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Effect of shiitake [*Lentinula edodes* (Berk.) Pegler] mushroom on laying performance, egg quality, fatty acid composition and cholesterol concentration of eggs in layer chickens

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Shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] has long been considered a delicacy as well as a medicinal mushroom in many Asian countries, as well as being dried and exported internationally. This study assessed the influence of shiitake mushroom on laying performance, egg quality, sensory properties, fatty acid composition and cholesterol concentration of eggs in laying hens. The dietary groups were control (basal diet), shiitake 0.25% (basal diet + 0.25% shiitake mushroom) and shiitake 0.5% (basal diet + 0.5% shiitake mushroom). Egg production was significantly increased ($p<0.05$) by the shiitake supplementation compared to the control, but other laying parameters were not affected ($p>0.05$). Haugh unit was significantly increased ($p>0.05$) in both shiitake groups, but a thinner egg shell was observed in the shiitake 0.25% group and thicker egg albumen in the shiitake 0.5% group compared to the control group. Dietary addition of shiitake mushroom did not induce any effect ($p>0.05$) on sensory evaluation of eggs. Among the fatty acid composition of egg yolk, linoleic acid as well as total n-6 and polyunsaturated fatty acid contents were increased ($p<0.05$), whereas palmitoleic acid and α -linolenic acid were decreased ($p<0.05$) in the shiitake 0.5% group. Cholesterol concentration of egg yolk was significantly decreased ($p<0.05$) in the shiitake 0.5% group compared to the control group. Dietary supplementation with shiitake mushroom up to 0.5% positively affects egg production, egg quality, fatty acid composition and cholesterol level reduction of eggs, and did not adversely affect sensory properties of eggs. Shiitake mushroom could be beneficial in layer diet.

Key words: Shiitake, egg production, egg quality, sensory evaluation, fatty acid, cholesterol, layer.

INTRODUCTION

Shiitake [*Lentinula edodes* (Berk.) Pegler] is a traditionally renowned mushroom in Far East countries (for example, Japan, China, and Korea) that has been used as a food and medicine for thousands of years. Many compounds have been isolated and their health

promotion activities demonstrated (Wasser, 1997; Hobbs, 2000). Shiitake is one of the five most cultivated edible mushrooms in the world. Its production (2 million tons) is second only to button mushroom *Agaricus bisporus* (Chang, 1999; Stamets, 2000). Grown mainly in East Asia, shiitake is now arousing market interest worldwide, because of its' exotic and well-appreciated taste, and demonstrated medicinal properties (Wasser, 2005).

Shiitake mushrooms have excellent nutritional value. Their raw fruit bodies include 88 to 92% water, protein,

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lipids, carbohydrates, vitamins, and minerals. Dried shiitake mushrooms are nutrient-rich, containing 58 to 60% carbohydrates, 20 to 23% protein (digestibility of 80 to 87%), 9 to 10% fiber, 3 to 4% lipids, and 4 to 5% ash. The mushroom is a good source of vitamins, especially provitamin D2. It also contains B vitamins, and minerals include Fe, Mn, K, Ca, Mg, Cd, Cu, P, and Zn (Mizuno, 1995; Hobbs, 2000). Shiitake is one of the best-known and best-characterized mushrooms used in medicine. It is the source of several well-studied preparations with proven pharmacological properties. In addition to glycogen-like polysaccharides, (1-4)-(1-6)-*a*-D-glucans and antitumor polysaccharides, lentinan, (1-3)-(1-6)-*b*-bonded heteroglucans, heterogalactans, heteromannans, and xyloglucans have been identified (Wasser, 1997; Hobbs, 2000). Mushrooms also contain various biologically active compounds such as gallic acid, protocatechuic acid, chlorogenic acid, naringenin, hesperetin, and biochanin-A (Alam et al., 2008, 2010). Lentinan stimulates various kinds of NK cell-, T cell-, B cell-, and macrophage-dependent immune reactivity. Of particular interest are extracts derived from various mushrooms, as they are known to modulate immune response and stress reduction on poultry (Dalloul et al., 2006; Lee et al., 2010). Various studies have confirmed that the mushrooms can lower blood pressure and free cholesterol in plasma (Hobbs, 2000; Yoon et al., 2011), as well as accelerate the accumulation of lipids in the liver by removing them from circulation, and can prevent cardiovascular disease (Guillamón et al., 2010). Xu et al. (2008) reported that the administration of polysaccharides from *L. edodes* significantly reduced serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, and enhanced serum antioxidant enzyme activity and thymus and liver index in high-fat rats.

Studies conducted in chickens have shown that polysaccharides derived from mushrooms show antibacterial (Guo et al., 2003; Hearst et al., 2009), antiviral (Liu et al., 1999; Yu and Zhu, 2000), and antiparasitic activities (Hu et al., 1998; Pang et al., 2000). Giannenas et al. (2010a) reported that dietary mushroom supplementation at the level of 20 g/kg increased lactobacilli counts in the ileum and both lactobacilli and bifidobacteria counts in the cecum of broilers, although the intestinal morphology at either level of inclusion remained unaffected. Similarly, Willis et al. (2007) noted enhanced beneficial bifidobacteria production from *L. edodes* extracts given to broiler chickens. Guo et al. (2004) investigated several mushroom and herb polysaccharides, as alternatives for an antibiotic, on growth performance of broilers, and found *L. edodes* to be a significant growth promoter in broilers. Several recent studies highlighted that medicinal mushrooms can reduce lipid peroxidation during refrigerated storage of broiler meat (Giannenas et al., 2010b) and turkey meat (Giannenas et al., 2011). In a layer experiment, Cho et al. (2010) concluded that fermented spent mushroom substrates could be used as a resource in laying hen feed at 5 to 15% without

adversely affecting egg-related performance and egg quality.

There is scant information on the effects of mushrooms and their extracts on layer performance and egg quality characteristics. To address this shortcoming, this study evaluated the effects of shiitake mushroom on the egg-related performance, egg quality, sensory evaluation, fatty acid composition, and cholesterol content of eggs in laying hens.

MATERIALS AND METHODS

Birds, feed, and experimental design

Fifty four, 22-week-old "Tetran Brown" layers were assigned to three dietary treatments in a completely randomized design with nine replicates per treatment. For this experiment, two layers were housed in one cage (cage dimensions, 24 × 38 × 45 cm) and considered as a replicate. The layers were acclimated for 1 week before the 8 week trial began. The three dietary treatments were control (basal diet without shiitake mushroom), and diets containing 0.25 and 0.5% shiitake mushroom. The basal diet was formulated (Table 1) to meet or exceed nutrient requirements of laying hens (NRC, 1994). The shiitake strain used in the experiment was *L. edodes* KFRI 405, which was maintained at the Korea Forest Research Institute (KFRI), Seoul, Korea. All parts of the mushrooms were air-dried and then ground to a fine powder using an electric blender. The shiitake diets were prepared separately using the same ingredients as in the basal diet, but enriched with shiitake at 0.25 and 0.5% inclusion levels on a weight: weight ratio basis. The experimental diets and drinking water were supplied *ad libitum*. The room temperature was kept at 21 to 23°C and the light cycle consisted of 16 h of light (incandescent lighting, 10 lux) and 8 h of dark.

Measurements and analysis

Laying performance

All the eggs produced each day were manually collected each day at 5 pm and counted. All the eggs produced on a certain day of the week were weighed individually. The egg production rate, egg weight, egg mass, and feed conversion ratio (FCR) were determined on a weekly basis. Egg production rate was calculated by dividing the total number of eggs by hen-day and was expressed in percent on weekly basis. Egg weight was measured by an Adventurer AR2140 electronic scale (OHAUS, USA). Egg mass was calculated by multiplying the average egg weight by egg production rate. Feed conversion ratio was calculated by dividing feed intake by egg mass.

External and internal quality of eggs

Nine eggs (one egg per replicate, total 27 eggs) from each treatment were selected for external and internal quality measurement. The eggs were weighted first and then shape index was measured (ratio of width to length of egg). The same eggs were used for internal quality measurement. Shell thickness was measured by Peacock dial pipe gauge FHK (Ozaki, Meg. Co., Ltd, Japan, Japan) and is presented as the average thickness of three locations on the egg (air cell, equator and sharp end). For yolk color determination, egg albumen, eggshell, and shell membranes were removed from broken eggs and the egg yolk color was measured

Table 1. Ingredients and chemical composition of basal diet (as fed basis).

Item	Amount (%)
Corn grain	65.59
Wheat bran	6.50
Soybean meal	16.00
Corn gluten meal	2.60
Salt	0.30
Tricalcium phosphate	0.87
Limestone	7.73
Vitamin mineral premix ¹	0.30
L-Lysine	0.04
Methionine	0.07
Total	100.00
Calculated composition	
ME (kcal/kg)	2,805
Crude protein	15.45
Calcium	3.25
Available phosphorus	0.25

¹ Vitamin mineral premix provided nutrients per kg of diet: Vitamin A, 27000 IU; Vitamin D3, 6300 IU; Vitamin E, 45 IU; Vitamin K, 6 mg; Vitamin B1, 4.5 mg; Vitamin B2, 12 mg; Vitamin B6, 9 mg; Vitamin B12, 0.045 mg; Calcium Pantothenate, 25.5 mg; Niacin, 60 mg; Biotin, 0.33 mg; Folic acid, 1.8 mg; Co, 0.9 mg; Cu, 10.5 mg; Mn, 16.5 mg; Zn, 120 mg; I, 1.8 mg; Se, 0.39 mg.

using a model CR-200 chromameter (Minolta, Japan). Haugh unit and albumen height were measured using a model EMT-5200 Multi Tester (Hikari Technology, Japan).

Sensory evaluation for boiled eggs

Nine eggs per treatment (total 27) produced at the end of the experimental periods were randomly selected for sensory evaluation tests. Ten trained panelists from the Department of Animal Science and Technology at Sunchon National University evaluated warm hard-boiled eggs after shelling and cutting them in half. Panelist score, on a 9-point scale (9, "like very much" to 1, "dislike very much") ranked the degree of preference concerning appearance, color, flavor, oily nature, and acceptability.

Fatty acid composition of egg yolk

The fatty acid composition of egg yolk was determined according to methyl ester extraction method with slight modification. Briefly, 1 g egg yolk was dissolved separately into 12 ml of Folch solution (chloroform: methanol, 2:1 v/v) and homogenized (9500 rpm) for 1 min and kept in ice for 10 s. The samples were flushed under a flow of nitrogen gas, filtered through Whatman No.1 filter paper, and kept at 4.5°C until it separated into two layers. After phase separation, the filtrate from the upper layer was collected in a syringe and added to 2.4 ml of 0.9% NaCl solution. The sample was centrifuged (3000 rpm, 15 to 20 min) and the bottom layer was collected into an ampoule. Each sample was dried under a flow of nitrogen and resuspended by the addition of three drops of benzene and 3 ml of 5% sulfuric acid methanol solution. Each sample

was flushed under a flow of nitrogen and sealed. The sealed ampoules were heated in a water bath at 95°C for 45 min and cooled. After breaking the ampoules, 3 ml of 5% Na₂CO₃ (anhydrous) solution was added and vortexed. The fatty acid methyl ester was extracted three times with 3 ml of petroleum ether, dried with nitrogen, dissolved in 100 µl of petroleum ether, and 1 µl volumes were injected and analyzed by gas chromatography using a model DS 6200 apparatus (Donam, Korea). Fatty acids were identified by matching their retention times with those of their relative standards (polyunsaturated fatty acid (PUFA)-2; Supelco, USA) and the Food Composition Table (NRLSI, 2002).

Cholesterol concentration of egg yolk

Cholesterol concentration was determined as described previously (AOAC, 1984). Briefly, approximately 1 g egg yolk, 500 µg of α-cholestane, and 15 ml water were homogenized with 200 ml of Folch solution (Chloroform: methanol, 2:1 v/v) and filtered. The filtrate was added to 50 ml of 0.5% sodium hydroxide. The sample was saponified at 85°C for 60 min with 10 ml of 2 M ethanolic potassium hydroxide solution (Adams et al., 1986). After cooling to room temperature, cholesterol was extracted with 1 ml of hexane. The process was repeated four times. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was redissolved in 1 ml of hexane and was stored at -20°C until analysis. Total cholesterol was determined on a silica fused capillary column (HP-5, 30 m × 0.32 mm I.D. × 0.25 µm thickness) using a Hewlett-Packard 5890A gas chromatography (Palo Alto, CA) equipped with a flame ionization detector. The injector and detector temperature was 280 and 280°C, respectively. The oven temperatures were programmed to advance from 200 to 300°C at 10°C/min, with a 20 min hold at 300°C. Nitrogen was used as a carrier gas at a flow rate of 2 ml/min with a split ratio of 1/50. Quantitation of cholesterol was done by comparing the peak areas with a response of an internal standard.

Statistical analyses

Data were analyzed using the general linear models of the SAS Institute (2003) to estimate variance components with a completely randomized design. Duncan's multiple comparison tests were used to examine significant differences among the treatment means. The level of significance was set at p<0.05.

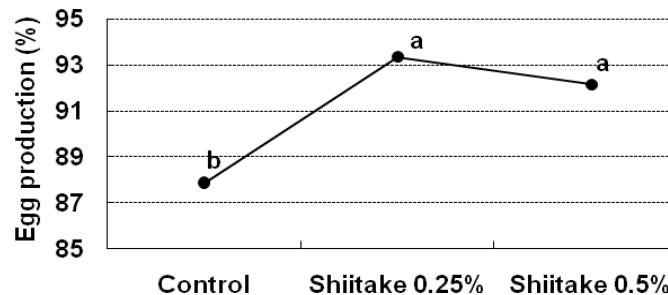
RESULTS AND DISCUSSION

Laying performance

Dietary supplementation of shiitake mushroom had an effect on egg production of layers (Figure 1). Egg production were increased significantly (p<0.05) in shiitake groups compared to the control group. Egg weight, feed intake, egg mass, and FCR were not different (p>0.05) among the groups, although higher egg mass and better FCR were observed in the shiitake groups (Table 2). Cho et al. (2010) conducted an experiment with 5 to 15% fermented spent mushroom substrate and found no effect on egg production, dissimilar with our results. However, the previous findings about egg weight, egg mass, and feed conversion was similar with our study. The hen day egg production of shiitake-fed hens in this study was

Table 2. Effect of dietary shiitake mushroom on the laying performance of hens.

Parameter	Control	Shiitake 0.25%	Shiitake 0.50%	SEM
Egg weight (g)	62.85	61.77	61.80	0.68
Feed intake (g)	125.73	124.87	124.99	1.83
Egg mass (g)	55.21	57.68	56.95	0.81
Feed conversion ratio (feed intake: egg mass)	2.28	2.17	2.20	0.04

**Figure 1.** Effect of dietary shiitake mushroom on egg production of layers. Means with different superscripts represent significant difference at $p<0.05$ by Duncan's multiple range tests.

92.16 to 93.35%, which was higher than the previous observation by Ogabe et al. (2009), who found 73.8% hen day egg production in *Ganoderma* mushroom-fed layers. Increased egg production was also reported by the supplementation of different green teas (Uuganbayar et al., 2006) and herbs (Awadein et al., 2010). In contrast to our study, Uuganbayar et al. (2005) reported a decreased in egg weight and egg mass when layers were fed a 0.5% green tea supplemented diet. However, Park et al. (2010) found a linear increase in egg weight and egg mass, but a cubical affect on feed consumption and feed conversion, by dietary *Epimedium koreanum* supplementation. Data from previous studies summarized by Windisch et al. (2008) suggested that the effects of phytogenic products on production performance of poultry vary widely with respect to botanical origin, processing procedure, composition, as well as animal species, animal age, and environmental hygiene.

External and internal quality of eggs

The effects of shiitake mushroom on external and internal quality of eggs are summarized in Table 3. Egg weight, shape index, and yolk color were not affected ($p>0.05$) by the dietary supplementation of shiitake. Shell thickness was decreased ($p<0.05$) in the shiitake 0.25% group and albumen height was increased ($p<0.05$) in the shiitake 0.5% group compared to the control group. The Hague unit was increased ($p<0.05$) in the shiitake groups, with a

higher value evident with 0.5% supplementation. Cho et al. (2010) observed no significant difference in egg shell thickness and Haugh unit measurement, but a deep color was achieved in the fermented spent mushroom substrate group, contrary to our findings. In addition, Mužić et al. (2005) found that feed supplementation with *L. edodes* did not affect egg quality. In a similar study with green tea, Uuganbayar et al. (2005) reported that shell thickness was decreased by the addition of different levels (0.5 to 2.0%) of green tea. However, Park et al. (2010) found that yolk color was linearly increased by *E. koreanum* supplementation. We observed a higher albumen height and Haugh unit in the shiitake groups. The results agree with those of Yamane et al. (1999) and Biswas et al. (2000), who reported that Japanese green tea inclusion in the layer diet improved the Haugh unit score of eggs. On the contrary, Deng et al. (2011) and Uuganbayar et al. (2006) reported no changes in albumen index and Haugh unit in alfalfa extract- and green tea-fed layer eggs. The improvement of Haugh unit score with shiitake feeding was accompanied by a greater albumen height and physical stability of egg albumen, as well as signs of good internal egg quality.

Sensory evaluation of eggs

Supplementation by shiitake did not exert any significant effect ($p>0.05$) on the sensory evaluation of eggs, although acceptability was higher in shiitake 0.25% group (Table 4). Uuganbayar et al. (2005) reported a significant improvement of boiled egg appearance and color at 1.5% level of the green tea supplemented group, but they were decreased at 2.0% level. They also observed that flavor was increased at 1.0% level, but decreased at 2.0% level in the green tea supplemented group. Paguia et al. (2011) noted that *Capsicum frutescens* could have influenced the acceptability score of eggs. Hayat et al. (2010) concluded that 10% flax seed in the layer diet resulted in production of eggs that are acceptable to health- and taste-conscious consumers. However, Leeson et al. (1998) reported that high (>10%) levels of flaxseed used in the layer's diet results in some decrease in overall egg acceptability as assessed by aroma and flavor. They suggested that these effects seem to be accentuated by the use of high levels of vitamin E in the layer's diet.

Table 3. Effect of dietary shiitake mushroom on the external and internal quality of eggs.

Parameter	Control	Shiitake 0.25%	Shiitake 0.50%	SEM
Weight	64.37	60.66	61.11	1.68
Shape index	76.84	76.41	77.74	1.11
Shell thickness (mm)	0.38 ^a	0.32 ^b	0.35 ^{ab}	0.01
Albumen height (cm)	3.80 ^b	5.04 ^{ab}	5.48 ^a	0.40
Yolk color	7.71	7.75	7.50	0.24
Hague unit	52.46 ^b	68.63 ^a	69.75 ^a	4.32

Means with different superscripts in the same row represent significant difference at p<0.05 by Duncan's multiple range tests.

Table 4. Effect of dietary shiitake mushroom on sensory evaluation of boiled eggs.

Parameter	Control	Shiitake 0.25%	Shiitake 0.50%	SEM
Appearance	6.43	6.00	6.25	0.502
Color	6.14	5.14	5.63	0.491
Flavor	5.71	5.57	5.63	0.501
Oily nature	5.57	5.86	5.63	0.429
Acceptability	4.57	5.00	4.85	1.084

Table 5. Effect of dietary shiitake mushroom on the fatty acid composition of egg yolk.

Fatty acid	Control	Shiitake 0.25%	Shiitake 0.50%	SEM
Myristic acid (C14:0)	0.45	0.41	0.43	0.03
Palmitic acid (C16:0)	25.64	25.28	25.05	0.30
Palmitoleic acid (C16:1n7)	4.27 ^a	3.75 ^{ab}	3.56 ^b	0.18
Stearic acid (C18:0)	8.94	9.32	8.79	0.25
Oleic acid (C18:1n9)	42.71	42.69	43.17	0.36
Linoleic acid (C18:2n6)	13.75 ^b	14.19 ^b	14.85 ^a	0.17
α-linolenic acid (C18:3n3)	0.42 ^a	0.39 ^a	0.34 ^b	0.02
Eicosenoic acid (C20:1n9)	0.68	1.09	0.73	0.24
Arachidonic acid (C20:4n6)	2.64	2.49	2.55	0.10
Eicosapentaenoic acid (C20:5n3)	0.09	0.10	0.10	0.01
Docosahexaenoic acid (C22:6n3)	0.41	0.30	0.46	0.05
SFA	35.02	35.00	34.27	0.41
MUFA	47.67	47.53	47.45	0.35
PUFA	17.31 ^b	17.47 ^b	18.29 ^a	0.19
n-3	0.92	0.79	0.89	0.04
n-6	16.39 ^b	16.68 ^b	17.40 ^a	0.17
n-6/n-3	18.03	21.12	19.88	1.17

Means with different superscripts in the same row represent significant difference at p<0.05 by Duncan's multiple range tests. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-3 = total omega-3 fatty acid; n-6 = total omega-6 fatty acid.

Fatty acid composition and cholesterol concentration of egg yolk

Fatty acid composition of egg yolk was evaluated and the findings are summarized in Table 5. Linoleic acid as well as PUFA and total n-6 content were significantly increased (p<0.05) in the shiitake 0.5% group compared to the shiitake 0.25% and control groups. Palmitoleic acid

was increased significantly (p<0.05) in the control group than in the shiitake 0.5% group, whereas α-linolenic acid was decreased (p<0.05) in the shiitake 0.5% group compared to the other groups. Total cholesterol content of egg yolk decreased (p<0.05) significantly in the shiitake 0.5% group compared to the control group (Figure 2).

The content of fatty acids in shiitake mushroom is

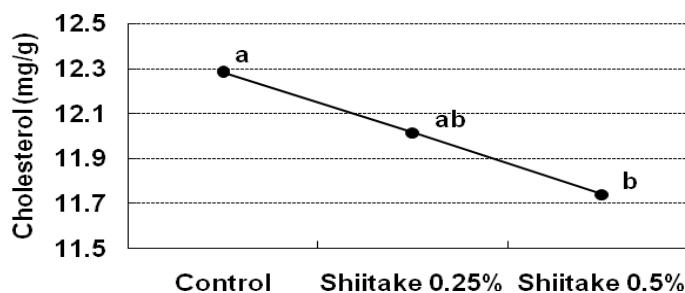


Figure 2. Effect of dietary shiitake mushroom on cholesterol concentration of egg yolk. Means with different superscripts represent significant difference at $p<0.05$ by Duncan's multiple range tests.

3.38% of the total lipids (Mizuno, 1995, 1996), comprising 72.8% linoleic acid, 14.7% palmitic acid, 3.0% oleic acid, 1.6% tetradecenoic acid, 0.9% stearic acid and 0.1% myristic acid (Wasser, 2005). The fatty acid pattern of mushroom affected the fatty acid composition of egg yolk in this study. We observed higher contents of linoleic acid, total n-6 fatty acid, and PUFA in the shiitake 0.5% group, reflecting the fact that shiitake contains 72.8% linoleic acid among the total fatty acids. However, these changes did not affect the n-6/n-3 ratio in egg yolk. The result coincides with the findings of Shimada et al. (2002), who reported that the proportion of linoleic acid in microsomal and plasma phosphatidylcholine was increased when rats were fed *L. edodes* supplemented diets. In addition, Uuganbayar et al. (2005) reported that palmitoleic acid was decreased and linoleic acid was increased in a 1.5% green tea supplemented group.

The use of edible mushrooms has been prescribed in oriental medicine due to their hypocholesterolemic effects (Sun et al., 2007). Similar with our result, various studies (Kim et al., 2001; Yang et al. 2002; Yoon et al., 2011) have confirmed that shiitake mushrooms lower blood serum cholesterol levels in rats and support the use of the mushrooms as a natural cholesterol lowering substances in the diet. In contrast, Mužić et al. (2005) found no effect of *L. edodes* on the cholesterol content of egg yolk. In addition, Daneshmand et al. (2011) noted that oyster mushroom did not affect antibody response against total cholesterol concentration, but decreased the high-density lipoproteins concentration of plasma in broilers. However, Wasser et al. (1997) and Hobbs (2000) reported have shown that mushroom can lower blood pressure and free cholesterol in plasma, as well as accelerate the accumulation of lipids in the liver by removing them from circulation (Kabir and Kimura, 1989). Uuganbayar et al. (2005) also reported that supplementation of 2% green tea powder decreased the cholesterol concentration of egg yolk compared to the antibiotic diet.

Eritadenine or lentinacin (or lentysine) is an adenosine analogue alkaloid or purine alkaloid [(2R,3R)-2,3-

dihydroxy-4-(9-adenyl)-butyric acid], which produces hypocholesterolemic effects in *L. edodes* (Chibata et al., 1969; Tokita et al., 1972). In one study, lentinacin reduced cholesterol levels in rats by 25% after 7 days of oral administration in a dose as low as 0.005% of feed intake (Chibata et al., 1969). The main cause of the hypocholesterolemic action of eritadenine seems to be associated with a modification of the hepatic phospholipid metabolism by inducing a phosphatylethanolamine N-methyltransferase deficiency (Sugiyama et al., 1995). Also, dietary eritadenine can alter the fatty acid and molecular profile of liver and plasma, suppressing the metabolic conversion of linoleic acid into arachidonic acid by decreasing the $\Delta 6$ -desaturase activity (Shimada et al., 2002), which can be affected through transcriptional regulation (Shimada et al., 2003). The microsomal enzyme 3-hydroxy-3-methylglutarylcoenzymeA (HMG-CoA) reductase is the major rate-limiting enzyme in cholesterol biosynthesis, which converts HMG-CoA to mevalonate. Therefore, inhibiting HMG-CoA reductase decreases intracellular cholesterol biosynthesis (Steinberg et al., 1989). Mevinolin is a pharmacological HMG-CoA reductase inhibitor; a high quantity has been found in mushroom fruiting bodies.

The fatty acid pattern of edible mushrooms seems to contribute to reduce serum cholesterol (Barros et al., 2007; Mauger et al., 2003). The presence of trans isomers of unsaturated fatty acids is associated with the strongest effects on raising the serum total cholesterol to high-density lipoprotein ratio, increasing cardiovascular disease risk (Mauger et al., 2003; Sun et al., 2007). The trans isomers of unsaturated fatty acids have not been detected in mushrooms (Barros et al., 2007). The results of some reports suggest that the hypocholesterolemic effects of some fruiting bodies of edible mushroom could be attributed to the dietary fiber supply (Guillamón et al., 2010). The soluble dietary fiber has shown healthy effects on serum lipid levels, reducing total cholesterol and low-density lipoprotein-cholesterol amounts (Erkkilä and Lichtenstein, 2006). The formation of viscous gels from soluble dietary fiber such as glucans might contribute inhibition of cholesterol and triglycerol absorption (Ikeda et al., 1989; Marlett et al., 2002).

Conclusion

The results indicate that the use of shiitake mushroom in the diet of layers has a positive effect on their egg production. Eggs of good quality can result from the addition of shiitake mushroom to the diet. The effective shiitake levels do not detrimentally affect the sensory properties of eggs. Fatty acid composition was improved and egg cholesterol concentration was reduced in the higher level of shiitake mushroom. It is suggested that shiitake could be used in layer diet up to 0.5%.

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REFERENCES

- Adams ML, Sullivan DM, Smith RL, Richter EF (1986). Evaluation of direct saponification method for determination of cholesterol in meats. *J. Assoc. Off. Anal. Chem.*, 69: 844-846.
- Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW, Lee TS (2008). Nutritional analysis of cultivated mushrooms in Bangladesh: *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiol.*, 36: 228-232.
- Alam N, Yoon KN, Lee KR, Shin PG, Cheong JC, Yoo YB, Shim MJ, Lee MW, Lee UY, Lee TS (2010). Antioxidant activities and tyrosinase inhibitory effects of different extracts from *Pleurotus ostreatus* fruiting bodies. *Mycobiol.*, 38: 295-301.
- AOAC (1984). Official Methods of Analysis. 14th edn. Association of Official Analytical Chemists. Washington, DC.
- Awadein NB, Eid YZ, Abd El-Ghany FA (2010). Effect of dietary supplementation with phytoestrogens sources before sexual maturity on productive performance of mandarah hens. *Egypt. Poult. Sci.*, 30: 829-846.
- Barros L, Baptista P, Correia DM, Sá Morais J, Ferreira ICFR (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of portuguese wild edible mushrooms. *J. Agric. Food Chem.*, 55: 4781-4788.
- Biswas MAH, Miyazaki Y, Nomura K, Wakita M (2000). Influences of long-term feeding of Japanese green tea powder on laying performance and egg quality in hens. *Asian-Aust. J. Anim. Sci.*, 13: 980-985.
- Chang ST (1999). World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Singer in China. *Int. J. Med. Mush.*, 1: 387-409.
- Chibata I, Okumura K, Takeyama S, Kotera K (1969). Lentinacin: a new hypcholesterolemic substance in *Lentinus edodes*. *Experientia*, 25: 1237-1238.
- Cho YJ, Choi EJ, Shin PG, Yoo YB, Cho YU, Kim HC, Gal SW, Moon YH, Cho SJ (2010). Effect of spent mushroom (*Pleurotus eryngii*) substrates addition on egg quality in laying hens. Proceedings of the 6th Meeting of East Asia for Mushroom Science, p. 139.
- Dalloul RA, Lillehoj HS, Lee JS, Lee SH, Chung KS (2006). Immunopotentiating effect of a *Fomitella fraxinea*-derived lectin on chicken immunity and resistance to coccidiosis. *Poult. Sci.*, 85: 446-451.
- Daneshmand A, Sadeghi GH, Karimi A, Vaziry A (2011). Effect of oyster mushroom (*Pleurotus ostreatus*) with and without probiotic on growth performance and some blood parameters of male broilers. *Anim. Feed Sci. Technol.*, Published online: 15 September 2011. doi:10.1016/j.anifeedsci.2011.08.008.
- Deng W, Dong XF, Tong JM, Xie TH, Zhang Q (2011). Effects of an aqueous alfalfa extract on production performance, egg quality and lipid metabolism of laying hens. *J. Anim. Physiol. Anim. Nutr.* Published online: 31 Jan 2011. Doi: 10.1111/j.1439-0396.2010.01125.x.
- Erkkilä AT, Lichtenstein AH (2006). Fibre and cardiovascular disease risk: How strong is the evidence? *J. Cardiovasc. Nurs.*, 21: 3-8.
- Giannenas I, Tontis D, Tsalί E, Chronis Ef, Doukas D, Kyriazakis I (2010a). Influence of dietary mushroom *Agaricus bisporus* on intestinal morphology and microflora composition in broiler chickens. *Res. Vet. Sci.*, 89: 78-84.
- Giannenas I, Pappas IS, Mavridis S, Kontopidis G, Skoufos J, Kyriazakis I (2010b). Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet. *Poult. Sci.*, 89: 303-311.
- Giannenas I, Tsalί E, Chronis Ef, Mavridis S, Tontis D, Kyriazakis I (2011). Consumption of *Agaricus bisporus* mushroom affects the performance, intestinal microbiota composition and morphology, and antioxidant status of turkey poulets. *Anim. Feed Sci. Technol.*, 165: 218-229.
- Guillamón E, García-Lafuente A, Lozano M, D'Arrigo M, Rostagno MA, Villares A, Martínez JA (2010). Edible mushrooms: role in the prevention of cardiovascular diseases. *Fitoterapia*, 81: 715-723.
- Guo FC, Williams BA, Kwakkel RP, Li HS, Li XP, Luo JY (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poult. Sci.*, 83: 175-182.
- Guo FC, Williams BA, Kwakkel RP, Verstragen MWA (2003). In vitro fermentation characteristics of two mushrooms, an herb, and their polysaccharide fractions, using chicken cecal contents as inoculum. *Poult. Sci.*, 82: 608-615.
- Hayat Z, Cherian G, Pasha TN, Khattak FM, Jabbar MA (2010). Sensory evaluation and consumer acceptance of eggs from hens fed flax seed and 2 different antioxidants. *Poult. Sci.*, 89: 2293-2298.
- Hearst R, Nelson D, McCollum G, Milar BC, Maeda Y, Goldsmith CE, Rooney PJ, Loughrey A, Rao JR, Moore JE (2009). An examination of antibacterial and antifungal properties of constituents of Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. *Complement. Therap. Clin. Pract.*, 15: 5-7.
- Hobbs CH (2000). Medicinal value of *Lentinus edodes* (Berk.) Sing. A literature review. *Int. J. Med. Mush.*, 2: 287-302.
- Hu TJ, Liang JL, Cheng FS (1998). The use of 8301 polysaccharide as an adjuvant in coccidial infected chickens. *J. Grass Livest.*, 6: 5-7.
- Ikeda I, Tomari Y, Sugano M. (1989). Interrelated effects of dietary fiber and fat on lymphatic cholesterol and triglyceride absorption in rats. *J. Nutr.*, 119: 1383-1387.
- Kabir Y, Kimura S. (1989). Dietary mushrooms reduce blood pressure in spontaneously hypertensive rats (SHR). *J. Nutr. Sci. Vitaminol.*, 35: 91-94.
- Kim BK, Shin GG, Jeon BS, Cha JY (2001). Cholesterol lowering effect of mushrooms powder in hyperlipidemic rats. *J. Korean Soc. Food Sci. Nutr.*, 30: 510-515.
- Lee SH, Lillehoj HS, Jang SI, Kim DK, Ionescu C, Bravo D (2010). Effect of dietary *curcuma*, *capsicum*, and *lentinus*, on enhancing local immunity against *Eimeria acervulina* infection. *J. Poult. Sci.*, 47: 89-95.
- Leeson S, Caston L, MacLaurin T (1998). Organoleptic evaluation of eggs produced by laying hens fed diets containing graded levels of flaxseed and vitamin E. *Poult. Sci.*, 77: 1436-1440.
- Liu YJ, Li QZ, Hao YH (1999). Effects of lentinan and astragaloside on IL-2 inductive activity and lymphocyte proliferative reaction in chicks infected with Marek's disease. *J. China Vet. Med.*, 25: 3-5.
- Marlett JA, McBurney MI, Slavin JL (2002). Position of the American Dietetic Association: Health implications of dietary fiber. *J. Am. Diet Assoc.*, 102: 993-1000.
- Mauger JF, Lichtenstein AH, Ausman LM, Jalbert SM, Jauhainen M, Ehnholm C (2003). *Am. J. Clin. Nutr.*, 78: 370-375.
- Mizuno T (1995). Shiitake, *Lentinus edodes*: functional properties for medicinal and food purposes. *Food Rev. Int.*, 11: 7-21.
- Mizuno TA (1996). Development of antitumor polysaccharides from mushroom fungi. *Food Food Ingred. Jpn. J.*, 167: 69-87.
- Mužić S, Janjević Z, Mesarić M, Svalina K (2005). Cholesterol content and quality of eggs from hens fed feed mixture supplemented with *Lentinus edodes* mushroom. *Stockbreeding*, 59: 271-279.
- NRC (1994). Nutrient Requirements of Poultry. 8th rev. edn. National Academic Press. Washington, DC.
- NRLSI (2002). Food Composition Table. 6th rev. ed. National Rural Living Science Institute. Rural Development Administration, South Korea.
- Obge AO, Ditse U, Echeonwu I, Ajodoh K, Atawodi SE, Abdu PA (2009). Potential of a wild medicinal mushroom, *Ganoderma* sp., as feed supplement in chicken diet: effect on performance and health of pullets. *Int. J. Poult. Sci.*, 8: 1052-1057.
- Paguia HM, Magpantay DO, Paguia RQ (2011). Laying performance of chicken (*Gallus domesticus* L.) fed diets supplemented with *Capsicum frutescens*. *Int. Confer. Asia Agric. Anim.*, 13: 44-49.
- Pang FH, Xie MQ, Ling HH (2000). The investigation of immunodulators

- tested for the results on the control of a coccidial infection. Chin. J. Vet. Parasitol., 8: 1-3.
- Park KM, Jin YH, Lee KT, Lee WI, Nam SW, Han YK (2010) Influence of *Epimedium koreanum* on the performance of laying hens, egg quality, and fat soluble vitamin and cholesterol contents in the yolk. J. Med. Plants Res., 4: 1971-1976.
- SAS (2003). SAS User's Guide, Version 9.1 edn. SAS Institute, Cary, NC.
- Shimada Y, Morita T, Sugiyama K (2002). Effects of *Lentinus edodes* on fatty acid and molecular species profiles of phosphatidylcholine in rats fed different levels of corn oil. Biosci. Biotechnol. Biochem., 66: 1759-1763.
- Shimada Y, Yamakawa A, Morita T, Sugiyama K (2003). Effects of dietary eritadenine on the liver microsomal delta 6-desaturase activity and its mRNA in rats. Biosci. Biotechnol. Biochem., 67: 1258-1266.
- Stamets P (2000). Growing Gourmet and Medicinal Mushrooms, 3rd edn. Ten Speed Press. CA, USA.
- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL (1989). Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N. Eng. J. Med., 320: 915-924.
- Sugiyama K, Akachi T, Yamakawa A (1995). Eritadenine-induced alteration of hepatic phospholipid metabolism in relation to its hypocholesterolemic action in rats. J. Nutr. Biochem., 6: 80-87.
- Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ, Rexrode KM, Willett WC, Hu FB (2007). A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. Circulation, 115: 1858-1865.
- Tokita F, Shibukawa N, Yasumoto T, Kaneda T (1972). Isolation and chemical structure of the plasma-cholesterol reducing substances from shiitake mushroom. Mushr. Sci., 8: 783-788.
- Uuganbayar D, Bae IH, Choi KS, Shin IS, Firman JD, Yang CJ (2005). Effects of green tea powder on laying performance and egg quality in laying hens. Asian-Aust. J. Anim. Sci., 18: 1769-1774.
- Uuganbayar D, Shin IS, Yang CJ (2006). Comparative performance of hens fed diets containing Korean, Japanese and Chinese green tea. Asian-Aust. J. Anim. Sci., 19: 1190-1196.
- Wasser SP (2005). Shiitake (*Lentinus edodes*). In: Encyclopedia of Dietary Supplements DOI: 10.1081/E-EDS-120024880. Marcel Dekker: New York, pp. 653-664.
- Wasser SP, Weis AL (1997). Medicinal Mushrooms. *Lentinus edodes* (Berk.) Singer. Nevo E. (eds.). Peledfus Publ. House: Haifa, Israel, p. 95.
- Willis WL, Isikhuemhen OS, Ibrahim S (2007). Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult. Sci., 86: 1856-1860.
- Windisch W, Schedle K, Plitzner C, Kroismayr A (2008). Use of phytopreparations as feed additives for swine and poultry. J. Anim. Sci., 86: E140-E148.
- Xu C, Yan ZH, Hong ZJ, Jing G (2008). The pharmacological effect of polysaccharides from *Lentinus edodes* on the oxidative status and expression of VCAM-1mRNA of thoracic aorta endothelial cell in high-fat-diet rats. Carbohydr. Polym., 74: 445-450.
- Yamane T, Goto H, Takahashi D, Takeda H, Otowaki K, Tsuchida T (1999). Effects of hot water extracts of tea on performance of laying hens. J. Poult. Sci., 36: 31-37.
- Yang BK, Kim DH, Jeong SC, Das S, Choi YS, Shin JS, Lee SC, Song CH (2002). Hypoglycemic effect of a *Lentinus edodes* exopolymer produced from a submerged mycelia culture. Biosci. Biotechnol. Biochem., 66: 937-942.
- Yoon KN, Alam N, Lee JS, Cho HJ, Kim HY, Shim MJ, Lee MW, Lee TS (2011). Antihyperlipidemic effect of dietary *Lentinus edodes* on plasma, feces and hepatic tissues in hypercholesterolemic rats. Mycobiol., 39: 96-102.
- Yu JG, Zhu LY (2000). The use of Astragalus polysaccharide against infectious bursa disease in chickens. J. Trad. Chin. Vet. Med., 6: 3-4.