Effect of Feeding Common (*Agaricus bisporus*) and Oyster (*Pleurotus ostreatus*) Mushrooms on Performance, Intestinal Microbiology and Morphology of Female Japanese Quails (*Coturnix coturnix japonica*)

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Abstract. *This research was performed to study the performance, intestinal microbiology and morphology of Japanese quails supplemented with two types of edible mushrooms including common (Agaricus bisporus) and oyster (Pleurotus ostreatus) powders. A total of 420 twenty-one day old female quail chicks were randomly allocated to seven experimental treatments. Each treatment consisted of 3 replicates of 20 birds. The birds within the control group were given the basal diet for the respective growth stage. The other six groups were fed experimental diets based on the basal diets containing 0.5%, 1%, and 2% of dried common or oyster mushroom powders. Birds were given free access to feed and water during the 84 days of the experimental period. During the experiment, performance characteristics were measured. Count of coli-form bacteria in the gut and intestinal morphological characteristics were studied at the age of 84 days. Egg weight, feed intake, egg mass and feed conversion ratio in 84 days were not significantly influenced by the supplementation of mushrooms. Bifidobacteria and Lactobacilli populations were significantly increased ($P < 0.05$) by 2% of mushrooms compared with the control. Total counts (Aerobes) and Escherichia coli were significantly decreased ($P < 0.05$) by 2% of mushrooms compared with the control. Crypt depth and papillae height in parts of 10%, 50% and 70% of the gut were positively influenced by the supplementation of mushroom ($P < 0.05$). Therefore, it seems that mushrooms could increase useful microflora and prove helpful in the fight against pathogenic organisms colonizing in the quail chicks gut.*

Introduction

Due to banning the use of antibiotics in animal nutrition, some additives such as herbal materials, probiotics, prebiotics and organic acids can be used instead of antibiotics in poultry diets (Fouladi et al., 2018). Recently, natural materials such as medicinal plants, mushrooms and herbs have been investigated. Wang et al. (1998) reported antimicrobial activities, immune enhancement and stress reduction in farm animals given natural medicinal products from fungi and herbs. Asadi Dizaji et al. (2014) reported that 2% of mushrooms (*Agaricus. bisporus*) in the diet positively affect performance parameters and some internal organs of quails. Mushrooms have long been appreciated as an important source of bioactive compounds of medicinal value (Breene, 1990). Use of 2% of mushrooms (*Pleurotus. ostreatus*) in the diet positively affects blood biochemical characteristics of quails (Asadi Dizaji et al., 2017). 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 & Buswell, 1996). Mushrooms may have a wide range of activities (Guo et al., 2003). For particular interest, extracts derived from various mushrooms 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 & Lillehoj, 2006; Dalloul et al., 2006). Guo et al. (2004a) reported that the population of *bifidobacteria* and *lactobacilli* were significantly increased with the addition of a shiitake mushroom extract (*Lentinusedodes*). It was found that the immunologically active components in medical mushrooms and plants may include polysaccharides, glycosides, alkaloids, volatile oils, and organic acids (Yang & Feng, 1998). Anti-microbial activity including anti-bacterial, anti-parasitic, anti-fungal and anti-viral agents is a widespread therapeutic effect reported in mushrooms (Wasser & Weis 1999; Kettering et al., 2005).

Intestinal microflora play an important role in the health status of host animals. Intestinal microflora constitute a dynamic ecosystem that is essential to the health of the chicken. In general, intestinal bacteria may be divided into species that exert either harmful or beneficial effects on host health (Macfarlane & Cummings, 1991). Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens (Rolfe, 1991). The composition and activity of intestinal macrobiotics can be altered by diet composition and dietary manipulations such as

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the use of feed additives and antibiotics (Coates et al., 1981; Jensen, 1993). Guo et al. (2004b) investigated several mushrooms and herb polysaccharides, as alternatives for an antibiotic, on growth performance of broilers, and found *Lentinula edodes* to be a significant growth promoter in broilers. Similarly, Willis et al. (2007) noted enhanced beneficial *bifidobacteria* production from a mushroom extract (*Lentinulaedodes*) given to broiler chickens. Mahfuz et al. (2020) also stated that supplementing *Agaricus bisporus* and *Pleurotus ostreatus* in broiler diets led to an increase *bifidobacteria* and *lactobacilli* in the caecum and the ileum.Therefore, the aim of this study was to investigate the effects of supplementation of different levels of dried powder of common and oyster mushrooms on the performance, intestinal morphology and microflora composition in female Japanese quails.

Materials and Methods

Birds and experimental design

A total of 420 twenty-one day old female quail chicks were randomly allocated to seven experimental treatments. Each treatment consisted of 3 replicates of 20 birds. The birds within the control group were given the basal diet for the respective growth stage. The other six groups were fed experimental diets based on the basal diets containing 0.5%, 1%, and 2% of dried common (*Agaricus. bisporus*) or oyster (*Pleurotus. ostreatus*) mushroom powders. Birds were given free access to feed and water during the 84 days of the experimental period. Each replicate was housed in separate stainless floor pens under controlled temperature and light conditions. Each pen was 100 × 100 cm. The lighting cycle was 23 h/day maintained at all growth times. The diets were formulated to meet the nutrient requirements of poultry as recommended by the National Research Council (NRC, 1994). Table 1 presents the ingredients and the composition of the basal diets fed in a mash form.

Preparation of mushroom diet

Fresh fruiting bodies of mushrooms were obtained from mushroom producers. The mushroom powders were obtained from oven dried mushrooms. The whole mushrooms were dried out at 60°C for 12 h

Table 1. Composition of experimental diets of female Japanese quails with or without mushroom powder (%)

Ingredients	Control	0.5% *Agaricus bisporus*	1% *Agaricus bisporus*	2% *Agaricus bisporus*	0.5% *Pleurotus ostreatus*	1% *Pleurotus ostreatus*	2% *Pleurotus ostreatus*
Yellow corn	53.31	52.00	52.00	52.08	52.00	52.00	52.18
Soybean meal	39.69	39.00	38.75	39.50	39.00	38.78	39.60
Corn gluten meal	3.07	4.20	4.00	2.50	4.20	4.00	2.50
Vegetable oil	1.00	1.38	1.33	0.98	1.38	1.30	0. 80
Oyster mushroom	-	0.50	1.00	2.00	-	-	-
Commom mushroom	-	-	-	-	0.50	1.00	2.00
Oyster shell	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Di calcium phosphate	0.77	0.77	0.77	0.77	0.77	0.77	0.77
L-lysine	0.06	0.06	0.06	0.06	0.06	0.06	0.06
DL-methionine	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Mineral-vitamin premix*	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated analysis							
ME (Kcal/Kg)	2900	2900	2900	2900	2900	2900	2900
CP (%)	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Calcium (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Phosphor (%)	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Sodium (%)	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Lysine (%)	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Methionine + Cycteine (%)	0.89	0.89	0.89	0.89	0.89	0.89	0.89

*Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; Biotin, 0.1 mg; Choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu,12 mg; I,1 mg;Se, 0.2 mg, and adequate antioxidant.

and were added to the experimental diets of chicks after carefully grinding. After drying, fruiting bodies were milled to a powder approximately less than 1 mm in particle size using a cyclotec grinder (Tecator, Hoganas, Sweden). The chemical composition of *Pleurotuso streatus* and *Agaricus Bisporus* powders was determined by the standard AOAC (Association of Official Analytical Chemists) methods outlined by Conniff (1995), as shown in Table 2.

Data Collection

At day 84 of age, the feed consumption and egg weight of each pen were used to calculate the feed conversion ratio (FCR) and some laying performance values (egg production percentage and egg mass).

At day 84 of age, two birds from each pen picked out randomly were killed by cervical dislocation in a germ-free isolation chamber sterilized by ultraviolet radiation (Fallah et al., 2016). The caecum was then removed from each bird, and the fresh excreta of the caecum were gently squeezed and carefully collected in sterilized 25-mL tubes, each tube containing pooled excreta for 5 birds (per pen). Three grams of fresh caecal contents were diluted with 10 mL of distilled water and vortexed before pH 6 and viscosity 7 were measured.

One gram of a wet sample was diluted with 10 mL of sterilized distilled water, of which 1 mL was transferred into 9 mL of sterilized distilled water. The samples were serially diluted from 10^{-1} to 10^{-7}. One-tenth milliliter of each diluted sample was plated on the appropriate medium for enumeration of microbial populations. Bacterial counts were performed using the appropriate dilution and plate culture techniques under aerobic or anaerobic conditions according to Barnes and Impey (1970). The results were expressed as colony forming unit's $\log_{10}$ per gram of fresh sample. The bacterial groups and species determined included total aerobes (nutrient blood agar), *Lactobacilli* (citromalic acid-enriched MRS agar), *Escherichia coli* (MacConkey agar), and *bifidobacteria* (*bifidobacterium* agar composed of tomato juice, 400 mL; dissoluble amylum, 0.5 g; peptone, 15 g; yeast extract, 2 g; glucose, 20 g; sodium chloride, 5 g; Tween-80, 1 mL, 5% cysteine, 0.5 mL; liver extract, 80 mL; agar powder, 20 g; and distilled water, 520 mL; pH = 7.0, at 37°C for 72 h).

Collection of intestinal tissue samples

At day 84 of age, two birds per replicate were randomly chosen, based on the average weight of the group and slaughtered, and the digestive tract was carefully excised. After removing the intestinal contents, approximately 5 cm lengths on 10%, 50% and 90% of the jejunum (the mid point of the jejunum) were removed for gut morphological measurements. The intestinal samples were immersed in formalin, before fixation in Bouin's solution and paraffin embedding. The samples were then transferred into 70% ethanol after 24 h.

Histology of the jejunum

Histological examinations were carried out according to the method of Iji et al. (2001).

Statistical analysis

Data were statistically analyzed using the general linear model (GLM) procedure of SAS (2005). The test of significance for the differences between the means of each classification was done by the Duncan multiple range test (Duncan, 1955).

Results and Discussion

Performance parameters

Egg weight, feed intake, egg mass and feed conversion ratio at 84 days of age were not significantly influenced by the supplementation of mushrooms at day 84 (Table 3). The results of the current study were in line with the findings of Cho et al. (2010) who conducted an experiment with a 5% to 15% fermented spent mushroom substrate and found no effects on egg production, egg weight, egg mass, and feed conversion ratio. Uuganbayar et al. (2005) reported a decrease in egg weight and egg mass when layers were fed a 0.5% green tea supplemented diet.

Increased egg production was also reported by the supplementation of herbs (Awadein et al., 2010). However, Park et al. (2010) found a linear increase in egg weight and egg mass. 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.

Table 2. Proximate analysis of common and oyster mushroom powders

Component	Oyster mushroom *(Pleurotus ostreatus)*	Common mushroom *(Agaricus bisporus)*
ME (Kcal/kg)	1898	1843
Moisture (%)	7.01	15.11
Ash (%)	6.55	12.18
Ether extract (%)	2.3	2.5
Crude protein (%)	21.86	23.21
NFE (%)	62.28	47

Morphometric analysis of the jejunum

Crypt depth and villus height of female quails given the common (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) mushrooms at the level of 2% were significantly higher than from the quails in the control group (Table 4). In other trial in broilers, Giannenas et al. (2010) reported that the use of *Agaricus bisporus* mushroom did not produce any significant effect on villus height and crypt depth. But other trials in turkey poults showed a villi height increased by *Agaricus bisporus* mushroom supplementation in all the intestinal section; however, the use of mushroom did not have any effect on crypt depth (Giannenas et al., 2011). An addition of probiotics in broiler diets caused an increase in villus height in the ileum (Nava et al., 2001).

Intestinal microflora composition

The effect of various levels of mushrooms on total counts (aerobes), *Bifidobacteria*, *Lactobacilli* and *Escherichia coli* populations is shown in Table 5.

Total counts of microflora (aerobes) and *Escherichia coli* populations from female quails given the mushroom common (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) 2% diet were significantly lower than the respective population values for quails in the control group. *Bifidobacterial* populations from female quails given the 2% common (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) mushroom diets (treatments 4 and 6) were significantly higher than the respective population values for quails in the control group (treatment 1); and for treatments 3 (1% *Agaricus bisporus*), 5 and 6 (0.5% and 1% *Pleurotus ostreatus*) were also significantly varied ($P < 0.05$), but not in treatment 2 (0.5% *Agaricus bisporus*).

Bifidobacteria are major species components of the chicken gut microflora (Mead, 1987) that may quantitatively and qualitatively influence the intesti-nal microflora. The results of the current study were in line with the findings of Mahfuz et al. (2020) who stated that supplementing *Agaricus bisporus* and *Pleurotus ostreatus* in broiler diets led to an increase in *bifidobacteria* and *lactobacilli* in the intestine.

Lactobacilli populations from female quails given the 2% common (*Agaricus bisporus*) and

Table 3. Effects of mushrooms on egg weight, feed consumption, egg production, egg mass and feed conversion ratio of female Japanese quails (day 84)

Treatments	Egg weight (g)	Egg production percentage	Feed consumption (g/day/per bird)	Egg mass (g)	Feed conversion ratio
Control	10.75	76.70	27.13	8.24	3.29
Agaricus bisporus 0.5%	10.43	76.14	27.00	7.94	3.41
Agaricus bisporus 1%	10.79	78.74	27.00	8.48	3.18
Agaricus bisporus 2%	10.87	76.40	27.07	8.28	3.26
Pleurotus ostreatus 0.5%	10.86	78.33	28.40	8.49	3.34
Pleurotus ostreatus 1%	10.88	68.33	26.78	8.30	3.23
Pleurotus ostreatus 2%	10.83	78.55	27.11	8.51	3.19
SEM	0.27	2.37	0.57	0.29	0.14
P value	0.9128	0. 1954	0.5338	0.8182	0.9008

Table 4. Effects of dietary supplementation of mushrooms on villus height and crypt depth in different parts of the jejunum at day 84 of age in female Japanese quails (μm)

Treatments	Crypt depth (10% jejunum)	Crypt depth (50% jejunum)	Crypt depth (90% jejunum)	Villus height (10% jejunum)	Villus height (50% jejunum)	Villus height (90% jejunum)
Control	64.30^{cd}	61.37^{c}	54.56^{d}	280.10^{c}	226.42^{b}	135.26^{d}
Agaricus bisporus 0.5%	65.31^{cd}	61.93^{c}	54.80^{d}	280.63^{bc}	223.87^{d}	137.07^{cd}
Agaricus bisporus 1%	64.50^{b}	63.16^{b}	55.51^{abc}	281.74^{ab}	225.60^{bc}	137.81^{cd}
Agaricus bisporus 2%	67.13^{ab}	63.93^{ab}	57.20^{b}	283.27^{a}	228.33^{a}	140.85^{ab}
Pleurotus ostreatus 0.5%	65.17^{d}	62.01^{c}	54.91^{d}	281.21^{bc}	224.50^{cd}	137.48^{cd}
Pleurotus ostreatus 1%	65.54^{bc}	64.14^{a}	56.41^{bc}	281.19^{bc}	225.67^{bc}	138.48^{bc}
Pleurotus ostreatus 2%	69.83^{a}	64.74^{a}	58.41^{a}	283.61^{a}	228.33^{a}	141.70^{a}
SEM	0.68	0.26	0.34	0.48	0.48	0.81
P value	0.0008	0.0001	0.0001	0.0010	0.0002	0.0009

Means with different superscripts in the same column represent significant difference at $P < 0.05$.

Table 5. Effects of dietary supplementation of mushrooms on intestinal microbiology of female Japanese quails at day 84 of age (log_{10} CFU/g)

Treatments	Total counts (Aerobes)	*Bifidobacteria*	*Lactobacilli*	*Escherichia coli*
Control	5.894^{a}	8.559^{cd}	7.552^{c}	6.790^{a}
Agaricus bisporus 0.5%	5.759^{abc}	8.527^{d}	7.707^{c}	6.670^{bc}
Agaricus bisporus 1%	5.647^{cd}	8.765^{bc}	7.930^{bc}	6.572^{d}
Agaricus bisporus 2%	5.500^{d}	9.217^{a}	8.492^{a}	6.140^{e}
Pleurotus ostreatus 0.5%	5.838^{ab}	8.772^{bc}	7.585^{c}	6.736^{ab}
Pleurotus ostreatus 1%	5.690^{bc}	8.939^{b}	7.887^{bc}	6.587^{cd}
Pleurotus ostreatus 2%	5.585^{cd}	9.155^{a}	8.293^{ab}	6.192^{e}
SEM	0.0535	0.0636	0.1268	0.0276
P value	0.0116	0.0006	0.0076	0.0001

Means with different superscripts in the same column represent a significant difference at $P < 0.05$.

oyster (*Pleurotus ostreatus*) mushroom diets were significantly higher than the respective population values for quails in the control group.

Quails given the 2% common (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) mushroom diets had the lowest intestinal *Escherichia coli* population count when compared with population values for quails in the control group. Guo et al. (2004b) indicated that mushroom and herb polysaccharide extracts stimulated beneficial bacteria (*bifidobacteria* and *lactobacilli*) while reducing the number of harmful bacteria. Willis et al. (2009) reported an increase in *bifidobacterial* populations and a reduction in *Salmonella* from broilers given the mushroom extract. The mechanism by which mushrooms stimulate *bifidobacterial* growth and survival in the gut of chickens is not known. However, it is sufficient to assume that the shiitake mushroom extract, which is known to be rich in β-glucans (polysaccharides), has components that may either act as quality and specific nutritional factors or create a buffered physical chemical environment in which *bifidobacteria* can survive and multiply compared with the levels in control samples.

Conclusion

Our results clearly demonstrate that tested mushrooms have greater abilities to decrease the fecal *Escherichia coli* population count. Also, the results of this study showed that *Bifidobacterial* and *Lactobacilli* population counts increased by supplementing diets with mushrooms. Mushroom powders exhibit potential benefits because they seem to stimulate health-enhancing *bifidobacteria*, thereby competitively reducing the *Escherichia coli* population. Mushrooms also cause an increase in crypt depth and villus height in male and female quails. This study shows that mushrooms could be helpful in the fight against pathogenic organisms colonizing and increase villus height of quail chicks. In an overall conclusion, the mushrooms could be a beneficial supplement in Japanese quail diet.

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Reference

1. Asadi-Dizaji, A., Aghdam Shahryar, H., Shaddel-Tili, A., Maheri-Sis, N., Ghiasi-Ghalehkand, J. Effect of common mushroom (*Agaricus bisporus*) Levels on Growth Performance and Carcass yields of Japanese quails (*Coturnix coturnix Japonica*). Bull. Env. Pharmacol. Life Sci. 2014. 3 (7), 01-05.
2. Asadi-Dizaji, A., Aghdam Shahryar, H., Maheri Sis, N. Effect of level of oyster mushroom (*Pleurotus. ostreatus*) on performance and blood biochemical characteristics in Japanese quails (*Coturnix Coturnix*). Iran. J. Appl. Anim Sci. 2017. 7(4), 185-188.
3. Awadein, N.B., Eid, Y.Z., AbdelGhany, F.A. Effect of dietary-supplementation withphytoestrogens sources before sexual maturityon productive performance of mandarah hens. Egypt. Poult. Sci. J. 2010. 30, 829-846.
4. Barnes E.M., Impey C.S. The isolation and properties of the predomiant anaerobic bacteria in the caeca of chickens and turkeys.Br. Poult. Sci.1970.11, 467–481.
5. Breene W.M. Nutritional and medicinal value of specialty mushrooms. J. Food Prot.1990. 53, 883–894.
6. ChangS.T., BuswellJ.A. Mushroom nutriceuticals. World J. Microbiol. Biotechnol. 1996. 12, 473–476.
7. Cho Y.J., Choi E.J., Shin P.G., Yoo Y.B., Cho Y.U., Kim H.C., Gal S.W., Moon Y.H., Cho S.J. Effect of spent mushroom (Pleurotuseryngii) substrates addition on egg quality in laying hens. Proceedings of the6th Meeting of East Asia for Mushroom Science, p. 139.2010.
8. Coates M. E., Cole C. B., Fuller R., Houghton S. B., Yokota H. The gut microflora and the uptake of glucose from the small intestine of the chick. Br. Poult. Sci. 1981. 22, 289–294.
9. Conniff P. Official methods of analysis. 16th ed. Arlington (Va): Association of Official Chemists. 1995.
10. Dalloul R. A., Lillehoj H. S. Poultry coccidiosis. Recent advancements in control measures and vaccine development. Expert Rev. Vaccines. 2006. 5, 143–163.
11. Dalloul R. A., Lillehoj H. S., Lee J. S., Lee S. H., Chung K. S. Immunopotentiating effect of a Fomitellafraxinea-derived lectin on chicken immunity and resistance to coccidiosis. Poult. Sci. 2006. 85, 446–451.

12. Duncan D B. Multiple range and multiple F tests. Biometrics. 1955.11, 1-42.
13. Fallah F., Ebrahimnezhad Y., Maheri-Sis N., Ghasemi-Sadabadi M. The effect of different levels of diet total volatile nitrogen on performance, carcass characteristics and meat total volatile nitrogen in broiler chickens. Arch. Anim. Breed. 2016. 59, 191–199.
14. Fouladi P., Ebrahimnezhad Y., Aghdam Shahryar H., Maheri Sis N., Ahmadzadeh A. Effects of organic acids supplement on performance, egg traits, blood serum biochemical parameters and gut microflora in female Japanese quail (*Coturnix coturnix japonica*). Braz. J. Poult. Sci. 2018. 20(1), 133-144.
15. Giannenas I., Tontis D., Tsaloe E., Chronis E. F., Doukas D., Kyriazakis I. Influence of dietary mushroom *Agaricus bisporus* on intestinal morphology and microflora composition in broiler chickens. Res. Vet. Sci. 2010.89,78-84.
16. Giannenas I., Tsalie E., Chronis E.F., Mavridis S., Tontis D., Kyriazakis I. Consumption of *Agaricus bisporus* mushroom affects the performance, intestinal microbiota composition and morphology, and antioxidant status of turkey poults. Anim. Feed Sci. Technol. 2011. 165, 218–229.
17. Guo, F.C., Savelkoul, H.F.J., Kwakkel, R.P., Williams, B.A., Verstegen, M.W.A. Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. Worlds Poult. Sci. J. 2003. 59, 427–440.
18. Guo F.C., Kwakkel R.P., Williams B.A., Parmentier H.K., Li W.K., Yang Z.Q., VerstegenM. W. A. Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of Eimeriatenella-infected chickens. Poult.Sci. 2004a. 83, 1124-1132.
19. Guo F.C., Williams, B.A., Kwakkel R.P., Li H.S., Li X.P., Luo J.Y., Li W.K., Verstegen M.W.A. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci. J. 2004b. 83, 175–182.
20. Iji P.A., Hughes R.J., Choct M., Tivey D.R. Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. Asian-Australasian. J. Anim. Sci. 2001. 14, 54-60.
21. Jensen B.B. The possibility of manipulating the microbial activity in the digestive tract of monogastric animals. Page 20 in 44th Annual Meeting. European Association for Animal Production, A° rhus, Denmark.1993.
22. Kettering M., Valdivia C., Sterner O., Anke H., Thines E. Seven novel diterpenoids from producing organism, fermentation, isolation and biological activities. J. Antibiotics. 2005.58, 390-396.
23. Macfarlane G.T., Cummings J.H. The colonic flora, fermentation and large bowel digestive function. Pages 51-92 in The Large Intestine: Physiology, Pathophysiology and Disease. Phillips S F, Pemberton J H and Shorter R G, ed. Raven Press, New York..1991.
24. Mahfuz S.U., Long S.F., Piao X.S. Role of medicinal mushroom on growth performance and physiological responses in broiler chicken. Worlds Poult. Sci. J. 76 (1), 1-18.
25. Mead G.C. Microbes of the avian caecum: Types present and substrates utilized. J. Exp. Zool. 1987.3, 48-54.
26. Nava G., Ledesma N., Priego A., Priego C., Sutton L., Tellez G. Effect of Aspergillus sp.and bacterial phytase containing broiler diets on body weight, gastrointestinal transit time and the crop and cecom pH of the broiler chick. Poult. Sci. 2001.80, 13-23.
27. NRC (National Research Council). Nutrient requirements of poultry. Ninth revised edition, National Academy Press, Washington DC, USA. 50-90 p. 1994.
28. Park K.M., Jin Y.H., Lee K.T., Lee W.I., Nam S.W., Han Y.K. Influence of Epimedium koreanum on the performance of laying hens, eggquality, and fat soluble vitamin and cholesterol contents in the yolk. J. Med. Plants Res.2010.4, 1971-1976.
29. Rolfe R.D. population dynamics of the intestinal tract. Pages 59–75 in Colonization Control of Human Enteropathogens in Poultry. L. C. Blankenship, ed. Academic Press, San Diego, CA. 1991.
30. SAS Institute. SAS Users Guide Statistics. SAS Institute Inc., Cary, NC. 2005.
31. Uuganbayar D, Bae IH, Choi KS, Shin IS, Firman JD and Yang CJ.Effects of green tea powder on laying performance and egg quality in laying hens. Asian-Australasian. J. Anim. Sci. 2005.18, 1769-1774.
32. Wasser S. P.,WeisA. L.Therapeutic properties of substances occurring in higher Basidiomycete mushrooms: A modern perspective. Crit. Rev. Immunol. 1999.19, 65-96.
33. Wang R.J., Li D.F., Bourne S. Can 2000 years of herbal medicine history help us solve probelms in the year 2000? Pages 273–291 in Biotechnology in the Feed Industry. Proc. Alltech's 14th Annu.Symp.University Pres, Nottingham, UK.1998.
34. Willis W., Isikhuemhen O.S., Ibrahim S.A. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult.Sci. 2007. 86, 1856–1860.
35. Willis W.L, King K., Iskhuemhen O.S., Ibrahim S.A. Administration of mushroom extract to broiler chickens for bifidobacteria enhancement and Salmonella reduction. J. Appl. Poult. Res. 2009. 18, 658–664.
36. Yang Y., Feng W.S. Brochures of isolation and extraction of chemical components from herbs China. Chinese Herbal Medicine Press, Beijing, China.1998.

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