of *Eimeria acervulina* (Weybridge strain), 1.25×10^4 sporulated oocysts of *Eimeria maxima* (Weybridge strain), and 7.5×10^3

sporulated oocysts of *Eimeria tenella* (Houghton strain). The oocysts were laboratory strains and were obtained from the Animal Health Service Ltd., Poultry Health Center (Deventer, the Netherlands). The sporulated oocysts were administered with 1 mL of tap water via a scaled 1-mL syringe directly into the crop. Likewise, the negative groups were given 1 mL of water only. To avoid cross-contamination between uninfected and infected groups, the sides of all cages were partially equipped with plastic. Birds of the uninfected groups were always fed and weighed before the infected groups. On d 21 of the experiment, 2 randomly selected birds per cage (16 or 20/experimental group) were euthanized by cervical dislocation, dissected, and the coccidial lesions scored.

Performance Measurements

Birds were weighed individually on d 15 and 21. 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 of feces was determined for excreta collected on d 6 postinfection (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% (wt/vol) feces suspension in a salt solution (151 g of 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). The suspension was then put into the McMaster chamber with a micropipette and the number of oocysts was counted and expressed per gram of feces (Peek and Landman, 2003).

The severity of coccidial lesions was scored on d 6 PI, with the investigator blinded to the treatment modality. The 0- to 4-point 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 by using multinomial transformation. Lesion scores for the various experimental groups were compared with the nonparametric Mann-Whitney *U*-test. Performance and oocyst data were subjected to the least significant differences test. The statistical program SPSS (SPSS Inc., Chicago, IL) was used. The level of statistical significance was preset at $P < 0.05$.

RESULTS

Growth Performance

Postinfection mortality was 1 or 2 animals per treatment group. Final BW of the uninfected broilers exposed to

Table 1. Postinfection (PI) growth performance of broilers in the 4 experimental groups

Day in experiment	Control ¹		EMF ²		Pooled SEM
	Uninfected	Infected	Uninfected	Infected	
BW (g)					
21 (6 d PI)	888 ^a	812 ^b	828 ^b	804 ^b	14.63
Weight gain (g/d)					
15-21 (1-6 d PI)	58.7 ^a	44.5 ^c	53.3 ^b	51.5 ^b	10.88
Feed intake (g/d)					
15-21 (1-6 d PI)	106 ^a	93 ^b	95 ^b	93 ^b	2.01
Feed conversion ratio					
15-21 (1-6 d PI)	1.67 ^a	1.94 ^b	1.65 ^a	1.68 ^a	0.13
Water intake (mL/d)					
16-21 (2-6 d PI)	162 ^{ab}	159 ^b	151 ^{bc}	147 ^c	2.75

^{a-c}Mean values within the same row with different superscript letters are different ($P < 0.05$).

¹n = 80 birds per experimental group.

²n = 64 birds per experimental group for the electromagnetic field (EMF)-treated group.

EMF was significantly lower than that of uninfected controls, but BW of the 2 infected groups were similar (Table 1). The PI daily growth rate of the infected EMF group was significantly higher ($P < 0.01$) than that of the infected control group. The uninfected birds exposed to EMF showed a significantly lower feed intake and lower weight gain than the uninfected control birds. Postinfection feed intake of the infected EMF birds was similar to that of the infected controls. Feed conversion in control birds was significantly raised by the infection, but there was no change in the EMF-treated birds (Table 1). The infection with coccidiosis had no effect on water intake as measured during d 2 to 6 PI (Table 1).

Coccidiosis Infection

The oocysts per gram of feces values were not different for the infected control and EMF-treated birds (Table 2). In excreta of the uninfected birds, no oocysts were detected.

Lesions caused by *E. acervulina* and *E. maxima* were significantly lower in the infected group exposed to EMF than in the infected controls (Table 3). Lesion scores related to the infection with *E. tenella* showed no influence of EMF treatment. No coccidial lesions were seen in the 2 uninfected groups.

DISCUSSION

It was clear that the infection with one single dose of the *Eimeria* mixture was successful in the control birds. The infection induced intestinal lesions, shedding of oocysts, and a reduction in growth performance. These effects of the infection have been described previously

(Conway et al., 1993; Adams et al., 1996; McDougald, 2003). The novel observation was that exposure of broiler chickens to EMF antagonized the effects of infection with the 3 *Eimeria* species. In the EMF-treated birds, the infection produced no effects on feed intake, weight gain, and feed conversion ratio. Furthermore, the severity of intestinal lesions mediated by *E. acervulina* and *E. maxima* was significantly less than in the infected controls. The EMF-mediated reduction of the severity of lesions in the duodenum and mid gut most likely was associated with less loss of digestive capacity, which in turn was reflected by the observed unchanged growth performance. In the infected control birds, on the other hand, the more severe lesions attributable to *E. acervulina* and *E. maxima* were mirrored by a decrease in weight gain and an increase in the feed conversion ratio. The invasion of the epithelial wall by *E. acervulina* and *E. maxima* is well known to result in morphological changes of the brush border and decreased activities of the digestive enzymes (Fernando and McCraw, 1973; Allen, 1987). The EMF treatment would also appear to have beneficial effects in laying hens. Keirs et al. (2005) reported that EMF exposure in commercial egg-layer flocks improved production, which may have important welfare and economic implications.

The lower intestinal lesion scores for *E. acervulina* and *E. maxima* in the infected birds exposed to EMF were not associated with less oocyst shedding and with lower lesion scores for *E. tenella*. The lack of effect of EMF on fecal oocyst numbers could relate to the fact that all oocysts were counted in excreta collected at only one time point (i.e., at 6 d PI). The counts probably mainly reflected the oocysts of *E. maxima* and *E. tenella* and few oocysts of *E. acervulina* because of the respective prepatent periods

Table 2. Number of oocysts per gram of feces, expressed as $\log_{10}(x + 1)$, for the 4 experimental groups¹

Day in experiment	Control		EMF		Pooled SEM
	Uninfected	Infected	Uninfected	Infected	
21 (6 d postinfection)	0 ^a	5.698 ^b	0 ^a	5.964 ^b	0.20

^{a,b}Mean values with different superscript letters are different ($P < 0.05$).

¹n = 10 replicates for the control group and 8 replicates for the electromagnetic field (EMF)-treated group.

Table 3. Lesion scores and frequencies for the 3 *Eimeria* species in the infected birds on d 21 in the experiment (d 6 postinfection)

Treatment	n	Species														
		<i>Eimeria acervulina</i>				<i>Eimeria maxima</i>				<i>Eimeria tenella</i>						
Mean lesion score																
Infected control	20	1.95 ^a ± 0.15 ¹				2.30 ^a ± 0.15				2.65 ± 0.13						
Infected EMF ²	16	1.19 ^b ± 0.1				1.50 ^b ± 0.13				2.00 ± 0.2						
P-value		<0.05				<0.05				0.21						
		Score														
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Lesion score frequency																
Infected control	20	—	5	11	4	—	—	2	10	8	—	—	1	5	14	—
Infected EMF ²	16	—	13	3	—	—	—	8	8	—	—	—	5	6	5	—

^{a,b}Mean ± SE values within the same column with different superscript letters are different ($P < 0.05$).

¹n = number of birds dissected.

²Electromagnetic field (EMF)-treated group.

(McDougald and Reid, 1991). Alternatively, the lack of effect of EMF on oocyst counts could be explained by the phenomenon that suboptimal levels of anticoccidial treatments caused the medicated infected animals to produce more oocysts than their unmedicated counterparts (Brackett and Bliznick, 1949; Barwick and Casorso, 1970; Williams, 1973; Reid, 1975). The suboptimal level of anticoccidial treatment is thought to reduce the burden of the coccidial infection so that the proportion of surviving sporulated oocysts has extra room in the intestinal epithelium and becomes more reproductive. Factors that might contribute to the differences in the responses of *E. tenella*, *E. acervulina*, and *E. maxima* to EMF include the site of parasite invasion, host immune reaction at the infection site, and parasite metabolism (Allen et al., 1997). Furthermore, *E. acervulina* and *E. maxima* are more immunogenic than *E. tenella* (Rose and Long, 1962).

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. Electromagnetic field exposure may have an antiinflammatory effect (Cronstein et al., 1999; Vallbona and Richard, 1999; Montesinos et al., 2000), and beneficial effects on the nervous system have been recognized (Kaszuba et al., 2005). The efficacy of EMF in increasing blood flow and wound healing has been documented (Cameron, 1961; Goldin et al., 1981; Gessi et al., 2000). Possibly, EMF treatment of the broiler chickens 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.

Further work on the effect of EMF exposure on coccidiosis infection in broiler chickens is needed. The infected

birds treated with EMF showed a lower feed conversion and higher weight gain than did the infected control birds. Whether 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, even though they were adjacent. As mentioned, the mechanism by which EMF exerts its anticoccidial effect is not known. However, we concluded that the exposure of the broilers to a low EMF could be useful in controlling coccidiosis. The potential of EMF signals on broilers during coccidiosis needs to be verified in controlled field trials. Perhaps EMF exposure could serve as an alternative to the anticoccidial drugs currently in use.

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