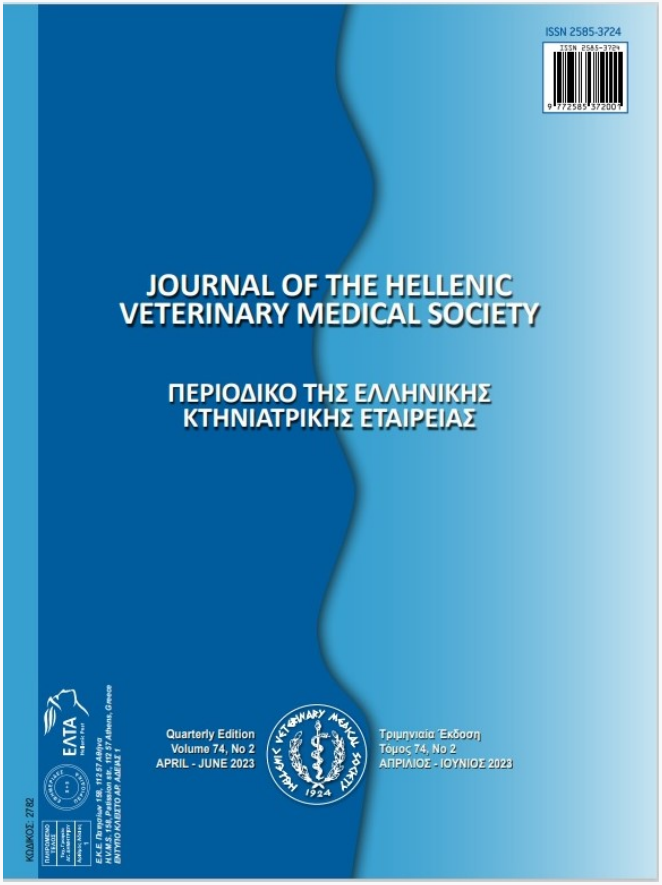
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Research article
Ερευνητικό άρθρο

Effects of Shiitake mushroom (*Lentinus edodes*) supplementation into quail diets on performance, blood serum parameters and intestine microbial populations

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ABSTRACT: This study was conducted to investigate the effects of Shiitake mushroom (*Lentinus edodes*) supplementation into quail diets on live weight, live weight gain, feed consumption, feed conversion ratio, blood serum biochemical parameters (total cholesterol, total protein, triglyceride, glucose) and intestine bacteria populations. A total of 220 Japanese quail (*Coturnix coturnix Japonica*) were used in present experiments. Quail were separated into 4 groups (one control and three treatment groups). Control group was fed with a basic ration and treatments groups were fed with 0.5, 1 and 2% shiitake mushroom-supplemented diets. Shiitake mushroom supplementations significantly influenced live weights ($P<0.01$), live weight gains ($P<0.05$), feed conversion ratios ($P<0.05$) and blood serum glucose levels ($P<0.01$).Differences in feed consumptions, blood serum total protein, total cholesterol and triglyceride levels and intestine bacteria populations of the treatment groups were not found to be significant ($P>0.05$). A linear decrease was seen in live weights with increasing Shiitake mushroom supplementation ratios into quail diets. Similarly, live weight gains linearly decreased with increasing doses of Shiitake mushroom. Therefore, feed conversion ratios were negatively influenced by Shiitake mushroom supplementation and feed consumptions of quail were not affected. Present findings revealed that increasing Shiitake mushroom supplementation ratios into quail diets worsened growth performance.

Keywords:*Shiitake; mushroom; blood parameters;intestinal microbiology; quail*

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INTRODUCTION

Animal-originated foodstuffs constitute essential nutrients in human nutrition and have great contributions to a healthy and balanced nutrition.Since animal proteins play an important role in human nutrition, researchers mostly focus on either improving available animal protein sources or new protein sources produced from animal products (Chadd et al., 2004).

The primary target of animal breeding operations is to achieve high yields with the minimum costs of feeding and maintenance. In poultry industry, quail breeding is getting popular each day and the primary target of quail operations is also to achieve desired performance with the minimum cost (Khieu,1999; Ravindran,2013).

In broiler operations, intensive feeding programs are applied to achieve a fast live weight gain in a short period of time. Therefore, nutritional values of rations are improved and animal diets are supplemented with growth promoting substances (Soares et al., 2003;Ulger et al., 2017; Cufadar et al., 2020).

Excessive and unnecessary use of antibiotics ends up with formation of resistant bacteria to these substances (Ekizoglu et al., 2020). Therefore, use of such antibiotic-originated growth factors in poultry industry has largely banned in European Countries since 1998-99 (Anadón and Martínez-Larrañaga, 1999; Casewell et al.,2003). Then, there is an ever-increasing search for natural growth-promoting substances as an alternative of antibiotics(Guo et al., 2003;Göçmen et al., 2016;Ulger, 2019;Najm and Cufadar, 2020).

Recent research mostly focused on mushroom species as alternative feed additives to promote growth in farm animals, however there is quite limited information in literature about the potential use of Shiitake mushroom in animal feeding (Guo et al., 2004; Buwjoom and Yamauchi, 2005; Daneshmand et al., 2011; Giannenas et al., 2011; Handayani et al., 2012; Willis et al., 2012; Kavyani et al., 2012; Shamsi et al., 2015a & 2015b; Khan et al., 2019). Previous researchers indicated that Shiitake mushroom had low calorie and high vitamin, protein and mineral contents and had various functional characteristics such as antitumor, antimicrobial, antioxidant and hypocholesterolemic effects (Wang et al., 1998;Manzi and Pizzoferrato, 2000; Emery,2005; Kitzberger et al.,2007; Dos Santos et al., 2010;Reis et al., 2012; Molz et al.,2014). With such attributes,Shiitake mushroom would be a possible candidate as a new and natural growth-promoter substance for poultry diets. Therefore, this study was conducted to investigate the effects of Shiitake mushroom supplementation into quail diets on growth performance, blood serum parameters and intestine microbial population.

MATERIALS AND METHODS

Experiments were conducted with the 12.10.2016 dated and 16/126 numbered decision of Erciyes University Local Ethics Committee.

Animal material

Experiments were conducted in the quail house of Animal Science Department of Erciyes University Agricultural Faculty. Quail chicks were supplied with sufficient heat and appropriate lighting before the initiation of the experiments.

As the animal material, 220 Japanese quail (*Coturnix coturnix Japonica*) chicks at the age of 10 days were randomly separated into 4 treatment groups in 5 replicates with 11 birds in each. Chicks were fed with total mixed rations prepared in accordance with the norms recommended by NRC (1994).One of the groups was fed with control mixed ration (control group) and the other groups were fed on mixed rations supplemented with Shiitake mushroom powder at different ratios (0.5, 1 and 2%).Sufficient heating and lighting were provided throughout the experiments. Feed and water were supplied *ad libitum*.Experiments were conducted in quail cages and lasted for 28 days.

Feed material

Constituents and chemical composition (calculated according to AOAC, 2005) of the concentrated feed used to prepare quail rations are provided in Table 1.Basal diets contain 24% crude protein and 2900 kcal/kg ME.

Shiitake mushroom powder was supplied from a commercial dealer and supplemented into above-specified concentrated feed homogeneously at different ratios (0.5, 1 and 2%). Chemical composition of Shiitake mushroom was analyzed according to the methods reported by AOAC (2005) in the laboratories of Animal Science Department of Erciyes University Agricultural Faculty and analysis results are provided in Table 2.

Table 1. Constituents and calculated chemical composition of concentrated feed

Feed ingredients	%
Maize	53.32
Soybean meal	39.69
Maize gluten	3.07
Vegetable oil	1.00
Marble powder	1.22
Dicalcium phosphate	0.77
Lysine	0.06
Methionine	0.12
Vitamin-Mineral Premix*	0.50
Salt	0.25
Calculated chemical composition **	**Value**
Metabolic Energy, Kcal/kg	2900
Dry matter, %	89.91
Crude protein, %	24.00
Calcium, %	0.81
Phosphorus, %	0.30
Sodium, %	0.12
Lysine, %	1.30
Methionine + Cysteine, %	0.89

*:Vitamin-Mineral premix is for per 2.5 kg: vitamin A, 12.500.000 IU; vitamin D3, 3.000.000 IU; vitamin E, 20.000 mg; vitamin K3, 3.000 mg; vitamin B1, 2.500 mg; vitamin B2, 7.000 mg; vitamin B6, 5.000 mg; vitamin B12, 20 mg; niacin, 20.000 mg; Cal-D-Pan, 15.000 mg; folic acid, 1.000 mg; biotin, 20 mg; vitamin C, 50.000 mg; choline chloride, 300.000 mg; manganese, 80.000 mg; iron, 70.000 mg; zinc, 50.000 mg; copper, 6.250 mg; iodine, 1.250 mg; cobalt, 200 mg; selenium, 150 mg; canthaxanthin, 0 mg; apo-carotenoic acid est., 0 mg; lasolosid sodium, 90.000 mg.

**: Apart from dry matter, values were calculated from the tables in NRC (1994).

Table 2. Chemical composition of shiitake mushroom powder

Parameters	%
Crude protein, DM%	20.33
Crude ash, DM%	5.53
Crude oil, DM%	1.05

DM: Dry matter.

Live weight and live weight gain

Live weights of the animals in each group were measured weekly with a precise balance (±0.01 g). The difference in weekly live weights (LW) were used to calculate live weight gains (LWG).

Feed consumption and feed conversion ratio

Feeds remained in feeders at the end of the 1, 2, 3 and 4thweeks were subtracted from the amount of feed supplied at the beginning of each week to get weekly feed consumptions.Weekly feed consumptions were divided by live weight gains to get feed conversion ratios.

Serum parameters

At the end of the experiments, blood samples were taken from 20 quail of each treatment group (two male and two female in each cage). Blood samples were centrifuged (Hettich Universal-320, Germany) to separate blood serums. Then serums were kept in a deep freezer at -20 °C until the analyses. Commercial kits (AMS Spa, Italy) were used to determine serum triglyceride, cholesterol, protein and glucose levels spectrophotometrically(Shimadzu Corp. UV–1601, Australia).

Intestine bacteria populations

Conventional methods were used for isolation and identification of bacterial agents. Quail intestine samples were sent to laboratory and subjected to microbiological analyses in the same day. Serial dilutions of the samples were prepared and sown into Plate Count Agar (Oxoid, United Kingdom) and total microorganism loads were determined.For isolation and identification, samples were inoculated to bloody agar supplemented with Chromagar mastitis Gram positive and Chromagar orientation (CHROMagar, USA), Mac Conkey Agar (Merck, Germany)and 7% sheep blood (Oxoid, United Kingdom). Broth media were incubated under aerobic conditions at 37°C for 18-24 hours. Suspicious isolates developed at the end of incubation were subjected to Gram staining, carbohydrate fermentation, catalase, coagulase, oxidase and nitrate reduction tests for phenotypic identification. Phenotypically identified microorganisms were preserved in cryotubes including 10%glycerin(Merck, Germany) Brucella Broth (Oxoid, United Kingdom) medium in a deep freezer at -80°C.

Statistical analysis

Experimental data were statistically analyzed by one-way ANOVA under General Linear Models of SPSS (1998) software. The means were compared by the Duncan's multiple range test.The results of statistical analysis were shown as mean values and standard error of means (SEM) in the tables. Significance level was considered as $P<0.05$.

RESULTS

Live weights of experimental groups fed with shiitake mushroom-supplemented diets are provided in Table 3. As can be inferred from the table, shiitake mushroom (SM) supplementations reduced live weights of the experimental groups in the 1, 2, 3 and 4th weeks ($P<0.01$).

Weekly changes in live weights of treatment groups throughout the experiments are provided in Table 4. SM supplementation into diet did not affect (P>0.05) LWG on weeks 2 and 3, but decreased the LWG on weeks 1 (P<0.01) and 4 (P<0.05). Also, inclusion of SM linearly decreased LWG of treatment groups throughout the experimental period (P<0.05).

Weekly feed consumptions of treatment groups throughout the experiments are provided in Table 5. The differences in feed consumptions of treatment groups were not found to be significant in all weeks (P>0.05).

Weekly feed conversion ratios of treatment groups throughout the experiments are provided in Table 6. Significantly greater feed conversion ratios were observed in SM-supplemented groups in the 1st and 4th week of the experiments (P<0.05); however, differences in feed conversion ratios were not found to be significant in the 2nd and 3rd week of the experiments (P>0.05).

Table 3. Live weights at the begging and throughout the experiments

	Live Weight (LW), g					
Period	Control	0.5% SM	1% SM	2% SM	SEM	P
Beginning	30.18	29.13	28.68	29.45	0.277	0.104
1stweek	74.59^{a}	68.56^{b}	67.20^{b}	67.13^{b}	0.615	0.001
2ndweek	112.38^{a}	106.26^{b}	106.18^{b}	105.16^{b}	0.731	0.001
3rd week	149.69^{a}	146.13^{ab}	142.88^{b}	142.14^{b}	0.864	0.006
4thweek	178.85^{a}	175.03^{ab}	167.63^{b}	164.39^{b}	1.408	0.001

$^{a, b}$: The means indicated with different letters in the same row are significantly different; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

Table 4. Weekly live weight gains of treatment groups throughout the experiments

	Live Weight Gain (LWG), g					
Period	Control	0.5% SM	1% SM	2% SM	SEM	P
1st week	44.41^{a}	39.43^{b}	38.52^{b}	37.68^{b}	0.651	0.009
2nd week	37.79	37.7	38.98	38.03	0.849	0.773
3rd week	37.31	39.87	36.7	36.98	1.062	0.327
4th week	29.16^{a}	28.9^{a}	24.75^{b}	22.25^{b}	1.589	0.029
General	148.67^{a}	145.9^{a}	138.95^{b}	134.94^{b}	1.460	0.012

$^{a, b}$: The means indicated with different letters in the same row are significantly different; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

Table 5. Weekly feed consumptions of treatment groups throughout the experiments

	Feed Consumption (FC), g					
Period	Control	0.5% SM	1% SM	2% SM	SEM	P
1st week	85.56	91.49	91.76	83.22	2.726	0.310
2nd week	118.24	119.16	118.26	113.77	2.401	0.394
3rd week	137.75	146.06	142.17	135.74	3.374	0.537
4th week	174.24	157.08	160.47	172.29	4.055	0.239
General	515.79	513.79	512.66	505.02	1.721	0.276

$^{a, b}$: The means indicated with different letters in the same row are significantly different; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

Table 6. Weekly feed conversion ratios of treatments groups throughout the experiments

	Feed Conversion Ratio (FCR), g/g					
Period	Control	0.5% SM	1% SM	2% SM	SEM	P
1st week	1.93^{b}	2.32^{a}	2.38^{a}	2.21^{a}	0.061	0.029
2nd week	3.13	3.16	3.03	2.99	0.098	0.113
3rd week	3.69	3.66	3.87	3.67	0.147	0.421
4th week	5.98^{c}	5.44^{c}	6.48^{b}	7.74^{a}	0.281	0.026
General	3.47^{b}	3.52^{b}	3.69^{a}	3.74^{a}	0.037	0.040

$^{a, b}$: The means indicated with different letters in the same row are significantly different; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

Effects of SM supplementations into quail diets on blood serum parameters are provided in Table 7. The differences in serum total cholesterol, total protein and triglyceride levels of the treatment groups were not found to be significant (P>0.05). However, the differences in blood serum glucose levels of the treatment groups were found to be significant (P<0.05).

Intestine bacteria populations (total bacteria, *Escherichia coli* and *Lactobacillus* bacteria) of treatment groups are provided in Table 8. The differences in intestine bacterial populations of treatment groups were not found to be significant (P>0.05).

Table 7. Serum biochemical parameters of treatment groups

	Treatment Groups					
Parameter	Control	0.5% SM	1% SM	2% SM	SEM	P
Total cholesterol, mg/dL	231.32	242.00	241.52	219.85	6.685	0.614
Glucose, mg/dL	274.52^{c}	308.17^{b}	322.39^{ab}	328.90^{a}	3.960	<0.001
Total protein, g/dL	2.02	1.81	1.79	1.72	0.044	0.092
Triglyceride, mg/dL	174.42	261.63	242.55	223.43	23.870	0.668

$^{a, b}$: The means indicated with different letters in the same row are significantly different; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

Table 8. Intestine bacteria populations of treatment groups

	Treatment Groups					
Microbial Population	Control	0.5% SM	1% SM	2% SM	SEM	P
Total bacteria, logx10^{6} CFU	33.06	31.84	33.01	31.12	1.963	0.903
Escherichia coli, logx10^{6} CFU	5.29	5.21	5.84	5.53	0.474	0.965
Lactobacillus bacteria, logx10^{6} CFU	4.59	4.08	4.49	4.01	0.390	0.940

CFU: Colony-forming unit; SM: Shiitake mushroom; SEM: Standard error of the mean; P: Probability.

DISCUSSION

The inclusion of mushroom *Lentinus edodes* (Shiitake in Japanese) into quail diets has resulted in a linear decrease in LWG. Therefore, the greatest LW was observed in the control group.Also, there was a numerically decrease in FC of SM supplemented groups. SM supplementation increased FCR and negatively affected feed utilization of quails. Supporting present findings,Daneshmand et al. (2011) showed that LW, LWG and FC were suppressed in broilers subjected to oyster mushroom (*Pleurotus ostreatus*). Such decreases were also reported by Buwjoom and Yamauchi (2005) in LWG and FC of broilers fed with SM stalk. Similarly, Kavyani et al. (2012) indicated that broilers receiving basal diet had higher LW than those fed on diets containing 20 or 30 g edible mushroom/kg and Fard et al. (2014) reported that inclusion of 2% dietary oyster mushroom wastes decreased LW of broilers. Willis et al. (2013) indicated that broilers responded differently to the mushroom type and level of inclusion and LW was negatively affected by dietary inclusion of %10 SM. Handayani et al. (2012) also indicated that the rats fed with the SM-enriched diet had lower BWG. In general, rodent studies have been previously reported that mushroom-enriched diets had an effect on body weight gain prevention (Kaneda and Tokuda, 1966; Kubo and Nanba, 1996; Kim et al., 2005; Neyrinck et al. 2009; Noh et al., 2011). Contrarirly, some researchers have reported improved performance and feed utilization in poultry with the inclusion of mushroom species into the diets (Shimadai et al., 2002; Giannenas et al., 2011;Willis et al., 2011; Shamsi et al., 2015b). In a similar study, Giannenas et al. (2010) showed improved performance of broiler chickens fed on edible mushroom-supplemented diets (*Agaricus bisporus*).However, Guo et al. (2004) concluded that different mushroom powder (*Lentinus edodes* and *Tremella fuciformis*) inclusion into broiler diets did not have any significant effects on animal performance.Differences from the present findings were mostly attributed to differences in animal species, supplementation dosages and the variety of mushroom (Handayani et al., 2012). Guo et al. (2003) reported that physicochemical properties of different mushroom polysaccharides, sugar composition, molar weights and structures exhibited a wide range of variation.

Extracts taken from SM have been demonstrated to have antibacterial (Hirasawa et al., 1999; Hatvani, 2001), antiviral (Chang, 1996), anti-tumor (Jong and Birmingham, 1993) and cholesterol-lowering properties (Kabir and Kimura, 1989; Fukushima et al., 2001). In this study, blood cholesterol was reduced numeri-

cally, but not significantly. Similarly, Buwjoom and Yamauchi (2005) indicated that SM stalk supplementation into broiler diets did not change blood cholesterol levels. SM contains some compounds affecting the blood glucose levels (Yang et al., 2002). In this study, serum glucose concentrations of the dietary SM-supplemented groups showed higher values than the control group. It is not clear why a much higher blood glucose concentration was observed in all SM-fed groups, but it may possibly depend on the difference in chemical composition of SM, including sugar composition and polysaccharide fractions (Shamsi et al.,2015b).

Previous researchers reported that mushroom supplementations into diets reduced *Escherichia coli* rates, but increased *Lactobacillus* bacteria rates in the intestines (Van Der Wielen et al., 2002; Guo et al., 2004; Willis et al., 2007, Shamsi et al., 2015b). However, in present study, SM supplementation did not resulted in significant changes of intestinal microbiota; but there was a slight decrease in intestine total bacteria count.This may be due to antibacterial activities of polysaccharides derived from mushrooms that shown in previous studies conducted onpoultry (Guo et al., 2003; Hearst et al., 2009).In this study, decreases were seen in *Lactobacillus* bacteria -which are usually considered as beneficial (Bolder et al., 1999) - and increases in *E. coli*. A well-balanced biota population in gastrointestinal tract of poultrymay lead to a greater efficiency in digestibility and feed utilization, or *vice versa* (Shamsi et al., 2015b). So, decreasein beneficial bacterial populations and increase in *E.coli* could explain the reduction in performance parameters of experimental groups.

CONCLUSIONS

Present findings revealed that inclusion of SM intoquail diets resulted in a linear decrease in LW. Similarly, LWG linearly decreased with increasing SM doses. Therefore, FCR was negatively influenced by SM supplementations; while FC of quail were not affected significantly.It appears that SM offered no potential as a growth promotingsubstance in poultry diets.Further research is recommended to validatepossible healthenhancement properties of different mushroom species.

AUTHOR STATEMENT

All the authors of this manuscript have contributed significantly towards the execution of this work.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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