

Journal of the Hellenic Veterinary Medical Society

Vol 74, No 2 (2023)



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doi: [10.12681/jhvms.30440](https://doi.org/10.12681/jhvms.30440)

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To cite this article:

Ulger, İ, Kaliber, M., Hizlisoy, H., & Ayasan, T. (2023). - Effects of Reishi Mushroom (*Ganoderma Lucidum*) Powder Inclusion into Quail Diets on Animal Performance, Carcass Traits, Intestinal Microflora and Serum Parameters. *Journal of the Hellenic Veterinary Medical Society*, 74(2), 5829–5836. <https://doi.org/10.12681/jhvms.30440> (Original work published July 6, 2023)

Effects of Reishi Mushroom (*Ganoderma lucidum*) Powder Inclusion into Quail Diets on Animal Performance, Carcass Traits, Intestinal Microflora and Serum Parameters

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ABSTRACT: This study was carried out to determine the effects of Reishi mushroom (*Ganoderma lucidum*) powder inclusion into quail diets on live weight, live weight change, feed consumption, feed conversion ratio, carcass traits, intestinal microflora and serum biochemical parameters of the animals. A total of 200 Japanese quail (*Coturnix coturnix Japonica*) were used in present experiments. Quail were separated into 4 groups (one control and three treatment). Control group was fed with a basic ration and treatments groups were fed with 0.5, 1 and 2% Reishi mushroom-supplemented diets. Differences in live weight changes, feed consumptions, feed conversion ratios, overall carcass parameters and blood serum total protein, total cholesterol, glucose and triglyceride levels of the treatment groups were not found to be significant ($P>0.05$). Also, there were not any significant differences in intestinal bacteria populations of the treatment groups ($P>0.05$). The results of the current study indicated that using Reishi mushroom in quail diet had not any negative effect on growth performance or other evaluated parameters. Also, further research is recommended to be conducted with greater ratios of Reishi mushroom in quail diets or with different poultry species.

Keywords: Reishi; mushroom; fattening performance; carcass characteristics; poultry

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Date of initial submission: 27-05-2022
Date of acceptance: 14-03-2023

INTRODUCTION

Number of poultry operations is continuously increasing to meet the deficit in animal protein and to solve nutritional problems in several countries. The efforts spend on poultry meat production mostly focus on diversification of poultry meat. In this sense, quail meat production has the second place after chicken meat in poultry meat production. As it was in broilers, the primary target in quail breeding is to achieve the greatest live weight with the least feed consumption in the shortest time. As compared to broilers, quail are quite motile, thus have a faster metabolism, therefore they need greater metabolic rates. Such a case requires the diets to be rich in nutrients (Guo et al., 2019).

Intensive feeding programs are applied in poultry breeding to achieve high live weight gain rates. To achieve such a target, poultry diets are either enriched in nutrients or growth promoting factors are supplemented into the diets (Wang et al., 2009; Ekizoğlu et al. 2020). Among the growth-promoting feed additives, antibiotics play a great role in animal nutrition (Kheiri et al., 2018). Anabolic effect of antibiotics was discovered the first in 1940s through supplementing chick diets with certain ratios of antibiotics and observing accelerated live weight gains with these supplementations (Gustafson and Bowen, 1997). Excessive and improper use of antibiotics result in development of antibiotic-resistant bacteria, therefore, use of antibiotic-originated growth factors in poultry breeding was largely banned in EU countries in 1998-1999 (Anadon and Martinez-Larranaga, 1999; Casewell et al. 2003). In recent years, researchers have been in search for alternative native and growth-promoting substances as an alternative of antibiotics (Shamsi et al., 2015a; Siadati et al., 2017; Fouladi et al., 2018; Asghar et al., 2022; Turgud and Nariç, 2022).

Among these additives, there is not much known about mushrooms. Mushrooms have antimicrobial activities, boost the immune system and reduce potential stress of animals (Wang et al., 1998; Smith et al., 2002). Therefore, such positive effects of mushrooms should be experimented as an alternative source of feed supplements to be used in poultry diets (Giannenas et al., 2010; Shamsi et al., 2015b). In present study, effects of Reishi mushroom (*Ganoderma lucidum*) powder inclusion into quail diets on animal performance, carcass traits, blood parameters and intestinal microflora were investigated.

MATERIAL AND METHOD

Ethical approval

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

Animal Material

As the animal material of the experiments, 200 Japanese quail (*Coturnix coturnix Japonica*) chicks at the age of 10 days were randomly separated into 4 treatment groups comprising 5 replicates of 10 birds each. During the fattening period, quail chicks were fed on mixed diets prepared based on norms recommended by NRC (1994). While one of the groups was fed on control mixture diet (control), the other groups were fed on 0.5%, 1% and 2% Reishi mushroom (RM) powder-supplemented diets. Required heating and lighting were supplied and feed and water were supplied ad libitum throughout the experiments. Cage experiments lasted for 4 weeks.

Feed Material

Composition of concentrate feed used in preparation of experimental diets and calculated chemical composition are provided in Table 1. Quail diets contain 24% crude protein (CP) and 2900 kcal/kg metabolic energy (ME).

Reishi 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 Reishi mushroom was analyzed according to methods reported by AOAC (2005) and presented in Table 2.

Live weight and Live Weight Gain

Animal live weights (LW) were weighed at the beginning of experiments and in 1st, 2nd, 3rd and 4th weeks of experiments. Weighing was performed with the use of precise balance (± 0.01 g). The difference between consecutive weighing was taken as live weight change (LWC).

Feed Consumption and Feed Conversion Ratio

The feed remaining in feeders in the 1, 2, 3 and 4th weeks were weighed and deducted from the quantity of feed supplied at the beginning of each week to get weekly feed consumption (FC) of each group. The feed consumption between two consecutive weighing was divided by live weight change of that period to get feed conversion ratios (FCR).

Table 1. Composition of concentrate feed and calculated chemical composition

Feed Materials, %	Composition
Maize	53.32
Soybean Meal	39.69
Corn Gluten	3.07
Vegetable Oil	1
Marble Powder	1.22
Dicalcium Phosphate	0.77
Lysine	0.06
Methionine	0.12
Vitamin-Mineral Premix*	0.50
Salt	0.25
Calculated Nutrients**	
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-carotenoid 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 Reishi mushroom powder

Parameters	Composition
Crude protein, DM %	17.21
Crude ash, DM %	4.33
Crude oil, DM %	1.14

DM: Dry matter

Slaughter

At the end of the experiments (39 days of age), final live weights of the animals were measured and 2 male and 2 female quail representing mean final live weight of each cage were slaughtered in a controlled fashion following standard procedure (Landy et al., 2012).

Hot Carcass Dressing Percentage

Following the completion of slaughter process, carcasses were weighed to get hot carcass weight. Hot carcass weight was then divided by slaughter weight to get hot carcass dressing percentage:

$$\text{Hot carcass dressing percentage (\%)} = [\text{Hot carcass weight (g)} / \text{Slaughter weight (g)}] \times 100$$

Liver, Heart and Visceral Organ Weights

Liver, heart, gizzard, empty intestine, spleen, bur-

sa and testes (only male quail) weights of slaughtered animals were measured with the use of a precise scale (± 0.01 g) and also calculated as a percentage of slaughter weight.

Serum Biochemical 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% glycerine (Merck, Germany) Brucella Broth (Oxoid, United Kingdom) medium in a deep freezer at -80 °C.

Statistical analysis

All data were analyzed using the General Linear Model procedures of SPSS (1998) software. Means were compared using Duncan test at 5% probability. The results of statistical analysis were shown as mean values and standard error of means (SEM) in the tables.

RESULTS

Live weights of experimental groups are provided in Table 3. As can be seen from Table 3, RM supplementation into quail diets did not influence live weights in 1st and 2nd weeks of the experiments ($P>0.05$). In the 3rd week of the experiments, the greatest live weight (152.28 g) was observed in the control and the lowest live weight (146.16 g) was observed in 2% RM-supplemented group ($P<0.05$); however, they have compensated the live weights in the 4th week ($P>0.05$).

Weekly and overall live weight changes (LWC) of treatment groups are provided in Table 4. The differences in weekly live weight changes of the treatment groups were not found to be significant ($P>0.05$). Also, RM supplementations into quail diets did not influence LWC of treatment groups throughout the experimental period ($P>0.05$).

Weekly and overall feed consumptions of treatment groups are provided in Table 5. Differences in weekly and overall feed consumptions of the treatment groups were not found to be significant ($P>0.05$).

Weekly and overall feed conversion ratios (FCR) of treatment groups are provided in Table 6. As can be seen from Table 6, RM supplementation into quail diets did not influence FCR in 1st, 3rd and 4th weeks and the general of the experiments ($P>0.05$). However, in the 2nd week of the study, higher feed conver-

Table 3. Mean live weights of treatment groups

Period	Live Weight, g				SEM	P
	Control	0.5% RM	1% RM	2% RM		
ILW	35.20	35.15	34.67	34.97	0.252	0.879
1st week	75.999	72.94	74.89	73.18	0.457	0.056
2nd week	115.87	113.40	112.38	107.18	0.566	0.886
3rd week	152.58 ^a	148.58 ^{ab}	148.60 ^{ab}	146.16 ^b	0.719	0.018
4th week	177.57	175.14	178.09	177.02	1.069	0.785

^{a, b}: Means indicated with different letters in the same row are significantly different; ILW: Initial live weight; RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

Table 4. Weekly and overall live weight changes of experimental groups

Period	Live Weight Change, g				SEM	P
	Control	0.5% RM	1% RM	2% RM		
1st week	40.68	37.36	40.53	38.21	0.541	0.068
2nd week	34.66	40.88	36.60	31.49	1.254	0.061
3rd week	36.71	35.18	36.00	38.98	1.255	0.178
4th week	24.99	26.56	29.49	30.86	1.703	0.067
General	133.88	138.85	135.45	133.55	2.101	0.803

RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

Table 5. Weekly and overall feed consumptions of the treatment groups

Period	Feed Consumption, g				SEM	P
	Control	0.5% RM	1% RM	2% RM		
1st week	95.00	98.78	92.84	91.44	3.447	0.645
2nd week	122.74	133.60	120.29	126.71	2.666	0.331
3rd week	134.42	114.68	131.00	120.91	3.459	0.155
4th week	163.29	155.80	155.74	161.79	3.426	0.828
General	515.40	502.88	499.88	500.84	5.584	0.415

RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

Table 6. Weekly and overall feed conversion ratios of the treatment groups

Period	Feed Conversion Ratio, g/g				SEM	P
	Control	0.5% RM	1% RM	2% RM		
1 st week	2.34	2.64	2.29	2.39	0.075	0.767
2 nd week	3.25 ^b	3.2 ^b	3.29 ^b	4.02 ^a	0.160	0.050
3 rd week	3.66	3.26	3.64	3.10	0.156	0.571
4 th week	6.53	5.87	5.28	5.24	0.327	0.201
General	3.85	3.62	3.69	3.75	0.040	0.251

^{a-b}: Means indicated with different letters in the same row are significantly different; RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

Table 7. Slaughter and carcass parameters of treatment groups

Parameters	Treatment Groups				SEM	P
	Control	0.5% RM	1% RM	2% RM		
Hot carcass weight, g	119.74	115.08	117.71	112.11	1.095	0.074
Cold carcass weight, g	118.61	113.95	115.88	111.32	1.074	0.101
Carcass loss, %	0.99	0.99	0.99	1.00	0.001	0.212
Dressing percentage, %	65.60	65.28	64.98	66.79	0.285	0.098
Hot carcass pH	5.67	5.76	5.70	5.76	0.024	0.462
Cold carcass pH	5.80	5.85	5.82	5.85	0.016	0.643

RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

sion ratio (4.02 g/g) was observed in 2% RM-supplemented group than the others (P=0.05).

Slaughter and carcass parameters of the treatment groups are provided in Table 7. RM supplementations into quail diets generally did not influence carcass parameters (P>0.05). It was also observed RM supplementations into mixed feed did not influence pre-slaughter live weights (Table 3). As it was in pre-slaughter live weights, RM supplementations in to mixed feed did not influence hot and cold carcass weights (P>0.05). Similarly, dietary RM did not have significant effects on carcass pH values (P>0.05).

Carcass color parameters of the treatment groups are provided in Table 8. Effects of RM supplementations in quail diets on carcass color parameters (L*, a* and b* values) were not found to be significant (P>0.05).

Visceral organ weights and ratios to slaughter weight of treatments groups are provided in Table 9. Differences in visceral organ weights of the treatment

groups were not found to be significant (P>0.05). Similarly, there were not any statistically significant differences in the ratio of intestine, heart, liver, gizzard, bursa and testes weights to slaughter weights between the experimental groups (P>0.05). However, ratio of spleen weight to slaughter weight was calculated as 0.07% in the control group, 0.07% in 0.5% RM-supplemented group, 0.09% in 1% RM-supplemented group and 0.07% in 2% RM-supplemented group (P<0.05).

Intestine bacteria populations of the treatment groups are provided in Table 10. Differences in intestine bacteria populations of the treatment groups were not found to be statistically significant (P>0.05).

Effects of RM-supplementations into quail diets on blood serum biochemical parameters are provided in Table 11. The differences in blood serum total cholesterol, total protein, glucose and triglyceride levels of the treatment groups at the end of the experiments were not found to be significant (P>0.05).

Table 8. Carcass color parameters of the treatment groups

Color Parameters	Treatment Groups				SEM	P
	Control	0.5% RM	1% RM	2% RM		
L*	51.40	51.63	53.87	52.86	0.398	0.098
a*	9.12	8.28	7.87	8.40	0.188	0.121
b*	6.59	5.17	6.89	6.16	0.233	0.080

RM: Reishi mushroom; L*: Brightness value; a*: Redness value; b*: Yellowness value; SEM: Standard error of the mean; P: Probability value.

Table 9. Visceral organ weights and ratios to slaughter weight of treatments groups

Organ Weights, g	Treatment Groups				SEM	P
	Control	0.5% RM	1% RM	2% RM		
Intestine	7.46	6.98	6.86	7.40	0.172	0.524
Heart	1.64	1.50	1.51	1.49	0.024	0.073
Liver	4.48	4.18	4.36	4.60	0.135	0.729
Gizzard	4.14	4.09	3.92	4.18	0.066	0.530
Spleen	0.13	0.12	0.15	0.11	0.006	0.055
Bursa	0.26	0.27	0.24	0.23	0.009	0.358
Testes*	2.78	2.47	2.74	2.27	0.133	0.624
Organ Weight / Slaughter Weight, %						
Intestine	3.98	4.10	3.96	4.23	0.079	0.618
Heart	0.88	0.82	0.82	0.78	0.024	0.485
Liver	2.39	2.32	2.36	2.47	0.078	0.913
Gizzard	2.23	2.38	2.26	2.39	0.032	0.170
Spleen	0.07 ^b	0.07 ^b	0.09 ^a	0.07 ^b	0.003	0.029
Bursa	0.14	0.16	0.14	0.14	0.005	0.534
Testes*	1.55	1.42	1.66	1.39	0.097	0.729

^{a,b}: Means indicated with different letters in the same row are significantly different; RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

*: Testes weights were taken from male quail.

Table 10. Intestine bacteria populations of the treatment groups

Microbial Population	Treatment Groups				SEM	P
	Control	0.5% RM	1% RM	2% RM		
Total bacteria, logx10 ⁶ CFU	25.04	25.57	29.91	28.18	1.094	0.341
<i>Escherichia coli</i> , logx10 ⁶ CFU	3.90	4.00	4.20	4.60	0.234	0.733
<i>Lactobacillus</i> bacteria, logx10 ⁶ CFU	5.07	6.13	7.89	6.73	0.415	0.099

CFU: Coliform unit; RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

Table 11. Serum biochemical parameters of the treatment groups

Parameter	Treatment Groups				SEM	P
	Control	0.5% RM	1% RM	2% RM		
Total Cholesterol, mg/dL	239.37	248.56	251.13	261.06	6.483	0.703
Glucose, mg/dL	332.00	335.07	312.83	335.77	4.942	0.283
Total Protein, g/dL	1.54	1.53	1.57	1.67	0.044	0.676
Triglyceride, mg/dL	151.47	155.08	145.39	163.91	7.716	0.879

RM: Reishi mushroom; SEM: Standard error of the mean; P: Probability value.

DISCUSSION

In present study, effects of Reishi mushroom supplementation (at 0.5, 1 and 2% ratios) into quail diets on live weight, live weight change, feed consumption, feed conversion ratio, carcass traits, carcass color pa-

rameters, visceral organ weights, intestine bacteria populations and serum biochemical parameters were investigated and values observed from treatment groups were compared with the values of a control group which fed with a basal diet.

Dietary RM supplementations did not influence LW of experimental animals, except 3rd week, and LW of RM supplemented groups slightly decreased in the 3rd week, but they have compensated the LW at the end of the experiment; so LWC did not influenced by dietary RM inclusion. As it was indicated by different researchers (Buwjoom and Yamauchi, 2005; Daneshmand et al., 2011; Kavyani et al. 2012; Fard et al., 2014), decreasing live weights were observed with mushroom supplementations into poultry diets; some other researchers have reported improved performance in poultry with the inclusion of mushroom species (Shimadai et al., 2002; Giannenas et al., 2011; Willis et al., 2011; Shamsi et al., 2015b). On the other hand, Guo et al. (2004) incorporated different mushroom powders to broilers diet and concluded that these additives had no significant effects on the birds' body weight. Similarly, Fard et al. (2014) reported that inclusion of mushroom wastes had no significant effect on the whole period LW and LWC of broilers. Similar to LW, different statements were found in the literature regarding to effects of mushroom supplementation to poultry diets on FC. While, Fard et al. (2014) reported that supplementation of 1% mushroom wastes to broiler diets led to the significant increase of FC; Buwjoom and Yamauchi (2005) and Daneshmand et al. (2011) showed that FC were suppressed in broilers subjected to dietary mushroom inclusion. In addition, Asadi-Dizaji et al. (2017) reported that the level of dietary mushroom had no significant effect on LW, FC and FCR of quail. The inconsistency of findings from different studies on the use of different mushroom species in poultry diets are also exists in terms of FCR (De Barros et al., 2012; Toghiani et al., 2012; Willis et al., 2013; Fard et al., 2014; Shamsi et al., 2015b). These results indicated that the birds responded differently to the mushroom type and dietary level. Guo et al. (2003) stated the wide variety of the physicochemical properties of different mushroom polysaccharides, such as sugar composition, molar weights, and structures.

Dietary RM supplementations into quail diets did not influence carcass weights and traits (like carcass pH and color parameters), also visceral organ weights of the treatment groups. However, antibacterial, antiviral and immune regulatory effects of mushrooms were reported in previous studies (Chang, 1996; Hirasawa et al., 1999; Hatvani, 2001; Shieh et al., 2001;

Berger et al., 2004). According to this, in our study, RM supplementation did not resulted in significant changes of intestinal microbiota; but there was a slight increase in intestine total bacteria count and *Lactobacillus* bacteria rates in the intestines. Such changes in intestinal microflora by mushroom inclusion to poultry diets were reported also by Guo et al. (2004) and Willis et al. (2007).

The differences in blood serum total cholesterol, total protein, glucose and triglyceride levels of the treatment groups at the end of the experiments were not found to be significant. While, literature does not contain many studies in this area; Shamsi et al. (2015b) reported that serum concentration of total protein, triglycerides and glucose levels did not affected by mushroom supplementation to the broiler diets. Similar findings reported by Daneshmand et al. (2011) for serum triglyceride and cholesterol levels in broilers fed with mushroom supplemented diets. Also, Buwjoom and Yamauchi (2005) indicated that Shiitake mushroom stalk supplementation into broiler diets did not change blood serum cholesterol and glucose levels.

CONCLUSIONS

The results of current study indicated that using up to 2% Reishi mushroom powder in quail diet had not any negative effect on animal performance or other evaluated parameters. It should be noted that greater levels of RM may offer potential as a growth promoting substance in poultry diets. Also, present findings on quail may also be valid for the other poultry species. Therefore, further research is recommended to be conducted with the other poultry species and different types of mushroom.

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.

ACKNOWLEDGEMENT

This study was supported by the Scientific Research Projects Department of Erciyes University, Turkey (Project no: FHD- 2017- 7347).

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