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Comparing the Feeding of Fungus Myceliated Grain with Other Anticoccidial Control Measures on Oocyst Excretion of *Eimeria* Challenged Broilers

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Abstract: An experiment was conducted to determine if dietary Fungus Myceliated Grain (FMG) as an alternative to other coccidiosis control measures would result in oocyst reduction in the presence of a field strain *Eimeria* challenge during grow out. A total of 144 broiler chicks were assigned to 8 treatment groups as follows: 1) Control (no coccidiosis protection - no challenge); 2) 5%-FMG (no challenge); 3) Inovocox (IC) (no challenge); 4) Coccivac-B (CB) (no challenge); 5) Control (no coccidiosis protection, challenged); 6) 5% FMG (challenged); 7) Inovocox (IC) (challenged) and 8) Coccivac-B (CB) (challenged). Broilers were challenged with a mixture of *E. acervelina*, *E. maxma* and *E. tenella* at 28 days of age. Fecal oocyst egg count, mortality and whole blood differentials were measured. The *Eimeria* counts 1 week post challenge were significantly higher for all challenged chickens (trts 5-8) as compared to unchallenged (trts 1-4). Oocyst counts at day 49 of challenged but unprotected chickens (trt 5) was significantly ($p>0.05$) greater and showed a higher percentage of mortality when compared to challenged treated groups (trt 6-8). Treatment 6 (5% FMG) led to a reduction in fecal oocyst counts and protected against mortality at rates comparable to the vaccine control methods. Additionally, there were comparable increases in macrophages, heterophils and lymphocytes in the 5% FMG and vaccine groups. The results from this study strongly suggest that a diet supplemented with 5% FMG can be used as an alternative to other coccidiosis control methods in reducing *Eimeria* oocyst numbers during grow out.

Key words: Broilers, fungus myceliated grain, coccidiosis, alternative control

INTRODUCTION

Each year, in the United States, the broiler industry either spends millions of dollars protecting birds from coccidiosis infection, or absorbs losses associated with this parasitic disease. The economic impact of coccidiosis on the world poultry industry is estimated to exceed $3 billion annually (Bal, 2009). Traditionally, most commercial poultry flocks receive anticoccidial medication as a feed additive to control coccidiosis. Despite these control measures and using rotation and shuttle programs with the feed additives, coccidiosis continues to be a major challenge for efficient poultry production. Particularly troublesome is the continual emergence of drug resistant strains of coccidiosis in the poultry industry. Therefore, there is great interest in developing alternative methods of control. Recently, the introduction of a new coccidiosis vaccine (Inovocox) has provided a new tool to help control one of industry's most costly diseases. Other approaches are gaining attention, including mushroom extracts as a treatment for coccidiosis. Most reports in the literature for these products are based on *in vitro* evidence, or studies conducted with humans, rodents and other species. Bioactive compounds in mushrooms that function as medicines to animals and humans have been reported in experiments (Li, 1998). Guo *et al.* (2004) reported that supplementation with mushroom and herb extracts resulted in enhancement of both cellular and humoral immune responses in *E. tenella*-infected chickens. In another study, Ogbe *et al.* (2009) reported that the wild mushroom (*Ganoderma lucidum*) used to treat *E. tenella* infected broilers resulted in a reduction in the number of *E. tenella* oocysts shed in the faeces and led to improved weight gain. There are some reports demonstrating positive effects from feeding fungal myceliated grain to chickens (Willis *et al.*, 2009; 2010) and these researchers have observed its potential to control coccidiosis. Therefore, using mushrooms to protect broiler chickens from coccidiosis and other diseases has a promising future. The restriction and removal of subtherapeutic antibiotics from poultry rations in many parts of the world has raised interest in natural health alternatives. As such, this experiment was conducted to investigate the effects of dietary fungal myceliated grain supplementation on *Eimeria* oocyst egg count and mortality of broilers with and without *Eimeria* challenge.

MATERIALS AND METHODS

Experimental design and husbandry: A total of 144 day-old Ross x Ross straight-run broiler chicks were obtained from a local commercial hatchery. The chicks

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were randomly assigned to 8 treatments as follows: 1) Control-no challenge; 2) 5% FMG -no challenge; 3) Inovo-coccivac (IC)-no challenge; 4) Coccivac B (CB) no challenge; 5) Control-challenged; 6) 5% FMG-challenged; 7) Inova-coccivac (IC) - challenged and 8) Coccivac B (BC) - challenged. Each treatment was comprised of 3 replicated cages with 6 birds each. Treatments 1 through 4 were housed in battery and finisher cages in separate rooms from treatments 5-8 within the same building. The chicks were fed a starter, grower and finisher diet supplemented with and without 5% fungus myceliated grain throughout the 49 day experiment. No withdrawal of the 5% fungus myceliated grain from the feed occurred in this experiment. The basal mash feed was free of drugs or medication (Table 1). The chicks were initially started at 35°C in battery cages, then the temperature was reduced by 5°C each week, until reaching 25°C. Continuous lighting was provided throughout the 49 day experiment with a reduction in intensity starting at three weeks. Feed and water were provided *ad libitum*. All broilers were vaccinated at the commercial hatchery for Infectious Bronchitis, Newcastle and Marek's Disease. A representative group of chicks received Coccivac B and Inovo-coccivac at the hatchery for this experiment. At three weeks, the replications of chicks undergoing treatment were transferred to finisher cages within the same building and rooms.

Table 1: Composition of the basal diets

	Amount		
Ingredients	Starter	Grower	Finisher
Corn	1167	1324	1410
Soybean meal	716	563	478
Corn micro-flush	19.94	20.73	20.30
Limestone fine	19.42	20.40	21.37
Dicalcium phosphate (18.5%)	41.77	36.92	31.47
Lysine (78.5%)	0.01	1.26	4.27
Methionine (99%)	3.80	2.67	2.01
Threonine	1.06	0.02	1.58
Salt	10.00	10.00	10.00
PX NCSU Br Mineral (TM90)	4.00	4.00	4.00
Choline chloride (60)	4.00	4.00	4.00
PX NCSU Br Vitamin (NCSU90)	1.00	1.00	1.00
Selenium Premix NCSU (0.02%)	2.00	2.00	2.00
Poultry fat (Miter)	10.00	10.00	10.00
Total batch weight	2000	2000	2000

Fungus myceliated grain preparation: Sorghum grain was soaked in water overnight, drained and 5 kg each was loaded in unicom bags, sterilized at 121°C for three hours, inoculated with Shiitake (*Lentinula edodes*) and incubated at 25°C for two weeks before use. This process resulted in the conversion of the sorghum grain into a fungal biomass and the accumulation of extra-cellular compounds. The resulting myceliated grain was processed by air drying at about 25°C for approximately six hours and ground into a powder that was used for supplementing the basal ration in the experiment.

Parasite preparation and administration: On day 28, chicks in respective treatments were challenged with an *Eimeria* mixture consisting of *E. acervulina*, *E. maxima* and *E. tenella*, with a target dose of 50,000 oocysts/chick. The mixture was placed directly into the crop, using an oral gavage needle. On the day of placement, chicks in treatments 4 and 8 were vaccinated by spray application of the commercially available live oocyst coccidiosis vaccine Coccivac-B, using a spraycox II machine at the hatchery. Conversely, chicks in treatments 3 and 7 were vaccinated at the hatchery via in ovo administration of a Inovocox vaccine®. The vaccine was administrated in ovo to 18 or 19-day old incubated broiler chicks eggs via an in ovo injection system. On days 35 and 49, the oocysts from droppings were counted using a McMaster's chamber slide (Hodgson, 1970).

Blood differential counts: Blood samples were collected from the broilers jugular veins in vacuum tubes containing EDTA to prevent clotting. Blood smeared slides were prepared, allowed to air day and then fixed and stained with HEMA3. A total of one hundred cells were counted and the results were expressed as percentages of macrophages, lymphocytes, heterophils, eosinophils and basophils.

Statistical analysis: The means were compared by Duncan's statistical analysis. Data analysis was carried out using SPSS version 17.0 (Spss Inc. Chicago, IL). Analysis of Variance (ANOVA) was performed to detect any significant differences among the eight treatments. The Duncan multiple range test was subsequently used to detect the source of difference in the ANOVA output. The critical level for null hypothesis rejection in all the statistical tests was 5% ($a = 0.05$).

RESULTS AND DISCUSSION

The analysis of results at 35 days post-challenge showed that *Eimeria sp.* counts were significantly higher, exceeding 399,000 eggs/g in the challenged broilers (treatments 5-8) as compared to the unchallenged controls (treatments 1-4) ($p<0.05$) Table 2. On day 49, however, the chickens exhibited a different response to the *Eimeria*. The counts of *Eimeria sp.* eggs increased in the unchallenged/unprotected birds (2,167.50±1796.58 vs. 7,087.50±5036.71), but decreased in the challenged/unprotected broilers (>399,000 vs. 8,550.00±7402.8). To differing extents, the number of eggs in treatments 6, 7 and 8 were not significantly different from the unchallenged control ($p>0.05$). In comparison to controls trt 1 (no protection, no challenge) and trt 5 (no protection, challenge) there was a 65% and 94.6% reduction in the fecal *Eimeria* counts of broilers that received 5% FMG (trt 2 and trt 6) respectively.

Table 2: *Eimeria* counts and percent mortality of broilers at 35 and 49 days

Treatment #	Day 35 (n = 6)	Day 49 (n = 4)	% mortality @ day
Control-No protection, no challenge	2,167.50±1796.58ab	7,087.50±5036.71bc	0
FMG-5% (No challenge)	175.00±165.20a	2,537.50±810.96ab	0
Inovocox (No challenge)	1,150.00±674.66ab	712.50±438.92a	0
Coccivac-B (No challenge)	16.66±16.66667a	1,025.00±958.84ab	0
Control-No protection, (challenged)	>399,000d	8,550.00±7402.84c	22.22
FMG-5% (challenged)	>399,000d	462.50±151.89a	16.67
Inovocox (challenged)	>399,000d	2,762.50±891.24ab	16.67
Spray coccivac-B (challenged)	>399,000d	937.50±431.74a	5.56

Mean values down the column having the same alphabets are not significantly different at p<0.05 according to the Duncan multiple range tests

Table 3: Hematological analysis of the chicken blood (n = 3)

Macrophages (%)	Lymphocytes (%)	Eosinophils (%)	Heterophils (%)	Basophils (%)
27.10±3.45ab	37.53±6.39ab	4.56±2.566ab	30.8±5.8879ab	0.00a
26.03±7.01ab	31.76±6.753ab	6.16±1.266ab	35.00±0.556b	0.66±0.617a
25.20±3.97ab	32.80±8.931ab	1.33±0.881a	40.63±5.633b	0.00a
28.46±1.38ab	33.63±4.201ab	1.63±0.857a	36.93±4.230b	0.00a
26.25±2.97ab	26.75±4.885a	1.97±1.329a	44.950±0.696b	0.00a
36.50±3.50b	41.50±9.500ab	4.00±1.000ab	15.50±11.50a	0.00a
19.10±1.786a	47.90±2.478b	2.30±1.457ab	30.33±1.836ab	0.00a
23.20±6.85ab	29.93±2.367ab	7.30±1.700b	39.23±4.1738b	0.03±0.03a

Mean values down the column having the same alphabets are not significantly different at p<0.05 according to the Duncan multiple range tests

In terms of mortality response, 100% of the unchallenged controls survived throughout the experiment, in contrast to the mortality observed in treatments 5-8. In our assessment of the affect of FMG, we observed that the 5% FMG challenged broilers (trt 6) had a decreased rate of mortality that was similar to that observed with the challenged birds that received the Inovocox vaccine (trt 6 vs trt 7) when compared to treatment 5 Table 2 . The live oocyst coccidiosis vaccine, coccivac B, had the lowest mortality, which is in agreement with the findings of Williams *et al.* (1999), who reported that vaccination led to significantly lower mortality rates when compared with medication. However, there remain concerns about the negative effects on the cumulative performance of broilers when utilizing live oocyst vaccines. Taken together these data suggest that feeding a diet supplemented with 5% FMG grain reduces the parasite burden and protects against mortality at a rate that is similar to the other methods of controlling coccidiosis.

In recent studies, mushroom lections have demonstrated immunoenhancing effects (Brochers *et al*., 2004) and have proven to be an immunostimulating agent in poultry during coccidiosis (Dalloul *et al*., 2006). Therefore, the analysis of white blood cells to assess the effect of FMG on the immune response against coccidiosis was performed and shows that when compared to the control no challenge (trt 1), treatments 2, 3 and 4 each had an increase in the levels of heterophils (Table 3). Both heterophils and macrophages exhibit microbicidal, phagocytic and chemotatic activities (He *et al*., 2003; Kogut *et al*., 2005) and represent the first line of defense against an inflammatory or etiologic agents (Kogut and Klasing, 2009). The data, therefore, suggests that each of these methods of control lead to improved innate immune responses against coccidiosis during natural infections. Moreover, it is known that T-lymphocytes are crucial for the immune response against intracellular parasitic gastrointestinal infections such as *Eimeria*. Interestingly, in the challenged birds both the 5% FMG and the Inovocox led to increases in the percentages of lymphocytes in the blood (41.50±9.500 and 47.90±2.478 respectively) suggesting a potential role in the enhancement of the adaptive arm of the immune response.

Conclusion: Birds challenged with *Eimeria sp*. that were given diets supplemented with 5% fungus myceliated grain exhibited a reduction in oocyst excretion, mortality and enhanced adaptive immunity. Hence, the utilization of fungus myceliated grain shows potential as an alternative to other methods in coccidiosis intervention in ameliorating clinical *Eimeria* infection in broiler chickens. The lack to date of published research, focusing on the effects of myceliated grain for the control of coccidiosis, makes this research of interest and serves to further the understanding of how mushrooms may be used as health promoting alternatives.

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