Research Note

Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet

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ABSTRACT In this study, we evaluated the growth performance and antioxidant status of broiler chicken supplemented with the edible mushroom *Agaricus bisporus*. Ninety 1-d-old female broiler chickens randomly allotted to 3 dietary treatments were given either a nutritionally balanced basal diet or the basal diet supplemented with 10 or 20 g of dried mushroom/kg of feed for 6 wk on an ad libitum basis. Body weight, feed intake, and feed conversion ratio values were monitored weekly. To evaluate the antioxidant status of broiler chicken, refrigerated liver, breast, and thigh tissues were assayed for levels of glutathione, reduced glutathione, glutathione reductase, glutathione peroxidase, and glutathione S-transferase, as well as malondialdehyde at 6 wk of age. Results showed that dietary mushroom supplementation at both inclusion levels was accepted well by the broiler chicken and improved feed efficiency compared with the control diet. Dietary mushroom inclusion at 20 g/kg improved both growth performance and feed efficiency compared with control diet at 42 d of age. Dietary mushroom at both inclusion levels reduced malondialdehyde production in liver, breast, and thigh tissues and elevated glutathione peroxidase, reduced glutathione, glutathione reductase, and glutathione S-transferase compared with the control treatment, the effects being dose-dependent. These results suggest that *A. bisporus* mushroom exerts both a growth-promoting and tissue antioxidant-protective activity when supplemented in broiler chicken diets.

Key words: mushroom, *Agaricus bisporus*, broiler, performance, antioxidant

INTRODUCTION

Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Breene, 1990). Some fungi have been used for centuries to combat disease outbreaks in many parts of the world and are still used in ethnoveterinary medicine in Asian and Mediterranean countries (Chang and Buswell, 1996). Mushrooms may have a wide range of activities (Guo et al., 2003). Of particular interest are extracts derived from various mushrooms because they are known to confer health-promoting benefits, due to a multitude of compounds with antioxidant, antibacterial, immune-enhancing, and stress reduction properties on farm animals (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).

The poultry industry would greatly appreciate natural antioxidants that could replace synthetic ones and satisfy consumer demands for food products without residues from substances that have the potential to harm human health. Oxidative damage is associated with free radical formation and oxidative stress causes health deterioration. Superoxide, hydrogen peroxide, and hydroxyl radicals, which are mutagens produced by radiation, are also by-products of normal metabolism (Sies, 1986; Wagner et al., 1992). Besides health deterioration, lipid peroxidation is also a major cause of meat quality deterioration, affecting color, flavor, texture, and nutritional value (Halliwell and Gutteridge, 1999). Synthetic antioxidants have been used in meat stabilization. The most commonly used synthetic antioxidants are butylated hydroxyanisole, butylated hydroxytoluene, and tert-butylated hydroxyquinone, which are added in fat and oily foods to prevent oxidative deterioration (Lolliger, 1991). Synthetic antioxidants are currently approved to control lipid oxidation in foods, but consumer concern over their use (Imaida et al., 1983; Okada et al., 1990; Botterweck et al., 2000) has created a need and prompted research for alter-

native antioxidants. Recent research on the potential applications of antioxidants from natural products, for stabilizing foods against oxidation, has received much attention (Aruoma et al., 1996; Madsen et al., 1996; Gu and Weng, 2001; Lim et al., 2001).

Lately, ergothioneine has been identified and quantified in various genera of mushrooms as the main antioxidant compound (Dubost et al., 2007), whereas the phenolic antioxidants, variegatic acid and dibiviquinone, are also found in mushrooms (Kasuga et al., 1995). Antioxidant activity of mushrooms has been documented in vitro as a radical activity scavenger (Akanmu et al., 1991; Hartman, 1998) and in vivo as a cellular protector against oxidative damage in rat liver microsomes (Aruoma et al., 1999; Chaudiere and Ferrari-Iliou, 1999). However, there is no evidence as to whether dietary supplementation of dried mushrooms can improve oxidative stability of chicken tissues. *Agaricus bisporus* mushroom is also considered as a good source of selenium (Vetter and Lelley, 2004). Consumption of *A. bisporus*, which is the most widely investigated edible mushroom, has been shown to retard the development of free radicals (Falandysz, 2008). On this basis, we hypothesized that inclusion of *A. bisporus* mushroom into chicken diets would have both growth-promoting and antioxidant benefits on the stability of chicken tissues. The aim of the present study was to investigate this hypothesis in 1-d-old chickens whose diets have been supplemented with this dried mushroom.

MATERIALS AND METHODS

The trial protocol was approved by the Institutional Committee for Animal Use and Ethics of the Veterinary Faculty of the University of Thessaly. Throughout the trial, the birds were handled according to the principles for the care of animals in experimentation (NRC, 1996).

Birds and Experimental Design

Ninety 1-d-old female broiler chicks (Ross 308) were randomly allocated to 1 of 3 experimental treatments. Each treatment consisted of 3 replicates of 10 birds each. 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). All birds were reared in the floor pens using wood shavings as litter at a commercial poultry farm (Kotopoula Barbagianni, Giannitsa, Greece). The lighting program was set at 40 to 60 W/20 m^2 during the first 2 wk and 15 W/20 m^2 thereafter, with 23 h of light per day. The temperature was set at 36°C during the first day, 34°C during the first week, and was gradually reduced by 3°C per week to reach a minimum 22°C at 28 d of age. Relative humidity was between 65 to 75%.

The experiment lasted for 42 d. To meet the nutrient requirements of the broiler chicken over this period, a complete basal diet was formulated for each of the 3 stages of growth: starter, grower, and finisher. The feeds, which contained no antibacterial or anticoccidial supplements, were based on corn-soybean meal and were formulated to meet NRC recommendations (NRC, 1994). Table 1 presents the ingredients and the composition of the basal diets that were in mash form. Proximate analysis, which was conducted according to AOAC International (1995), showed no major deviation from calculated values.

The birds within the control group (**CON**) were given the basal diet for the respective growth stage. The other 2 groups were given experimental diets based on the basal diets but contained an additional 10 g (**M10**) or 20 g (**M20**)/kg of ground dried *A. bisporus* mushroom at the expense of ground yellow corn. Access to feed and water was provided on an ad libitum basis. Feeds were prepared every second day.

Mushroom Preparation and Supplementation

Mushrooms (*A. bisporus*) were obtained from a local mushroom producer (Ippotur-Lazarina S.A., Lazarina Trikala, Greece). The intact mushrooms were dried overnight at 60°C and ground through a 5-mm sieve before being incorporated into the feed by thorough hand mixing. For chemical analyses, mushrooms were freeze-dried at −76°C and 0.023 mbar in a vacuum for 30 h by Telstar Cryodos (Telstar, Barcelona, Spain). Dried mushrooms were milled through a 1-mm sieve (Polymix Kinematica PX-MFC90D, Littau, Switzerland) before analysis for protein, fat, fiber, and ash according to the procedures described by AOAC International (1995). Total protein content was determined by Kjeldahl (method no. 984.13), crude fat content was extracted from the samples with petroleum ether in a Soxhlet apparatus (method no. 920.39), CF content was analyzed in a Dosi Fiber (Selecta, Barcelona, Spain) apparatus (method no. 962.09), and ash (method no. 942.05) was measured by incinerating dried samples at 600°C for about 6 h in a furnace (Selecta) and moisture by oven drying (method no. 934.01). Starch and glucose- and fructose-monosaccharide contents were determined by Megazyme kits (Megazyme International, Wicklow, Ireland). Water-soluble polysaccharides in mushroom extracts were determined according to Guo et al. (2004a) and sugars of the polysaccharide fraction were determined according to the method of Dubois et al. (1956). Table 1 presents the proximate analysis and total sugar content of the polysaccharide extract of the mushroom preparation.

Mushroom Total Phenolic and Selenium Content

Total phenolic content in mushrooms was measured using Folin-Ciocalteu reagent (Merck, Darmstadt, Ger-

Table 1. Composition of experimental diets and mushroom (g/kg)

Item	Starter diet (1 to 14 d)			Grower diet (15 to 28 d)			Finisher diet (29 to 42 d)		
	CON[1]	M10[1]	M20[1]	CON	M10	M20	CON	M10	M20
Ingredients									
Corn grain	525.1	515.1	505.1	542.5	532.5	522.5	571.6	561.6	551.6
Dried mushroom	0.0	10.0	20.0	0.0	10.0	20.0	0.0	10.0	20.0
Soybean meal	340.0	340.0	340.0	340.0	340.0	340.0	320.0	320.0	320.0
Soybean oil	38.0	38.0	38.0	35.0	35.0	35.0	35.0	35.0	35.0
Coconut fat[2]	0.0	0.0	0.0	12.5	12.5	12.5	25.0	25.0	25.0
Fish meal	35.0	35.0	35.0	25.0	25.0	25.0	15.0	15.0	15.0
Corn gluten meal	25.0	25.0	25.0	12.5	12.5	12.5	0.0	0.0	0.0
Limestone	10.1	10.1	10.1	9.8	9.8	9.8	11.4	11.4	11.4
Monocalcium phosphate	14.1	14.1	14.1	12.7	12.7	12.7	14.2	14.2	14.2
L-Lysine	3.5	3.5	3.5	2.3	2.3	2.3	0.5	0.5	0.5
DL-Methionine	2.5	2.5	2.5	2.2	2.2	2.2	2.2	2.2	2.2
L-Threonine	0.5	0.5	0.5	0.4	0.4	0.4	0.0	0.0	0.0
Sodium chloride	1.9	1.9	1.9	2.0	2.0	2.0	2.1	2.1	2.1
Sodium bicarbonate	3.2	3.2	3.2	2.0	2.0	2.0	1.9	1.9	1.9
Vitamin-trace-mineral premix[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Natuphos phytase[4]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chemical analysis[5]									
DM	891.3	891.2	891.2	892.2	892.0	892.1	890.3	890.1	890.0
CP (N × 6.25)	230.2	230.2	230.3	221.0	221.1	221.3	205.1	204.9	205.2
Crude fat	54.4	54.3	54.3	71.1	71.0	71.1	87.7	87.5	87.4
Crude fiber	32.1	32.1	32.1	35.1	35.1	35.2	34.7	34.8	34.8
Ash	54.3	54.3	54.3	55.2	55.2	55.2	55.3	55.4	55.2
Calculated analysis									
Ca	9.3	9.3	9.3	9.1	9.1	9.1	9.0	9.0	9.0
P (total)	7.0	7.0	7.0	7.0	7.0	7.0	6.7	6.7	6.7
Lysine	14.0	14.0	14.0	13.0	13.0	13.0	11.5	11.5	11.5
Methionine + cystine	11.2	11.2	11.2	10.1	10.1	10.1	9.5	9.5	9.5
ME, kcal/kg	3,060	3,060	3,060	3,180	3,180	3,180	3,200	3,200	3,200
Mushroom-*Agaricus bisporus* composition[6]									
Moisture, g/kg				896.8					
Total protein (N × 6.25), g/kg				30.7					
Ether extract, g/kg				2.2					
Crude fiber, g/kg				17.8					
Ash, g/kg				8.7					
Nitrogen-free extract, g/kg				43.8					
Starch, g/kg				6.9					
Free sugars (monosaccharides), g/kg				2.4					
Total phenolic content,[7] mg of GAE/g				8.85					
Selenium content, mg/kg				0.12					
Yield of water-soluble polysaccharide extract,[8] g/kg				155					
Dry matter of the polysaccharide extract,[9] g/kg				962					
Total sugar content of the polysaccharide extract,[10] g/kg				643					

[1]CON, M10, and M20 represent diet supplemented with dried *Agaricus bisporus* mushroom at the level of 0, 10, or 20 g/kg of feed, respectively.

[2]Bergafat HTL-306, Berg & Schmidt GmBH & Co, Hamburg, Germany.

[3]Supplying per kilogram of feed: vitamin A (retinyl acetate), 12,000 IU; vitamin D$_3$, 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 30 mg; menadione, 3 mg; thiamine, 1 mg; riboflavin, 8 mg; pyridoxine, 3 mg; vitamin B$_{12}$, 0.02 mg; niacin, 20 mg; pantothenic acid, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; vitamin C, 10 mg; choline chloride, 480 mg; zinc, 100 mg; manganese, 120 mg; iron, 40 mg; copper, 20 mg; cobalt, 0.2 mg; iodine, 1 mg; and selenium, 0.3 mg.

[4]BASF, Ludwigshafen, Germany.

[5]According to AOAC International (1995).

[6]All data are means of 3 samples analyzed in duplicate.

[7]Phenolic content is expressed as dry weight basis in milligrams of gallic acid equivalents per gram (mg of GAE/g).

[8]Yield of polysaccharide is expressed on the basis of the DM of the intact mushroom (Guo et al., 2004a).

[9]The DM content of the extract was determined by freeze-drying.

[10]Total sugar content of the extract was determined using the phenol-sulfuric method (Dubois et al., 1956).

many) according to the method of Fu et al. (2002). The trace element selenium in dried mushroom was determined using inductively coupled plasma mass spectrometry (Agilent 7500s, Agilent Technologies, Waldbronn, Germany) according to Nisianakis et al. (2009). Table 1 presents total phenolic content and selenium concentration of the mushroom preparation.

Chicken Performance Measurements

Body weight and feed intake were monitored on a pen basis weekly, whereas weight gain and feed conversion ratio values were subsequently calculated. Mortality was also recorded on a daily basis in each pen. Chickens were killed by cervical dislocation at the end

of the trial. Six birds per pen were randomly selected for tissue sampling. After slaughter, tissues of liver, breast muscle (pectoralis major), and thigh muscle (biceps femoris) were collected by removing skin, fat, and connective tissue. Samples were vacuum-packed and stored at $-40°$C. For antioxidant measurements, all tissues were thawed and stored at $4°$C for 5 d.

Refrigerated Storage of Tissues and Antioxidant Status Determination

All excised tissues were assayed for the levels of reduced glutathione (**GSH**), glutathione peroxidase (**GSH-Px**), glutathione S-transferase (**GST**), glutathione reductase (**GR**), and malondialdehyde (**MDA**) formation according to the procedures described below. To assess the effect of dietary treatment on lipid oxidation of raw muscle during refrigerated storage, samples were thawed, wrapped in transparent oxygen-permeable polyvinyl chloride film (6,000 to 8000 cm^3/m^2 × 24 h), placed in a nonilluminated refrigerated cabinet at $4°$C for 5 d, and submitted to determination of antioxidant enzyme activities and lipid oxidation at 0 and 5 d of storage.

Reagents and Equipment

5,5′-Dithiobis-(2-nitrobenzoic acid); TBA; 1,1,3,3-tetraethoxypropane; GR; GSH; NAD phosphate and Coomassie Brilliant Blue G-50; and butylated hydroxytoluene were purchased from Sigma (St. Louis, MO). Sodium azide, tert-butyl-hydroxyperoxide, and BSA were purchased from Fluka (Steinheim, Germany). Potassium and sodium phosphate, Folin-Ciocalteu reagent, and ethanol were purchased from Merck. The spectrophotometer used was a Hitachi U-1900 model (Hitachi, Tokyo, Japan), the tissue homogenizer was Ultraturrax IKA T18 basic (IKA, Jacqvepagua, Brazil), and the centrifuge was a Centurion model by Scientific Ltd. Company (West Sussex, UK). The enzyme kits for starch and sugar monosaccharide determination, containing α amylase, β glucosidase, glucose, and starch standards were purchased from Megazyme International. The freeze-drier apparatus was by Telstar Cryodos (Telstar).

Oxidative Enzyme and Protein Determination Assays

Glutathione peroxidase activity was assayed with the method of Paglia and Valentine (1967). Reduced glutathione was measured by an adaptation of the method by Tietze (1969). Glutathione reductase activity was determined by the method of Staal et al. (1969). Glutathione S-transferase activity was measured with the method of Habig et al. (1973). Malondialdehyde was used as a marker of lipid peroxidation using the method described by Buege and Aust (1978). Tissue proteins were determined by the method of Bradford (1976), using BSA as a standard.

Statistical Analysis

Experimental data were analyzed as a randomized block design. Broilers were allocated by initial BW and the pen was the experimental unit. All data were subjected to 1-way ANOVA by the GLM procedure using the SPSS 12.00 statistical package (SPSS Ltd., Surrey, UK). Levene's test was performed to check homogeneity of variances and Tukey's test was carried out to assess any significant differences at the probability level of $P \leq 0.05$ among the experimental treatments.

RESULTS AND DISCUSSION

The present study was designed to evaluate the effect of sustained consumption of a natural product such as *A. bisporus* mushroom, rich in antioxidant polyphenols and polysaccharides, and its potential application as dietary supplement. Previous studies had shown putative beneficial effects of different mushrooms on broiler chicken performance and in particular immune-enhancing benefits in *Eimeria*-challenged chicken (Guo, 2003). In our study, the amount of dried mushrooms added to the basal diet corresponds to the relatively low dietary inclusion level of 0.1 or 0.2%. Moreover, it was consumed as a part of the usual feeding regimen of broiler chicken. No mortality was observed throughout the experiment. The performance, food intake, and feed conversion ratio values of the control and supplemented chicken are shown in Table 2. Food intake was not affected by mushroom inclusion. No significant effects of treatment were seen on BW values up to d 28 of age. At d 42 of age, the M20 group had BW and weight gain values that were greater ($P \leq 0.05$) than the CON group. The M10 group BW values did not statistically differ from M20 or CON, although they were numerically between the two. At d 42 of age, feed efficiency values were greater ($P \leq 0.05$) in M20 and M10 groups compared with CON but did not differ between each other. In conclusion, incorporation of the dried mushrooms in chicken diets improved both BW and feed efficiency compared with the unsupplemented treatment.

In Figure 1A, it is shown that liver and thigh tissues of chicken presented GST activity that did not differ among groups on d 0; however, the CON group presented significantly decreased ($P \leq 0.05$) GST activity compared with mushroom-supplemented groups on d 5 during refrigerated storage. Breast tissue presented significantly decreased ($P \leq 0.05$) GST activity compared with mushroom-supplemented groups at both time points. In Figure 1B, it is shown that thigh and breast tissues of the CON group presented significantly decreased ($P \leq 0.05$) GSH activity compared with the M20 group on both d 0 and 5 during refrigerated storage. Liver tissue presented GSH activity that did not

differ among groups at both time points. In Figure 1C, it is shown that liver and breast tissues of chicken of the CON group presented significantly decreased ($P \leq$ 0.05) GR activity compared with mushroom-supplemented groups on both time points. In thigh tissue, GR activity in CON and M10 groups did not differ among each other; however, GR activity in the M20 group was significantly increased ($P \leq 0.05$) compared with the CON and M10 groups at both time points (Figure 1C). In Figure 1D, it is shown that thigh tissue of the CON group presented significantly decreased ($P \leq 0.05$) GSH-Px activity compared with mushroom-supplemented groups at both time points. Although liver tissue presented GSH-Px activity that did not differ among groups on d 0, GSH-Px activity of the CON group presented significantly decreased ($P \leq 0.05$) activity compared with mushroom-supplemented groups during refrigerated storage Breast tissue presented GSH-Px activity that did not differ among groups at both time points (Figure 1D).

In Figure 1E, it is shown that liver and thigh tissues of chicken presented MDA values that did not differ among groups on d 0; however, the CON group presented significantly increased ($P \leq 0.05$) MDA values compared with mushroom-supplemented groups on d 5 during refrigerated storage. Breast tissue of the CON group presented significantly increased ($P \leq 0.05$) MDA values compared with mushroom-supplemented groups at both time points. On d 5, the M20 group had lower ($P \leq 0.05$) MDA values compared with the M10 group. This finding suggests that dietary mushroom exerted an antioxidative effect, which was dose-dependent.

Agaricus bisporus mushroom used in our study is easy to obtain because it is the most commonly cultivated on an industrial scale in various countries, including temperate ones. Mushroom stipes that have the same composition as the rest of the mushroom are discarded for aesthetic reasons and marketed as a relatively cheap by-product of this industrial production. The low cost of this material encouraged its study use as a promising and sustainable feed additive. The chemical composition of *A. bisporus* mushroom was in accordance with the values reported in literature (Vetter, 1993; Manzi et al., 2001). Total sugar content of the polysaccharide extracts was similar to the values of Guo et al. (2004a) for other mushroom species. Total phenolic content was also in accordance with values reported in literature (Manzi et al., 2001; Dubost et al., 2007) and selenium content was similar to published values (Vetter and Lelley, 2004). The physicochemical properties of the mushroom composition in relation to the phenolic content and polysaccharide fractions could be the basis for the observed results. Guo et al. (2003) have suggested that water-soluble polysaccharides of the *Lentinus edodes* and *Tremella fuciformis* mushrooms can enhance growth performance in broiler chicken. They stated that differences in response between different mushroom extracts were logical because of the large variation in the physicochemical properties of these polysaccharides, such as sugar composition, molar weights, and structures (Guo et al., 2003).

Our data are consistent with the work of Guo et al. (2004a) that showed increased weight gain in broilers through the use of a mixture of polysaccharide extracts of *L. edodes* and *T. fuciformis* and *Astragalus membranaceus* herb. Willis et al. (2007) also found increased BW gains with the supplementation of mushroom *L. edodes* extract to 21 d of age. However, this supplementation did not sustain improved weight gains up to d 49 when the trial was completed, possibly due to its removal from the feed on d 22 of age. In a recent study, Willis et al. (2008) also found that dietary mushroom *L. edodes* extract improved egg production in layer hens and lowered *Salmonella* population during molt induc-

Table 2. Body weight, weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) values of broiler chickens in response to diet and age[1]

Age of chickens	CON[2]	M10[2]	M20[2]	Pooled SEM	*P*-value
0 d					
BW, g	43.4	43.2	43.1	0.25	0.116
14 d					
BW, g	383.1	395.4	406.0	5.78	0.299
WG, g	339.7	352.2	362.9	5.84	0.309
FI, g	400.8	408.4	409.9	4.31	0.710
FCR	1.18	1.16	1.13	0.010	0.145
28 d					
BW, g	1,308.7	1,320.0	1,340.3	9.72	0.462
WG, g	1,265.3	1,276.8	1,297.2	9.77	0.466
FI, g	2,041.3	2,017.2	2,061.6	9.01	0.118
42 d					
BW, g	2,011.2[b]	2,116.3[ab]	2,204.7[a]	35.11	0.048
WG, g	1,967.8[b]	2,073.1[ab]	2,161.6[a]	35.15	0.048
FI, g	3,777.8	3,854.2	3,976.4	40.7	0.121
FCR	1.92[a]	1.86[b]	1.84[b]	0.014	0.015

[a,b]Values in the same row with a different superscript differ significantly at $P \leq 0.05$.

[1]Results are given as means of groups (n = 3 subgroups).

[2]CON, M10, and M20 represent a group of broiler chickens fed basal diet supplemented with ground, dried *Agaricus bisporus* mushroom at the level of 0, 10, or 20 g/kg of feed.
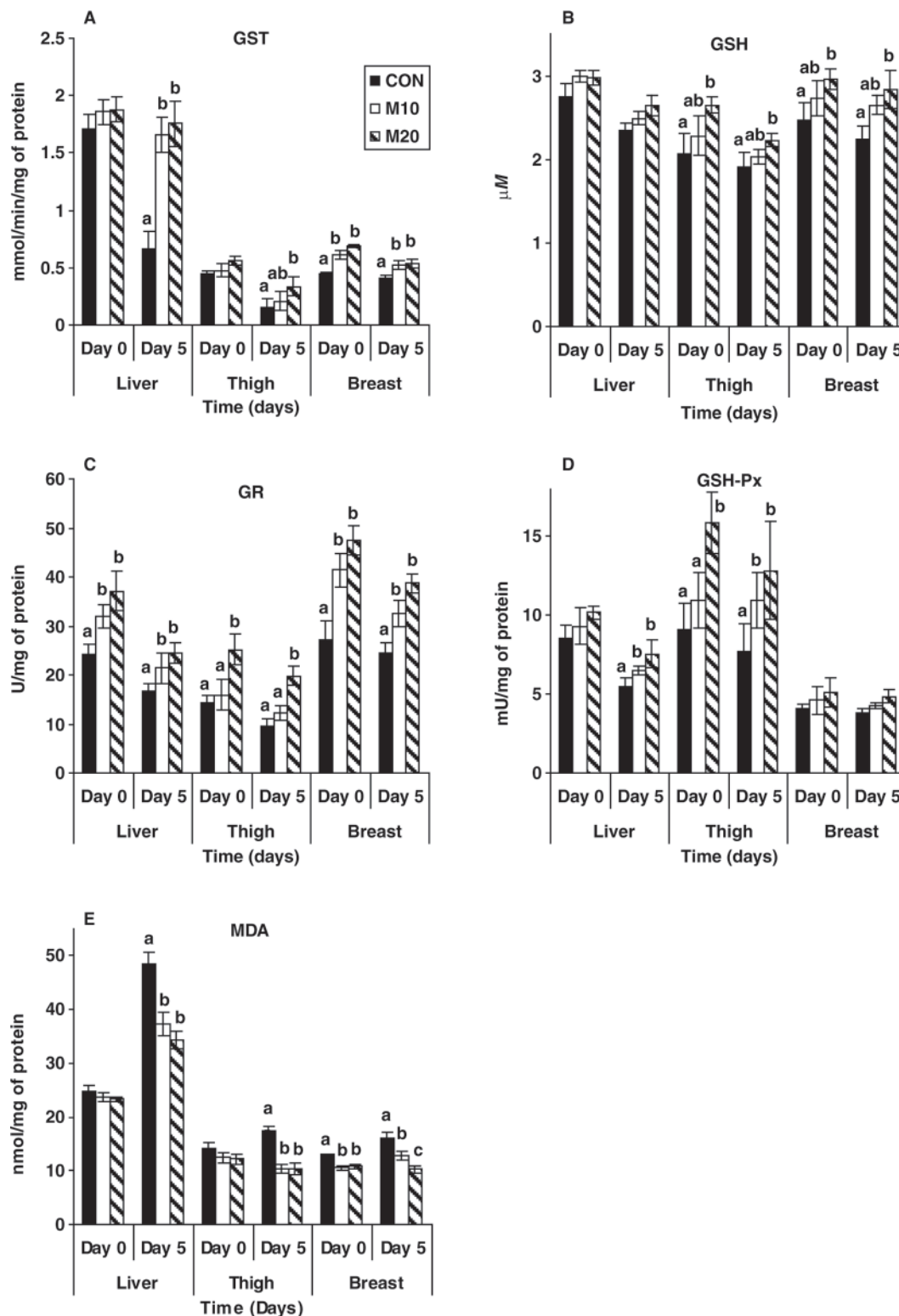
Figure 1. Effect of dietary *Agaricus bisporus* mushroom supplementation on activity of (A) glutathione S-transferase (GST), (B) reduced glutathione (GSH), (C) glutathione reductase (GR), (D) glutathione peroxidase (GSH-Px), and concentration of malondialdehyde (MDA) of liver, breast, and thigh tissues of broiler chicken during refrigerated storage at 0 or 5 d. [a–c]Values within the same day with a different letter differ significantly at $P \leq 0.05$. Results are given as means of groups (n = 3 subgroups). CON, M10, and M20 represent a group of broiler chickens fed basal diet supplemented with ground, dried *A. bisporus* mushroom at the level of 0, 10, or 20 g/kg of feed.

tion. It has to be emphasized, however, that not all of these mushrooms are available on an industrial scale.

Growth performance of broiler chicken is improved through the use of antimicrobial growth promotants

through various established routes (Jensen, 1993). Limited evidence exists on the mechanisms through which mushrooms exert their growth-promoting activities. Guo (2003) suggested that the effect of mushroom

polysaccharide extract was more pronounced under infectious conditions rather than that under "normal" ones. Polysaccharide extracts increased the activity of intestinal microflora and fermentation end products, such as volatile fatty acids, and increased proliferation of the gastrointestinal tract. Dalloul et al. (2006) suggested that mushroom and mushroom-derived lectin enhance innate immunity in broiler chicken challenged with *Eimeria acervulina.*

The second objective of the study was to investigate the antioxidant effects of mushroom when supplemented into feed. The literature is prolific with evidence of in vitro antioxidant activity of mushrooms. Mushrooms contain various polyphenolic compounds recognized as antioxidants due to their ability to scavenge free radicals by single-electron transfer in vitro (Hirano et al., 2001). Some common edible mushrooms, including *A. bisporus*, have been found to possess significant in vitro antioxidant activity (Cheung et al., 2003; Lo and Cheung, 2005), which was well correlated with their total phenolic content (Mau et al., 2002a,b; Yang et al., 2002, 2004). Various concentrations of methanolic extracts from mushrooms exhibited scavenging activity to free radicals (Yang et al., 2004). However, there are no in vivo studies about these effects on animal models and broiler chicken in particular. Certain soluble, low molecular weight polyphenolic compounds can be absorbed by the intestine, reaching the plasma and target organs. Although their levels in the circulation are low, with a reduced net absorption and relatively fast excretion half-lives, consumption of polyphenolics has been accompanied by increased total antioxidant activity (Youdim and Deans, 2000; Botsoglou et al., 2002, 2004; Jiang et al. 2007; Monino et al., 2008). Numerous studies have shown a postprandial antioxidant capacity of phenolic compounds from various foods (Cao et al., 1998; Duthie et al., 2000) or feedstuffs (Botsoglou et al., 2004; Jiang et al., 2007). However, when biomarkers of the redox status are measured after polyphenolic substance consumption, the results obtained are often contradictory (Lopez-Bote et al., 1998; Papageorgiou et al., 2003; Monino et al., 2008).

In our study, we observed an increased activity of the 4 selenoenzymes in the mushroom-supplemented groups compared with control birds. The high mushroom content in selenium (Vetter and Lelley, 2004) might contribute to this desired property. The depletion in overall glutathione activity, observed within 5 d after refrigerated storage, indicated an ongoing process of oxidative stress in the examined tissues. The antioxidant protective effect was more pronounced at the higher level of mushroom supplementation. Elevated antioxidant enzyme activities could be due to active induction of glutathione synthetic enzymes due to higher selenium uptake or passive sparing of glutathione by decreasing the oxidative load on the cells. Although the latter seems more plausible because MDA formation was found to be reduced in mushroom-supplemented groups, additional studies are required to determine which mechanism is responsible. It is possible that the antioxidant properties of mushrooms (Dubost et al., 2007) are being used by the cells, thus sparing the intracellular antioxidant systems such as GSH and GSH-Px.

Data in the literature yield contradictory results on the effect of dietary natural antioxidants on the activity of antioxidant enzymes (Lin et al., 1998). Young et al. (2000) showed an increased activity of erythrocyte GR and GSH-Px in human volunteers consuming a polyphenolic-rich grape skin extract. Dietary supplementation with thyme oil and thymol, rich in antioxidant terpenoids, prevented the age-induced decline of GSH-Px activity in rat brain (Youdim and Deans, 2000). On the other hand, Breinholt et al. (1999) observed a decreased activity of GSH-Px and GR after gavage administration of natural flavonoids to rats. Mushroom substances might also influence other cellular systems, suggesting that a more detailed examination of further antioxidant parameters is required.

To our knowledge, no previous attempt has been made to test the protective role of mushroom-enriched diets against oxidation. Further research, including dietary supplementation with certain mushroom extracts and specified mushroom constituents during certain periods of feeding, is necessary to elucidate the in vivo regulation of the antioxidant defenses in the liver as well as the response of the system to acute oxidative stress.

In conclusion, the results presented in this paper show a growth-promoting activity and an increase in the antioxidative capacity of broiler chicken tissues as a result of dietary mushroom supplementation. Inclusion of 20 g of *A. bisporus* mushroom/kg of diet was more effective in both performance enhancement and delaying lipid oxidation of poultry meat than to the inclusion level of 10 g/kg. Future experiments are required to determine whether the mushrooms can be used as alternatives for growth promoters in healthy or challenged birds, elucidate the mechanisms for potential enhanced growth performance and antioxidant activity, and determine the components responsible for these activities. It has to be emphasized, however, that this research should concentrate mainly on edible mushrooms because not all available mushrooms are safe because they may contain harmful bioactive compounds.

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