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Research Article

Effect of Resveratrol Supplemented to Japanese Quail *(Coturnix coturnix japonica)* Rations on Performance and Some Biochemical Parameters

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Abstract

This experiment was conducted to evaluate the changes on growth performance, carcass and some biochemical parameters of Japanese quail fed supplemental Resveratrol (Res). A total of 200 quails were distributed at equal body weights into four experimental groups, each consisting of five replicate pens having ten birds/replicate pen: 1- basal diet without Res (Control), 2- basal diet + 100 mg/kg Res, 3- basal diet + 200 mg/kg Res, and 4- basal diet + 400 mg/kg Res. There were no significant effects of the supplementation of resveratrol on body weight gain and carcass traits (P>0.05). Inclusion of Res in quail rations improved feed consumption (P<0.05) and feed conversion ratio during the period from 3 to 5 weeks and the throughout (0-5 wks) of the treatment (P<0.05). Malondialdehyde decreased as compared with the control group (P<0.05), but glutathione and nitric oxide did not change by the effect of Res (P>0.05). Glucose, triglyceride, total protein was not influenced by the supplementation of Res (P>0.05). As a result, the findings of this study suggest that Res can be used safely as a growth promoter up to 400 mg/kg in quail rations.

Keywords: *Antioxidant, Japanese quail, Performance, Polyphenolic compounds, Resveratrol*

Japon Bıldırcın *(Coturnix coturnix japonica)* Rasyonlarına İlave Edilen Resveratrol'ün Performans ve Bazı Biyokimyasal Parametreler Üzerine Etkisi

Öz

Bu çalışmada Japon bıldırcın rasyonlarına ilave edilen resveratrolün (Res) büyüme aktivitesi ile ilişkisinin belirlenmesi amaçlandı. Çalışmada eşit canlı ağırlığa sahip 200 adet bıldırcın civcivi beşer tekrarlı (10 civciv) olacak şekilde ve her grupta 50 adet hayvan bulunan dört deneme grubuna ayrıldı. Resveratrolün Japon bıldırcınlarında performans, karkas özellikleri, serum antioksidan ve bazı biyokimyasal parametreleri üzerine etkisini belirlemek için 1- Res içermeyen bazal diyet (Kontrol), 2- bazal diyet + 100 mg/kg Res, 3- bazal diyet + 200 mg/kg Res ve 4- bazal diyet + 400 mg/kg Res gruplarına ayrıldı. Resveratrol ilavesinin canlı ağırlık artışı ve karkas parametrelerini etkilemediği belirlendi (P>0.05). Japon bıldırcın rasyonlarına Res ilavesi ile 3-5 haftalık periyotta ve çalışmanın tamamında (0-5 hafta) yem tüketimi ve yem dönüşüm oranı önemli derecede etkilendi (P<0.05). Resveratrolün etkisiyle malondialdehit kontrol grubuna göre azalırken (P<0.05), glutatyon ve nitrik oksit seviyeleri değişmedi (P>0.05). Resveratrol ilavesinin glikoz, trigliserit ve total protein üzerine etkisinin olmadığı tespit edildi (P>0.05). Sonuç olarak, elde edilen bulgulara göre Res'in bıldırcın rasyonlarında 400 mg/kg'a kadar büyüme destekleyici bir katkı maddesi olarak güvenle kullanılabileceği kanaati oluşmuştur.

Anahtar sözcükler: *Antioksidan, Fenolik bileşikler, Japon bıldırcın, Performans, Resveratrol*

INTRODUCTION

Antimicrobial feed additives have played an important role in the economic development of the poultry industry for many years by increasing the growth rate, improving the feed conversion ratio, and reducing the risk of disease [1]. However, the use of antibiotics in farm animals has caused significant concerns since they remain in meat and eggs

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over time. Because of this, resistant bacterial populations have developed. As a result of these concerns, the use of antibiotics as a growth promoter for poultry has been banned in the European Union [2]. In addition to antibiotic prohibition in animal feeding, studies have focused on prebiotic, probiotic, medicinal and aromatic plants and phenolic compounds derived from them to minimize problems in poultry health and increase performance [3-7]. Resveratrol (Res) is a natural polyphenolic compound (3, 5, 4' - trihydroxytrans - stilbene) found in high concentrations in *Polygonum cuspidatum*, grape peel, hazelnut, Yucca schidigera and wine [8-11]. Resveratrol has antioxidant, anti-aging, and anti-inflammatory effects, and it plays a role in regulating energy metabolism [10,12-16]. Resveratrol or its derivatives exhibit antimicrobial properties and prevent cancer cell growth [12]. By protecting DNA from oxidative damage, malondialdehyde (MDA) and heat stress are reduced [12,17]. Resveratrol is considered an important monomeric bioactive compound that exhibits a strong antioxidant capacity to scavenging free oxygen and lipid radicals [18,19]. Resveratrol inhibits the formation of glutathione (GSH) disulfide and inhibits cellular damage produced by free radical reactions by maintaining GSH [18]. Resveratrol can prevent and treat cardiovascular diseases while preserving vascular endothelium, as well as cure micro-circulatory disorders. It can raise the level of cellular nitric oxide (NO) and also inhibits platelet aggregation [20,21]. Several animal and poultry experiments have been conducted to determine the dependence and functionality of Res and its supplementation in mammalian and poultry rations. Zhang et al.[9] found that Res (200, 400 or 800 mg/kg) supplementation in broiler chickens had a positive effect on their body weight compared to the control group. Sridhar et al.[22] reported that chicks given Res at 0.5% and 1.0% levels of ration help to increase antioxidative enzyme activities, at the same time contributing to the improvement of protein and total antioxidant capacity in plasma, and the degree of liver lesions in Res groups is much lower. It has been reported that the supplementation of grape seed extract (400 ppm) containing Res to broiler rations can improve serum biochemistry and lipid profile [23]. It is stated that dietary supplementation of Res, in addition to improving growth performance, gut morphology, and microbial balance, increased immunity in *E. coli* fighting broilers [24]. Therefore, the objective of this study was to determine the effects of dietary supplementation of Res on growth performance, antioxidant, and some serum biochemical parameters of Japanese quail.

MATERIAL and METHODS

Ethical Approval

The experimental protocol and animal care in this study were approved by animal experiments from the local ethics committee (KAÜ-HADYEK/2018-026) of Kafkas University.

Experimental Design

In this trial, the effect of 4 different levels of Res supplementation to Japanese quail rations on performance, carcass characteristics, serum antioxidant parameters and biochemical profile were investigated. The animal material of the study consisted of 200 Japanese quails *(Coturnix coturnix japonica)* of one day age in mixed gender, each consisting of five replicates having ten birds in each replicate. Quails were randomly divided into four treatment groups, 50 in each, and placed in cages. Research rations were prepared to take into account the criteria specified in the National Research Council (NRC) [25]. Ration containing 23.89% crude protein (CP) and 2929.28 kcal ME/kg was fed to all groups for 35 days in the trial. The raw material and nutrient contents of the ration used in the experiment are shown in *Table 1*. Quails were fed with ration containing 0 (control), 100, 200, and 400 mg/kg Res (50%, Resveratrol, Solgar, Istanbul, Turkey). The product contained naturally derived Res from 200 mg *Polygonum cuspidatum* (root). The experiment lasted for 35 days.

Growth Performance

Body weight gain (BWG) and feed consumption (FC) were recorded weekly as performance indicator parameters during the trial and the feed conversion ratio (FCR) was calculated. Feed and water were given as *ad libitum* and group feeding was applied to animals.

Table 1. *Nutrient and chemical composition of quail basal diet*

Ingredients	**Ratio, %**
Corn	48.45
Soybean meal (45% CP)	44.00
Wheat bran	1.50
Vegetable oil	2.80
Limestone	1.65
Dicalcium phosphate	0.80
Salt	0.25
DL-methionine	0.15
L-lysine sulfate	0.15
Vitamin mix[1]	0.15
Mineral mix[2]	0.10
Analysis of nutrient contents	
Crude protein (%)	23.89
Metabolizable energy (Kcal/kg)	2928.28
Calcium (%)	1.03
Available phosphorus (%)	0.58

[1] Vitamin mix makes per kilogram of ration: vit. D_3, 0.025 mg; menadione (menadione sodium bisulfate) 1.1 mg; vit. B_2, 4.4 mg; vit. B_1, 1.1 mg; vit. B_6, 2.2 mg; B_3, 35 mg; vit. B_{12}, 0.02 mg; folic acid, 0.55 mg; choline, 125.000 mg; d - biotin, 0.1 mg; calcium D-pantothenate, 10 mg; vit. A, 12.000 IU; vit. D_3, 2.400 IU; vit. E, 30 mg; vit. K_3, 5 mg
[2] Mineral mix makes per kilogram of ration: Se, 0.15 mg; Mn, 40 mg; Fe, 12.5 mg; Zn, 25 mg; Cu, 3.5 mg; I, 0.3 mg; Co, 0.1 mg

Carcass Parameters

At the end of the trial, 8 quails (a total of 32) were slaughtered from each group and their internal organs were exenterated. The weights of the carcasses and internal organs (heart, liver, and gizzard) were determined. The dressing percentage was calculated by proportioning the carcass weight to the body weight before slaughtering [26].

Blood Collection and Serum Separation

Blood samples were collected from each quail randomly selected for slaughtering (8/group, 32 in total). Blood samples taken from *Vena brachialis* during slaughtering were centrifuged for 10 minutes at 3000 rpm and stored at - 20°C a month until analysis.

Serum Antioxidant and Biochemical Parameters

Serum samples were dissolved at room temperature, antioxidant parameters as MDA, GSH, and NO and biochemical profile as glucose (GL), triglyceride (TG), and total protein (TP) concentrations were measured using commercial ELİSA kits (MyBioSource® Company, San Diego/USA) in the spectrophotometer device.

Statistical Analysis

For statistical analysis, the SPSS portable 18 (SPSS, Chicago, IL) statistical package program was used, and the data was analyzed with one-way analysis of variance (ANOVA). It was analyzed in terms of linear (L), quadratic (Q), and cubic (C) effects depending on the linear increase in the Res level (100, 200, and 400 mg/kg). Due to the significance is different, the P values for L, Q, and C effects are given in the tables. The mean separation among groups was performed utilizing Tukey's test. The means and standard errors of each group are indicated in the result tables. The significance level (P) was assessed as 0.05.

RESULTS

The effects of Res on BWG, FC and FCR in Japanese quails are presented in *Table 2*. It was determined that the differences between the groups in terms of BWG were not significant during the experiment (L=0.129). In the experiment, the average FC's belonging to the groups were significantly affected by increasing levels of dietary supplementation of Res.

In the trial, Res 100 group lower FC has occurred in comparison to control groups in 3-5. wks (Q=0.012; C=0.017) and 0-5. wks (Q=0.008). The effect of Res groups on FCR was significant (L=0.050; C=0.028). In the 3-5. wks of the trial, a cubic increase was observed in the FCR in Res 100 and 400 groups (C = 0.005). The carcass characteristics of the control and treatment groups thus obtained are presented in *Table 3*. There were no differences statistically among the treatment groups in terms of slaughter weight, dressing, heart, liver, and gizzard weights (L=0.119; L=0.630; L=0.814; L=0.968; L=0.816).

The effects of supplementation of Res on MDA, GSH, and NO are shown in *Table 4*. MDA level in serum was remarkably reduced by Res 200 and 400 groups (L=0.041 and L=0.047). Furthermore, no significant change was observed in the concentration of GSH (L=0.610) and NO (L=0.184) among the groups.

Supplementation with Res did not affect the levels of GL (L=0.723), TG (L=0.865) and TP (L=0.865) compared with the control group *(Table 5)*.

DISCUSSION

Based on the results of the present study, quail diets containing 100 mg/kg of Res were more effective in enhancing growth performance than the control group. In

Table 2. *Performance values of quails fed supplemental resveratrol (Mean±SEM[1])*

Parameters[2]	Weeks	Groups[3]				Contrasts[4]		
		Control	RES 100	RES 200	RES 400	L	Q	C
BWG (g)	0-3	20.04±0.09	19.88±0.05	19.98±0.16	19.95±0.01	0.716	0.482	0.129
	3-5	34.51±0.14	34.89±0.19	34.64±0.15	35.00±0.03	0.069	0.935	0.727
	0-5	27.27±0.09	27.39±0.11	27.31±0.04	27.48±0.02	0.129	0.062	0.213
FC (g)	0-3	51.09±0.34	50.82±0.13	50.52±0.17	50.78±0.30	0.276	0.306	0.607
	3-5	135.26±0.18^{a}	133.15±0.48^{b}	134.410.38^{ab}	134.46±0.45^{ab}	0.517	0.012	0.017
	0-5	93.18±0.20^{a}	91.98±0.21^{b}	92.47±0.21^{ab}	92.62±0.29^{ab}	0.258	0.008	0.064
FCR	0-3	2.55±0.01	2.56±0.01	2.53±0.02	2.54±0.01	0.457	0.811	0.247
	3-5	3.92±0.02^{a}	3.82±0.03^{b}	3.88±0.01^{ab}	3.84±0.01^{b}	0.058	0.102	0.005
	0-5	3.42±0.01^{a}	3.36±0.02^{b}	3.39±0.01^{ab}	3.37±0.01^{ab}	0.050	0.090	0.028

[1] SEM = Standard error mean; [2] BWG = Body weight gain; FC = Feed consumption; FCR = Feed conversion rate; [3] RES 100 = Resveratrol 100 mg/kg; RES 200 = Resveratrol 200 mg/kg; RES 400 = Resveratrol 400 mg/kg; [4] L = Linear; Q = Quadratic; C = Cubic; [a,b] Means within the same row with different superscript (P<0.05)

Table 3. *Carcass characteristics of quails fed supplemental resveratrol (Mean±SEM[1])*

Parameters[2]	Groups[3]				Contrasts[4]		
	Control	RES 100	RES 200	RES 400	*L*	*Q*	*C*
SW (g)	169.84±0.20	170.08±0.09	169.99±0.60	170.86±0.50	0.119	0.451	0.484
Dressing (%)	70.01±0.02	70.00±0.01	70.12±0.17	70.05±0.14	0.630	0.788	0.522
Heart (g)	1.03±0.03	1.02±0.03	1.03±0.00	1.02±0.03	0.814	0.895	0.639
Liver (g)	3.49±0.20	3.41±0.10	3.48±0.19	3.45±0.14	0.968	0.895	0.742
Gizzard (g)	3.61±0.09	3.42±0.11	3.76±0.11	3.45±0.21	0.816	0.671	0.078

[1] SEM = Standard error mean; [2] SW= Slaughter weight; [3] RES 100 = Resveratrol 100 mg/kg; RES 200 = Resveratrol 200 mg/kg; RES 400 = Resveratrol 400 mg/kg; [4] L = Linear; Q = Quadratic; C = Cubic

Table 4. *Serum antioxidant parameters of quails fed supplemental resveratrol (Mean±SEM[1])*

Items[2]	Groups[3]				Contrasts[4]		
	Control	RES 100	RES 200	RES 400	*L*	*Q*	*C*
MDA (mg/dL)	0.75±0.30[a]	0.31±0.08[a]	0.23±0.08[b]	0.23±0.10[b]	0.041	0.208	0.721
GSH (mg/dL)	0.07±0.00	0.06±0.00	0.06±0.00	0.06±0.00	0.610	0.275	0.785
NO (mg/dL)	10.63±2.49	13.28±2.59	23.70±4.95	23.44±7.48	0.184	0.780	0.736

[1] SEM = Standard error mean; [2] MDA = Malondialdehyde; GSH = Glutathione; NO = Nitric oxide; [3] RES 100 = Resveratrol 100 mg/kg; RES 200 = Resveratrol 200 mg/kg; RES 400 = Resveratrol 400 mg/kg; [4] L = Linear; Q = Quadratic; C = Cubic; [a,b] Means within the same row with different superscript (P<0.05)

Table 5. *Serum biochemical profile of quails fed supplemental resveratrol (Mean±SEM[1])*

Items[2]	Groups[3]				Contrasts[4]		
	Control	RES 100	RES 200	RES 400	*L*	*Q*	*C*
GL (mg/dL)	197.17±0.28	196.27±0.79	197.09±0.43	196.62±0.44	0.723	0.689	0.208
TG (mg/dL)	173.15±0.47	173.47±1.02	173.20±0.47	173.070.47	0.865	0.736	0.807
TP (g/dL)	3.20±0.08	3.20±0.12	3.20±0.11	3.22±0.10	0.865	0.942	0.982

[1] SEM = Standard error mean; [2] GL = Glucose; TG = Triglycerides; TP = Total protein; [3] RES 100 = Resveratrol 100 mg/kg; RES 200 = Resveratrol 200 mg/kg; RES 400 = Resveratrol 400 mg/kg; [4] L = Linear; Q = Quadratic; C = Cubic

the study, although BWG was not affected, the decrease of FC significantly improved FCR. He et al.[27] reported that the supplementation of Res at different levels (200, 350, 500 mg/kg) in broilers under heat stress does not affect the average daily feed consumption and improves body weight. Zhang et al.[28] reported that the average daily feed consumption, average body weight, and feed utilization rate improved with the supplementation of Res to broiler rations under heat stress. It has been stated that the Res supplementation of 0.5% and 1.0% to the ration of broiler chicks exposed to aflatoxin caused lower body weight and FC during five weeks period but did not affect FCR [22]. In a different trial, it was reported that the addition of different levels (200-400 and 800 mg/kg) of Res to broiler rations did not affect the performance values [9]. The observed decrease in feed intake in 100 and 400 mg/kg Res groups during the late period and whole experiment period may be due to the blandness of Res, leading to a direct reduction in FC [29]. The improved FCR in quails fed 100 mg/kg of Res could be attributed to the effects of resveratrol, which mediated reduction of pathogens and improved utilization of nutrients in the digestive system [30].

Kim et al.[31] stated that Res did not affect carcass efficiency and internal organ weights in a similar way to this study, but decreased liver weight. Abdel - Wahab et al.[32] revealed that grape seed does not affect carcass yield in quail. Sridhar et al.[22] reported that the supplementation of resveratrol decreased liver weight (P>0.05), but did not affect heart and liver weight in broilers.

It has been reported that the supplementation of Res to broiler rations helps chickens increase their antioxidant activities, and also contributes to the improvement of protein and total antioxidant capacity in plasma [22]. Also, Res could increase the expression of various antioxidant enzymes and reduce MDA content [28,33]. It has been determined that with the addition of Res, serum MDA level was decreased and serum GSH level in broilers was increased [27]. Sahin et al.[34] have noticed that serum MDA level was not affected in laying quail, differ from this study. Liu et al.[18] stated that adding 400 mg/kg Res to

chick rations increased antioxidant capacity and decreased MDA content. NO has a vital role as diverse physiological including, vasodilation and inflammation in cells [35]. Limited availability of studies was found about the effect of resveratrol on serum NO levels in poultry. In the current study, the effect of Res on serum NO can be considered as an initiator study investigating.

The inclusion of Res in quail diets did not affect GL, TG, and TP as compared to the control group. Zhang et al.[28] reported that with the addition of Res to broiler rations, triglyceride and total protein levels were affected and glucose levels were decreased compared to the control group. He et al.[27] reported that the supplementation of Res reduced serum glucose and total protein levels and increased the level of triglycerides. It has been reported that although the total protein level increased with the effect of Res, triglyceride and glucose levels did not change in broilers [22].

In conclusion, the study showed that Res has a positive effect on quail performance parameters as FC and FCR. Also, dietary res may reduce the serum MDA level that negatively affects health status of quails. So, Res can be supplemented to Japanese Quail rations as a feed additive (up to 400mg/kg). Besides, it is recommended to research using Res at different levels to reveal its antioxidant and biochemical effect in prospective poultry experiments.

Conflict of Interest

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Author Contributions

Experimental Design; MÖ, TŞ and MM, collecting data for analysis; ÖK and MM, analysis of samples; MM, Software; MÖ and MM, Writing-original draft; MÖ, Writing-review and editing; TŞ.

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