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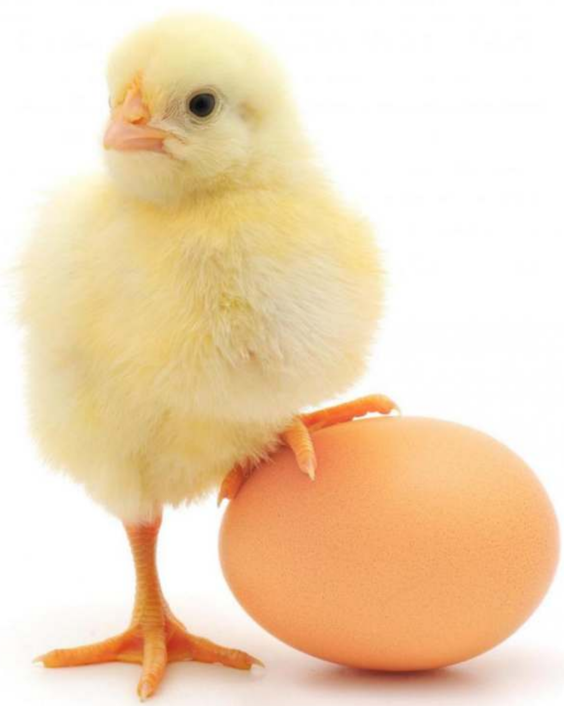

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Effect of Different Mushrooms Fed to *Eimeria*-Challenged Broilers on Rearing Performance

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Abstract: An experiment was conducted to evaluate the feeding of four mushrooms separate and combined via Fungus Myceliated Grain (FMG) on broiler chicken performance after an *Eimeria* challenge at 14 days of age. Male broilers (n-180) were randomly allocated to 1 of 12 treatments, replicated 3 times with 5 chicks each in a battery cage system for 28 days as follows: 1) Control-1 (no FMG, unchallenged), 2) Control-2 (no FMG, challenged), 3) Shiitake (S) 5% FMG, (unchallenged), 4) Shiitake (S) 5% FMG, (challenged), 5) Reishi (R) 5% FMG, (unchallenged), 6) Reishi (R) 5% FMG, (challenged), 7) Cordyceps (C) 5% FMG, (unchallenged), 8) Cordyceps (C) 5% FMG, (challenged), 9) Oyster (O) 5% FMG, (unchallenged), 10) Oyster (O) 5% FMG, (challenged), 11) S+R+C+O 5% FMG, (unchallenged) and 12) S+R+C+O 5% FMG, (challenged). Live weight and feed intake were measured at day 7, 14, 21, 28 and oocyst excretion, mortality and bursa weights at day 28. Coccidiosis infection induced depressed body weights in challenged birds at 21 and 28 days. *Eimeria* challenged birds in treatment four given Shiitake 5% FMG had body weights that were significantly higher than the other challenged birds at 21 days. The 28-day weight did not differ from each other in the challenged and unchallenged except for treatment seven (Cordyceps-unchallenged) which was significantly lower. While *Eimeria* counts at day 28 showed significant ($p \leq 0.05$) differences amongst challenged groups, significant differences were not found in the unchallenged treatment groups. There were significant ($p \leq 0.05$) differences in *Eimeria* counts and bursa weights between challenged and unchallenged birds at day 28. The results from this study demonstrate that Shiitake was superior and Cordyceps, at the 5% inclusion level, depressed body weight in birds whether they were challenged or unchallenged but data suggest that Cordyceps may reduce oocyst shedding.

Key words: Broiler chickens, performance, coccidiosis, mushrooms

INTRODUCTION

Avian coccidiosis is a major parasitic disease of poultry that cost the industry millions of dollars annually due to poor feed utilization, reduced growth performance and mortality (Lillehoj *et al*., 2004). The cost associated with prevention via drugs in the feed adds to the economic hardship of producers. Moreover, the increased drug-resistant problems occurring in poultry production has heightened public concern. In some countries, the use of drug/antibiotics in production has been severely regulated or banned altogether. Therefore, alternative control methods are being researched with great intensity. One such alternative that is receiving much interest is the mushroom family and the lectin associated with them. For instance, Dalloul *et al*. (2006) found that the growth performance of *Eimeria acervulina*-infected chickens was significantly improved by injecting *Fomitella fraxirea* lectin into 18-d-old embryos. In addition, oocyst shedding after oral infection with live parasites was significantly reduced. Interest in lectins has increased since they have been shown to induce some very important biological activities, such as immunomodulatory activities (Wang *et al*., 1996; 2002; She *et al*., 1998; Lima *et al*., 1999). Research conducted by Guillot and Konska (1997) revealed that only a few mushrooms have been shown to possess immunomodulatory properties. Recently, Giannenas *et al*. (2010) reported that *Agaricus bisporus*, the common white mushroom, exerted both growth-promoting and tissue antioxidant-protective activity when supplemented in broiler chicken diets. In 2004, Borchers *et al*., demonstrated the immune enhancing effects of mushrooms in promoting health. In other related research, Guo *et al*. (2004 and 2005) suggested that some mushroom extracts had immune-enhancing properties to abate coccidiosis in poultry. Although many mushrooms exhibit immune-stimulating potential, not all have been found to exert this ability when chickens are challenged with coccidiosis. More recently, the polysaccharide extract from the mushroom *Pleurotus*

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ostreatus was shown to have immunomodulating effects in chickens (Selegean *et al.*, 2009). Their findings led them to believe the extract and bural disease vaccines resulted in stimulating the production of infectious bursal disease antibodies in the period of the chicken first days of life. In other related research published by Ogbe *et al.* (2009), some effectiveness against *Eimeria tenella* challenged broilers was demonstrated with a wild mushroom *Ganoderma lucidum*.

Infection with *Eimeria* is known to stimulate a protective immune response in chickens (Rose, 1996; Yun *et al.*, 2000). Very few mushroom forming fungi types, especially fed as fungus myceliated grain have been studied for their ability to enhance health in chickens. Furthermore, far less is known of dose related feeding on immune enhancement by individual mushrooms or a combined mix of different species. Therefore, this experiment was undertaken to investigate the performance and health promoting effects of various mushrooms in broiler chickens.

MATERIALS AND METHODS

One hundred eighty (n = 180) day-old male broiler chickens (Ross x Ross) were obtained from a commercial hatchery. The chicks were weighed and randomly distributed into 12 different treatment groups that were fed different medicinal mushrooms separate or in a combined four-way mixture and were either unchallenged or challenged with an *Eimeria* mixture as follows: 1) Control-1 (no FMG, unchallenged); 2) Control-2 (no FMG, challenged); 3) Shiitake (S) 5% FMG, (unchallenged); 4) Shiitake (S) 5% FMG, (challenged); 5) Reishi (R) 5% FMG, (unchallenged); 6) Reishi (R) 5% FMG, (challenged); 7) Cordyceps (C) 5% FMG, (unchallenged); 8) Cordyceps (C) 5% FMG, (challenged); 9) Oyster (O) 5% FMG, (unchallenged); 10) Oyster (O) 5% FMG, (challenged); 11) S+R+C+O 5% FMG, (unchallenged) and 12) S+R+C+O 5% FMG, (challenged). The mushrooms utilized in this study were Shiitake (*Lentinus edodes*), Reishi (*Ganoderma lucidum*), Oyster (*Pleurotus ostreatus*) and Cordyceps *(Cordyceps sinensis)*. All mushrooms were used at a 5% inclusion level and incorporated into a balanced basal meal ration. The unchallenged chicks were housed in a separate room within the same building as those that had been challenged and were raised under conditions to prevent any possible accidental infection from occurring. All chicks were vaccinated against Marek's Disease, Infectious Bronchits and Newcastle Disease. The chicks were fed a balanced basal meal starter and grower ration supplemented with and without 5% (FMG) throughout the 28 day period. Feed and water were free of any medications and provided ad libitum. Lighting of the environment was provided for 24 hrs. Challenged birds in treatment groups 2, 4, 6, 8, 10 and 12 were gavaged with 49,000/1 ml of an *Eimeria* oocyst mixture consisting of *E. acervulina*, *E. maxima* and *E. tenella* on day 14. Body weights were recorded weekly and mortality daily. Fecal samples were obtained on day 28 to conduct *Eimeria* oocyst count using the McMaster Counting Chamber System (Hodgson, 1970). Bursas were removed from sacrificed birds on day 28 for weight determination.

Mushroom cultivation and basal diet: Shiitake, Cordyceps, Reishi and Oyster were cultivated by the Mushroom Biology and Fungal Biotechnology Laboratory at North Carolina A&T State University. Sterile sorghum grain was separately inoculated with selected fungus and incubated at 25°C for 2 weeks before use (Willis *et al.*, 2010). The resulting myceliated grain was processed by air-drying at about 25°C for approximately 6 hrs and ground into a powder that was used for supplementing the basal ration in the experimental trials. The basal diet composition is shown in Table 1. The four different mushrooms were put into the basal diet at a 5% level, mixed thoroughly and placed into trough feeders in respective treatment cages.

Table 1: Composition of basal diets

	Amount		
Ingredients	Starter	Grower	Finisher
Corn	1167.00	1324.00	1410.00
Soybean meal	716.00	563.00	478.00
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 NSCU 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	2000.00	2000.00	2000.00

***Eimeria* fecal analysis:** Fecal composite samples were collected on day 28 from cage pans of each treatment. The samples were transported to the laboratory where 2 grams of feces per tube was measured out onto a small scale and the placed into a sterile container in which 30 ml sodium nitrate was conjugated. The mixture of solution and fecal matter was thoroughly mixed, strained through cheese cloth and transferred to the chambers of a McMaster's slide. A total of 5 min elapsed in order for the eggs to reach the surface of chambers of the slide. A microscope using 10x lens and 10x eye piece was used to read the McMaster's slide. The total number of eggs in the two chambers was multiplied by 50, giving the eggs per gram for each sample (Hodgson, 1970).

Table 2: Body weights of *Eimeria* challenged broilers subjected to mushroom feeding at 21 and 28 days

	Body weights (kg)	
Treatments	Day 21	Day 28
1. Control, unchallenged (No FMG)	0.69±0.05a	0.98±0.06a
2. Control, challenged (No FMG)	0.41±0.03c	0.68±0.04b
3. Shiitake 5%, unchallenged FMG	0.68±0.02a	0.98±0.05a
4. Shiitake 5%, challenged FMG	0.56±1.13b	0.71±0.02b
5. Reishi 5%, unchallenged FMG	0.62±0.07ab	0.92±0.09a
6. Reishi 5%, challenged FMG	0.41±0.03c	0.66±0.05b
7. Cordyceps 5%, unchallenged FMG	0.55±0.03b	0.78±0.07b
8. Cordyceps 5%, challenged FMG	0.36±0.02c	0.62±0.05b
9. Oyster 5%, unchallenged FMG	0.69±0.00a	0.96±0.01a
10. Oyster 5%, challenged FMG	0.43±0.02c	0.67±0.02b
11. S+R+C+O 5%, unchallenged FMG	0.70±0.01a	0.96±0.02a
12. S+R+C+O 5%, challenged FMG	0.38±0.01c	0.73±0.03b

Note. S = Shiitake, R = Reishi, C = Cordyceps, O = Oyster. Mean values down the column having the same alphabets are not significantly different at p≤0.05 according to the Duncan's Multiple Range tests

Statistical analysis: Differences between experimental treatments were tested by ANOVA using the GLM Procedures (SAS Institute, 2001) and were considered significant at p<0.05 by the Duncan Multiple Range test.

RESULTS AND DISCUSSION

Body weights: The results of body weights at 21 and 28 days are presented in Table 2. Weights were not significantly (p≤0.05) different for days 1, 7 and 14 (data not shown). However, there were significant differences (p≤0.05) for body weights on day 21 and 28. Weights for the 21 day-old unchallenged broilers in treatments 1, 3, 9 and 11 differed significantly from treatment 7 (unchallenged + Cordyceps) and all challenged treatments 2, 4, 6, 8, 10 and 12. However, treatment 4 (Shiitake) did not differ at the same level as treatments 2, 6, 8, 10 and 12, which indicated that it was far superior among mushroom types and compatible with some of the unchallenged mushroom treatments for body weight maintenance. This finding is supported by a study conducted by Willis *et al.* (2011) that showed an increase in the body weights of broilers given Shiitake (FMG) at a 5% inclusion level in the starter feed. Significant differences were observed on day 28 for body weights amongst treatment groups. Broilers belonging to unchallenged treatments 1, 3, 5, 9 and 11 did not differ significantly from each other, but did differ from treatment 7 (unchallenged + Cordyceps) and all other challenged broiler treatments. The inability of the Cordyceps to sustain body weight continues to follow the pattern observed at the 21 day body weight assessment. It is not known why this occurs and more research is needed to elucidate the mechanism involved in this process. There were some numerical trend improvements for treatments 4 (Shiitake 5%) and 12 (S+R+C+O) which had the higher body weights of challenged broilers on day 28, but these weights were not statistically significant. The four-way mixture appeared to reduce the negative body weight impact of the Cordyceps utilized alone in the basal ration.

Table 3: *Eimeria* oocyst count of broilers on day 28

Treatments	Eimeria Counts
1. Control, unchallenged (no FMG)	50.00±50.00c
2. Control, challenged (no FMG)	10333.33±3633.79a
3. Shiitake 5%, unchallenged FMG	66.67±44.10c
4. Shiitake 5%, challenged FMG	3500.00±1976.32bc
5. Reishi 5%, unchallenged FMG	600.00±600.00c
6. Reishi 5%, challenged FMG	6583.33±2735.16ab
7. Cordyceps 5%, unchallenged FMG	16.67±16.67c
8. Cordyceps 5%, challenged FMG	4188.33±2702.52bc
9. Oyster 5%, unchallenged FMG	33.33±33.33c
10. Oyster 5%, challenged FMG	13116.66±10469.65a
11. S+R+C+O 5%, unchallenged FMG	16.67±16.67c
12. S+R+C+O 5%, challenged FMG	6816.67±664.79ab

Note. S = Shiitake, R = Reishi, C = Cordyceps, O = Oyster. Mean values down the column having the same alphabets are not significantly different at p≤0.05 according to the Duncan's Multiple Range tests

***Eimeria* oocyst counts:** *Eimeria* counts conducted on day 28 showed significant (p≤0.05) differences amongst challenged but not unchallenged treatments in Table 3. Large numbers of oocysts were produced in the feces of challenged birds that had received the primary infection challenge. By contrast, few oocysts were produced in unchallenged birds that had not been infected. This indicates that some exposure may have occurred even with birds being raised on wire flooring; however, it did not result in a clinical manifestation. Birds in treatment 2 (Control challenged no FMG) and 10 (Oyster 5% challenged) showed the highest significant oocyst counts. This data clearly shows that birds challenged with no protection (FMG) were adversely affected by this protozoan. Broilers in treatment groups 4 (Shiitake 5%) and 8 (Cordyceps 5%) showed the lowest count that differed from treatment 4 and 8, but not each other. The severity of experimental *Eimeria* infection in chickens has been shown by loss of body weight gain, excretion of fecal oocysts and the presence of bloody diarrhea (Ogbe *et al.*, 2009). The result obtained in this study demonstrates the effectiveness of Shiitake and Cordyceps in reducing the shedding of oocyst.

Table 4: Live and bursa weights of broilers on day 28

Treatments	Live Wt. (kg)	Bursa Wt. (Absolute)	Bursa Wt. (Relative)
1. Control, unchallenged (no FMG)	1.313±0.120a	1.633±0.240ab	0.262±0.198a
2. Control, challenged (no FMG)	1.016±0.101bc	1.433±0.145abcd	0.158±0.074abc
3. Shiitake 5%, unchallenged FMG	1.333±0.037a	1.6±0.2ab	0.195±0.148ab
4. Shiitake 5%, challenged FMG	1.003±0.067bc	1.633±0.133ab	0.185±0.140ab
5. Reishi 5%, unchallenged FMG	1.226±0.068ab	1.5±0.404abc	0.157±0.332abc
6. Reishi 5%, challenged FMG	0.94±0.090c	1.066±0.166bcd	0.107±0.156cd
7. Cordyceps 5%, unchallenged FMG	1.173±0.049abc	1.366±0.066abcd	0.116±0.093cd
8. Cordyceps 5%, challenged FMG	0.88±0.083c	0.8±0.115d	0.085±0.160d
9. Oyster 5%, unchallenged FMG	1.343±0.059a	1.566±0.218ab	0.181±0.133ab
10. Oyster 5%, challenged FMG	0.996±0.008bc	1.4±0.1abcd	0.156±0.081abc
11. S+R+C+O 5%, unchallenged FMG	1.283±0.021a	1.933±0.266a	0.267±0.177a
12. S+R+C+O 5%, challenged FMG	0.98±0.119bc	0.9±0.115cd	0.175±0.062ab

Note. S = Shiitake, R = Reishi, C = Cordyceps, O = Oyster. Mean values down the column having the same alphabets are not significantly different at $p \leq 0.05$ according to the Duncan's Multiple Range tests

Table 5: Mortality of broilers on day 28

Treatments	#No. of Birds Starting	#No. of Birds Ending	Mortality percent (no.)
1. Control, unchallenged (no FMG)	15	14	7% (1)
2. Control, challenged (no FMG)	15	15	0%
3. Shiitake 5%, unchallenged FMG	15	15	0%
4. Shiitake 5%, challenged FMG	15	12	20% (3)
5. Reishi 5%, unchallenged FMG	15	15	0%
6. Reishi 5%, challenged FMG	15	15	0%
7. Cordyceps 5%, unchallenged FMG	15	15	0%
8. Cordyceps 5%, challenged FMG	15	14	7% (1)
9. Pleurotus 5%, unchallenged FMG	15	15	0%
10. Pleurotus 5%, challenged FMG	15	14	7% (1)
11. S+R+C+O 5%, unchallenged FMG	15	14	7% (1)
12. S+R+C+O 5%, challenged FMG	15	13	13.4% (2)

Note. S = Shiitake, R = Reishi, C = Cordyceps, O = Oyster

Furthermore, it showed that Cordyceps naturally causes a reduction in body weight without challenge, but offered good protection against coccidiosis. In unpublished studies at this station, Cordyceps was found to exhibited higher immunoglobins (IgG, IgA) levels than other mushrooms. From this previous work, it was decided that a mixture using this specific species of fungi would be better, because it exhibit good immunity enhancement. In another study with mushroom feeding, Guo *et al.* (2004) showed the beneficial effects of different mushrooms that enhanced immunity in *Eimeria* challenged chickens. Also, these findings are in agreement with previous findings of Willis *et al.* (2010) that showed diets supplemented with a 5% Shiitake mushroom (FMG) exhibited a reduction in oocyst excretion and mortality in *Eimeria* challenged broiler chicks. The challenged dosage of *Eimeria spp.* given in this experiment is likely substantially higher than those levels in which commercial flocks are exposed. Therefore, it is believed that utilizing the combined mixture of mushrooms would be beneficial to the overall health of the chicken.

Bursa weights: The data for bursa weights are found in Table 4. Bursa weights of broiler chicks varied amongst treatments with significant ($p \leq 0.05$) differences. Broilers in the control, unchallenged treatment 1 and the combination mixture treatment 11 had the highest significant and numerical relative bursa weights, whereas, treatment 7 unchallenged had the lowest significant bursa weight. For the challenged broilers, the bursa weights were adversely affected by treatment 7 (Cordyceps) more so than any other treatment and were directly correlated with low body weight. On the positive side for the mushroom challenged broilers, the Shiitake (treatment 4) and the combination mixture (treatment 12) were compatible with the non-challenged control in treatment 1 and 11 for higher bursa weights. The results from this data clearly suggest that certain mushrooms exert positive health attributes as reflected by bursa weights.

Mortality rates: Mortality rates for treatments are in Table 5. The highest mortality rate of 20% (3 birds) were observed in treatment 4 (Shiitake) which was challenged. Although it seemed large, it is quite low based on the overall number. Since there was positive data from other parameters measured for this Shiitake treatment, it is not clear whether the mortalities are directly attributed to the Shiitake mushroom, seeing that Willis *et al.* (2010) observed no mortality problems with Shiitake in a similar study.

Conclusion: The data from this experiment clearly demonstrates that certain mushrooms have greater abilities to provide performance enhancement protection to chickens with *Eimeria sp.* challenges. It is conclusive that the Shiitake mushroom outperformed the other mushrooms as a stand alone or in combination with regards to performance and *Eimeria* protection. Perhaps Cordyceps alone at the 5% inclusion level should not be fed in rations for broiler chickens due to the significant repression of body weight but instead in combination with others, or at a reduced level, due to its superior protection against *Eimeria spp.* These results suggest that dietary FMG supplementation could improve growth performance in coccidia-infected broilers possibly through enhanced immune function. This research adds to the small body of knowledge for utilizing different mushrooms via FMG as alternatives to drug/antibiotics and hormone replacement in poultry rearing.

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