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Full Length Research Paper

Evaluation of dried powder of mushroom (*Agaricus bisporus*) as an antibiotic growth promoter substitution on performance, carcass traits and humoral immune responses in broiler chickens

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This experiment was conducted to evaluate the effects of different levels of edible mushroom (*Agaricus bisporus*) in comparison with an antibiotic growth promoter (flavophospholipol) on performance, carcass characteristics and immune responses of broiler chicks. In these trial 180 nine day old mix sexes broiler chicks (Ross 308) were weighed and randomly allocated to 1 of 6 experimental treatments. Each treatment consisted of 3 replicates of 10 birds. The 6 treatments were as follow: Basal diet (control); Basal diet + antibiotic (4.5 mg flavophospholipol/kg diet); and basal diet supplemented with either levels of 5, 10, 20 or 30 g of dried mushroom/kg of feed. Body weights (BW) of broilers were determined at day 9, 21, and 45, feed intake was determined at the same periods, and feed conversion ratio (FCR) was calculated accordingly. At day 45, two birds per replicate were slaughtered for determination of carcass and organ weights. Antibody titers against Newcastle (NDV), and sheep red blood cells (SRBC) were determined. The results showed that the use of 30 g mushroom/kg diet led to the highest antibody titers against SRBC ($p < 0.05$). The treatments had no effect on antibody titers against NDV. In grower and total period of the trial the highest amount of DFI was seen in the groups receiving 10 g mushroom/kg diet ($p < 0.05$). The BW obtained in birds fed the basal diet none significantly was greater than other groups at 45 day of age ($P > 0.05$). Broilers receiving flavophospholipol had lower FCR compared to broilers receiving 10 or 20 g mushroom/kg during total period of trial ($P < 0.05$) but it was not different from broilers fed the basal diet or basal diet supplemented with 5 or 30 g mushroom/kg. FCR of broilers in other periods was not affected. Internal organ weights and carcass traits were not influenced by the dietary treatments at day 45. In conclusion, the results indicated that supplementing broiler diet with 30 g mushroom/kg could induce favorable influences on immune responses of broilers without any adverse effects on performance criteria.

Key words: *Agaricus bisporus*, broilers, carcass characteristics, immune responses, mushroom, performance.

INTRODUCTION

For the past several decades, Sub-therapeutic dosages of antibiotics have been used extensively as growth promoters in poultry feeds. Antibiotic growth promoters (AGP) were supposed to increase growth rate as a result of improved gut health, resulting in better nutrients utilization and improved feed conversion (Visek, 1978). However, in the last few years, one of the main concerns of the poultry industry is the ban on antibiotic growth promoters by the European Union. These products are now considered as human health risk factors for their possible role in the emergence of microbial resistance

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(Nollet, 2005; Cervantes, 2006; Michard, 2008; Toghyani et al., 2010a). Now, restrictions to the use of antibiotic growth promoters have stimulated the search for alternative additives (Nasir and Grashorn, 2006). Phytogenic feed additives, also known as called as phytobiotic products are plant derived products, used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Toghyani et al., 2011b).

Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Breene, 1990). The fungi have a wide range of activities and have been used for centuries to combat disease outbreaks in many parts of the world such as Asian and Mediterranean countries (Chang and Buswell, 1996; Guo, 2003). Mushrooms and its different derivatives contain a variety of active substances like ergothioneine (Dubost et al., 2007), phenolic antioxidants, variegatic acid and dibiviquinone (Kasuga et al., 1995). Effective compounds, present in the mushrooms possess antioxidant, antibacterial, immune-enhancing, and stress reduction activities (Dalloul and Lillehoj, 2006; Dalloul et al., 2006). Recently, it has been reported that the combined use of Chinese herbal and mushroom extracts can operate as alternatives to antibiotic growth promoters in broiler chicken (Guo et al., 2004a,b). Also, Giannenas et al. (2010) observed the beneficial influence of Mushroom (*Agaricus bisporus*) on broilers performance and tissue antioxidant-protective activity. However, there have been few reports on the effect of the *A. bisporus* in chickens.

Therefore, the aim of this study was to investigate the effects of supplementation of different levels of dried powder mushroom *(A. bisporus)* as an antibiotic growth promoter substitution on performance, carcass traits and humoral immune responses in broiler chickens.

MATERIALS AND M ETHODS

Birds and experimental design

180 nine day old mix sexes broiler chicks (Ross 308) were randomly allocated to 1 of 6 experimental treatments. Each treatment consisted of 3 replicates of 10 birds. Each replicate was housed in separate stainless floor pens under controlled temperature and light conditions. Each pen was 100 × 100 cm (1 m^2 per 10 birds). The lighting cycle was 23 h/day maintained at all breeding times. The ambient temperature in experimental house was maintained at 29°C during the first week and after than gradually decreased by 3°C in the third week, and finally fixed at 22°C thereafter. The experiment lasted for 36 day. To meet the nutrient requirements of the broiler chicken over this period, a complete basal diet was formulated for each of the 2 stages of growth; starter and grower. The diets were formulated to meet the nutrients requirements of broilers as recommended by the National Research Council (NRC, 1994). Table 1 presents the ingredients and the composition of the basal diets fed in mash form. The birds within the control group were given the basal diet for the respective growth stage. The other 5 groups were given experimental diets based on the basal diets but contained an additional 5, 10, 20, or 30 g/kg of ground dried *A. bisporus* mushroom at the expense of corn in comparison broilers fed basal diet supplemented with 4.5 g/kg flavophospholipol. Birds were allowed to free access to have feed and water during the 36 day of growth period.

Mushroom preparation and supplementation

The Mushrooms (*A. bisporus*) were purchased from Golden Mushroom Institute in Iran (Golden Mushroom, Mazandaran, Iran). The whole mushrooms were dried out at 60°C and were added to experimental diets of broilers after carefully grinding. For chemical analyses, mushrooms were freeze dried at −76°C and 0.023 mbar in a vacuum for 30 h by Telstar cryodos. Dried mushrooms were milled through a 1 mm sieve before analysis for protein, fat, fiber, and ash according to the procedures described by AOAC International (1995). Total protein content was measured by Kjeldahl, crude fat content was extracted from the samples with petroleum ether in a Soxhlet apparatus, crude fiber content was analyzed in a Dosi fiber apparatus, and ash was determined by incinerating dried samples at 600°C for about 6 h in a furnace and moisture by oven drying.

Quantification of flavonoids

The total phenolic contents were determined using Folin-ciocalteu reagent (Merck, Darmstadt, Germany) according to the method reported by Kujala et al. (2000). Table 1 presents total phenolic content of the mushroom preparation.

Analytical procedures

Performance of broilers was evaluated by recording body weight (BW), daily body weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) during the 36 day of experimental period. Body weights (BW) of broilers were determined at 9, 21 and 45 days of age. Feed consumption and weight gain were recorded in different periods and feed conversion ratio (feed intake/weight gain) was calculated. Mortality was recorded as it occurred. Chicks were vaccinated against infectious bursal disease at day 14 and 21, Newcastle disease (B_1) at day 9 and Newcastle disease (Lasota) at day 21. At 28 day of age, two male broilers from each replicate of treatments were randomly selected. Blood samples were taken by puncture of the brachial vein for analysis of antibody titers against Newcastle disease virus (NDV). Serum antibody titers against NDV were measured by the hemagglutination inhibition test (HI), and HI antibodies were then converted into $\log_2$ (Cunningham, 1971). At 25 day of the trial, 2 birds per each group were injected in the right wing vein with 1 ml 1% Sheep red blood cell (SRBC). At 6 day post-SRBC, blood samples were taken and plasma was collected. Antibody titers against SRBC were measured by the microtiter procedure described by Wegmann and Smithies (1966).

At 45 day of age, 2 birds per replicate were randomly chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter. Eviscerated weight, breast weight and thigh weight were weighed and calculated as a percentage of live weight. Liver, abdominal fat pad were also removed, weighed and calculated as a percentage of live weight was calculated.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the general linear model procedures of SAS (SAS Inst. Inc., Cary,

Table 1. Composition of dietary treatments and mushroom (g/kg).

Item	Starter diet (9 to 21 days)					Grower diet (22 to 45 days)				
	Basal diet	5 g mushroom/kg	10 g mushroom/kg	20 g mushroom/kg	30 g mushroom/kg	Basal diet	5 g mushroom/kg	10 g mushroom/kg	20 g mushroom/kg	30 g mushroom/kg
Ingredients										
Corn grain	584.2	578.9	574.1	564.1	553.7	584.8	579.9	574.8	564.8	554.6
Dried mushroom	0	5	10	20	30	0	5	10	20	30
Soybean meal	315.8	314.2	312.5	309.2	306.0	300.7	299.0	297.4	294.1	290.8
Soybean oil	25.2	27.1	28.9	32.7	36.6	55.0	56.9	58.8	62.6	66.4
Fish meal	45	45	45	45	45	30	30	30	30	30
Mineral-Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin-Premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	1.5	1.5	1.4	1.4	1.3	0.9	0.8	0.8	0.7	0.6
L-Lysine	0.4	0.5	0.5	0.5	0.5	0	0	0	0.1	0.1
Dicalcium phosphate	9.3	9.3	9.2	9.0	8.9	8.0	7.9	7.8	7.7	7.6
$Caco_3$	10.6	10.5	10.4	10.1	10.0	11.7	11.6	11.5	11.2	11.0
NaCl	2.0	2.0	2.0	2.0	2.0	2.9	2.9	2.9	2.9	2.9
Oyster shell	1	1	1	1	1	1	1	1	1	1
Calculated composition										
Metabolisable energy (kcal/kg)	3000	3000	3000	3000	3000	3200	3200	3200	3200	3200
Crude protein (g/kg)	215.6	215.6	215.6	215.6	215.6	200.0	200.0	200.0	200.0	200.0
Calcium (g/kg)	9.7	9.7	9.7	9.7	9.7	9.0	9.0	9.0	9.0	9.0
Available phosphorus (g/kg)	4.2	4.2	4.2	4.2	4.2	3.5	3.5	3.5	3.5	3.5
Methionine + cysteine (g/kg)	8.4	8.4	8.4	8.4	8.4	7.2	7.2	7.2	7.2	7.2
Lysine (g/kg)	12.7	12.7	12.7	12.7	12.7	11.2	11.2	11.2	11.2	11.2
Mushroom-*Agaricus bisporus* composition[3]										
Total protein (N × 6.25), g/kg								230.0		
Total phenolic content,[4] mg of GAE/g								8.83		
Lysine (g/kg)								9.0		
Methionine (g/kg)								9.0		
Cysteine (g/kg)								6.6		
Calcium (g/kg)								13.3		
Total phosphorus (g/kg)								12		
Sodium (g/kg)								0.33		
Chlorine (g/kg)								0.66		

1-To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2 mg, riboflavin 4. 20 mg; vitamin B12 0.01 mg; pantothenic acid 5 mg; nicotinic acid 20 mg; f acid, 0.5 mg. 2- To provide the following per kg of diet: ; choline 3 mg; Mg 56 mg; Fe 20 mg; Cu, 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg. 3- All data are means of 3 samples analyzed in duplicate. 4- Phenolic content is expressed as dry weight basis in milligrams of gallic acid equivalents per gram (mg of GAE/g).

Table 2. Body weight, daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) values of broiler chickens in response to diet at different ages.

Days	Control	Flavophospholipol	5 g mushroom/kg	10 g mushroom/kg	20 g mushroom/kg	30 g mushroom/kg	SEM[4]
				DFI[1]			
9-21	79.10	77.40	77.33	78.62	79.44	80.31	0.919
22-45	143.5^{b}	133.0^{b}	149.6^{b}	183.6^{a}	146.6^{b}	143.2^{b}	6.137
9-45	121.6^{b}	114.0^{b}	124.8^{b}	147.9^{a}	124.0^{b}	121.5^{b}	4.480
				FCR[2]			
9-21	1.95^{cd}	2.04^{bc}	1.92^{d}	1.95^{cd}	2.19^{a}	2.15^{ab}	0.038
22-45	2.06^{b}	1.74^{c}	1.91^{bc}	2.42^{a}	2.07^{b}	1.96^{bc}	0.086
9-45	2.03^{bc}	1.80^{c}	1.91^{bc}	2.32^{a}	2.10^{ab}	1.99^{bc}	0.229
				BW[3](g)			
21	643.0^{a}	611.0^{bc}	637.3^{ab}	638.2^{ab}	593.0^{c}	598.2^{c}	9.219
45	2723.2	2607.5	2623.0	2617.2	2524.5	2611.0	78.206

$^{a-d}$Mean values followed by the same letters in the row do not differ according to Duncan test, [1] Daily feed intake (g per bird per day). [2] Feed conversation ratio (g/g). [3]Body weight. [4]Standard error of mean.

NC). The mean differences among different treatments were separated by Duncan's multiple range tests. A level of ($P < 0.05$) was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

Performance and carcass traits

Data on performance parameters are summarized in Table 2. Broilers receiving basal diet had higher body weights than those fed diet containing antibiotic, 20 or 30 g mushroom/kg at 21 day of age ($P < 0.05$). The BW obtained in birds fed the basal diet was greater than other groups at 45 day of age ($P > 0.05$). In starter period treatments failed to induce any marked effect on DFI. In grower and total part of the trial the highest amount of DFI were seen in the groups receiving 10 g mushroom/kg diet ($p < 0.05$). No differences because of treatment effects were observed on mortality. The lowest FCR in starter period obtained in broilers fed diet containing 5 g mushroom/kg.

The FCR obtained in bird fed diet containing 5 g mushroom/kg was lower than those fed diet containing antibiotic, 20 or 30 g mushroom/kg at starter period ($P < 0.05$). The lowest FCR in grower period and total part of trial obtained in broilers fed diet containing 4.5 mg flavophospholipol /kg. The FCR obtained in bird fed diet containing 4.5 mg flavophospholipol/kg was lower than those fed basal diet or basal diet supplemented with 10 or 20 g mushroom/kg at grower period ($P < 0.05$). Broilers receiving flavophospholipol had lower FCR compared to broilers receiving 10 or 20 g mushroom/kg during total period of trial ($P < 0.05$) but FCR of broilers in other periods was not affected but it was not different from broilers fed the basal diet or basal diet supplemented with 5 or 30 g mushroom/kg. Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks gut (Ferket, 2004). These effects may be due to interference in cell wall synthesis, changes in the permeability of the cytoplasmic membrane, interference in chromosome replication, and interference in cell protein synthesis (Mellor, 2000). A more balanced biota population in the gastrointestinal tract of poultry could lead to a greater efficiency in digestibility and utilization of feed, resulting in an enhanced growth and improved FCR (Bedford, 2000). Similar to the results obtained in this experiment, Performance enhancer effect of antibiotic growth promoters confirmed by several studies (Coates et al., 1963; Visek, 1978). Growth performance of broiler chicken is improved through the use of antimicrobial growth promoters through various

Table 3. Effect of experimental diets on carcass traits and internal relative organ weight of broilers at 45 days.

Characteristics	Dietary treatments						SEM[2]
	Control	flavophospholipol	5 g mushroom/kg	10 g mushroom/kg	20 g mushroom/kg	30 g mushroom/kg	
Eviscerated weight (%)[1]	74.23	72.51	74.10	73.81	74.89	73.29	0.87
Abdominal fat pad (%)	2.42	2.86	2.36	1.99	2.24	2.87	0.28
Liver (%)	1.98	1.96	2.29	2.11	2.14	2.02	0.09
Thigh weight (%)	25.76	25.44	25.75	25.46	25.88	26.36	0.44
Breast weight (%)	25.12	24.40	24.10	25.14	24.17	24.38	0.76

[a-b]Mean values followed by the same letters in the row do not differ according to Duncan test. [1]Percentage of live weight. [2]Standard error of mean.

established routes (Jensen, 1993). Although, the effects of mushrooms in broiler performance has not been clearly defined. Dalloul et al. (2006) reported that mushroom and mushroom-derived lectin increase innate immunity in broiler chicken challenged with *Eimeria acervulina*.

Also, Guo (2003) reported beneficial effects of feed supplementation with different mushrooms on broiler chicken performance and in particular immune-enhancing benefits in coccidian challenged chicken (Guo, 2003). Guo (2003) suggested that the effect of mushrooms was more pronounced under infectious conditions rather than that under normal ones. In this experiment, supplementation of broiler diet with *A. bisporus* mushroom had not any significant effect on BW of broilers at d 45 of age ($P > 0.05$). So, it is probably due to the normal condition in our study. Table 3 shows carcass, abdominal fat, breast, thigh and liver weights as a percentage of live weight at slaughter as a function of treatments. In the present study carcass traits and internal relative organ weight of broilers were not significantly influenced by the dietary treatments. These results are consistent with those reported by Willis et al. (2007) who did not find any differences among the control treatment and those receiving mushroom (*Lentinus edodes*) extract water additive on carcass, fat pad, and bursa weights as a percentage of live weights of broilers.

Also, Landy et al. (2011a) reported that carcass traits and internal organ weights of broilers fed dies supplemented with neem (Azadirachta indica) or antibiotic were not affected by dietary treatments.

Immune responses

The results for serum antibody titers against NDV, and SRBC in broilers are presented in the Table 4. The treatments had no effect on antibody titers against NDV. Use of antibiotic, flavophospholipol, failed to have any effect on antibody titer against NDV, and SRBC in comparison with control groups. Consistent with our results in other trial use of flavophospholipol had not any effect on antibody titer against NDV, AIV and SRBC in comparison with control group (Landy et al, 2011a, b). Also, Dafwang et al. (1985) reported no effect of dietary supplementation with oxytetracycline, lincomycin, penicillin, bambermycins or tylan on the antibody production against SRBC in broiler chicks. Although the contribution of gut microflora to the development and physiological status of the humoral and cellular mucosal immune system is well understood (Lu and Walker, 2001) but, the influence of enteric conditioners and their effects on gut microflora could be mainly limited to the mucosal immune complement and not the systemic portion of the immune system (Landy et al., 2011a). The highest SRBC antibody titer was observed in the group receiving 30 g mushroom/kg diet ($p < 0.05$).

The SRBC antibody titer observed in birds fed the diet containing 30 g mushroom/kg diet significantly was greater than those fed diets containing 5 g mushroom/kg diet or 4.5 mg flavophospholipol/kg diet ($P < 0.05$). As mushrooms have been reported to have antibacterial and stress reduction activities (Dalloul and Lillehoj, 2006; Dalloul et al., 2006) and the major components of mushrooms ergothioneine and phenolic antioxidants, variegatic acid and dibiviquinone have been indicated to possess potent antioxidant properties (Kasuga et al., 1995), an increase in immune responses of chicks was anticipated. Antioxidant activity of mushrooms has been documented in vitro as a radical activity scavenger (Akanmu et al., 1991) and *in vivo* as a cellular protector against oxidative damage in rat liver microsomes (Aruoma et al., 1999; Chaudiere and Ferrari-Iliou,

Table 4. Effect of experimental diets on antibody titers against Newcastle disease virus (NDV) at day 28 and sheep red blood cells (SRBC) at day 31.

Treatments	Antibody titer	
	NDV[1] (log_2)	SRBC[2] (log_2)
Control	3.33	8.00[ab]
Flavophospholipol	3.17	7.50[b]
5 g mushroom/kg	2.50	6.75[b]
10 g mushroom/kg	3.20	8.33[ab]
20 g mushroom/kg	3.33	8.00[ab]
30 g mushroom/kg	3.67	10.16[a]
SEM[3]	0.39	0.74

[a-b]Mean values followed by the same letters in the column do not differ according to Duncan test. [1]Antibody titers by the hemagglutination inhibition test (log_2). [2]Antibody titers by the hemagglutination test (log_2). [3]Standard error of mean.

1999). Also, in other trial Giannenas et al. (2010) showed that *A. bisporus* mushroom may potentiate the tissue antioxidant-protective activity when supplemented in broiler chicken diets. *A. bisporus* mushroom is also considered as a good source of selenium (Vetter and Lelley, 2004).

Selenium is important due to its function as antioxidant in organism, it neutralizes the free radicals that are resultant from many factors but especially by immune response. In poultry, the nutritional requirements for all nutrients and even selenium was normally calculated based on experimental trial using health animal in very low challenge conditions. However, on practical way animals are continually exposed to different infection challenges and intense vaccine program increasing immune system activation.

So, humoral immune activity can be improved by selenium source supplementation (Saad et al., 2009). Droke and Loerch (1989) showed that parental administration of selenium may potentiate the humoral immune responses in steers. In other study Arshad et al. (2005) showed that selenium supplementation may increase the antibody titers against infectious bursal disease in broiler chicks. Also, Guo (2003) showed that different mushrooms may provide protective immune-stimulation in *Eimeria*-challenged chicken. In addition, result of this study showed that application of 30 g mushroom/kg had significant effect on immune responses against SRBC in broiler chickens.

In conclusion, the results indicate that supplementing broiler diet with 30 g mushroom/kg could induce favorable influences on humoral immune responses of broilers without any adverse effects on performance indices.

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