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Effects of mulberry leaves on growth performance, carcass characteristics, and meat quality of Japanese quail

A.M. Ustundag#, M. Ozdogan
Department of Animal Science, Faculty of Agriculture, Adnan Menderes University, Aydın, Turkey

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Abstract

This experiment was conducted to determine the effects of mulberry leaves on performance, carcass characteristics, and meat quality of Japanese quail. A total of 240 one-day-old Japanese quail chicks were randomly allocated to three experimental groups and fed a basal diet (control) and the basal diet plus 50 g/kg and 100 g/kg mulberry leaf meal (MLM) for 42 days. By the end of the study, performance parameters of quail fed the 100 g/kg MLM-supplemented diet were affected negatively. The highest carcass weight and carcass yield levels were exhibited in the control group. In terms of meat quality, the shear force of the breast meat of quail fed the diet with 100 g/kg supplemented MLM was higher than the other groups. The highest L* and hue angle values of breast meat were found in the 50 g/kg MLM group. Released water from the thigh meat in the control group was higher than in the experimental groups. The current study indicates that 50 g/kg MLM can be used easily in quail rations without any negative effects.

Keywords: carcass parameters, meat quality, mulberry leaf meal, performance, quail
Corresponding author: austundag@adu.edu.tr

Introduction

The poultry industry is a very important sector that provides the cheapest animal protein source for human consumption within the shortest production period. Quail are a potentially good source of quality animal protein since quail are very resistant to different diseases, achieve sexual maturity at the age of 6 weeks, and adapt quickly to different rearing conditions (Kamruzzaman *et al*., 2014, 2018; Mnisi *et al.,* 2021; Shehata *et al.,* 2021). Fast growing quail require 24% protein to fully express their genetic potential, and soybean meal is used as the main protein source to meet this requirement. However, the cost of protein sources such as soybean and fish meal account for 15–40% of the total feed cost. Feed costs constitute a major proportion of 60–70% of poultry production costs and the poultry industry is heavily dependent on feed costs. The price of soybean is increasing daily due to its high demand by the food, biofuel, and animal sectors and this precludes economic production. Nevertheless, the growing demand for organic farming has developed consumer resistance against soybean derived from genetically modified crops. Therefore, finding economical, cost-effective, and locally-available feed alternatives to conventional feed sources can help to reduce poultry feed costs (Olarotimi & Adu, 2017; Ezeakacha *et al*., 2018; Kamruzzaman *et al*., 2018; Sebola *et al*., 2018; Tilahun *et al*., 2018 ; Ait-Kaki *et al.,* 2021; Mnisi *et al.,* 2021; Siddiqui *et al.,* 2022).

Plant leaves are potential alternative sources to protein-rich feedstuffs, especially sought after by smallholders and large-scale poultry farmers where there are feed deficits and high feed costs (Sugiharto & Ranjitkar, 2019; Gulizia & Downs, 2020; Sugiharto, 2021). Despite that the nutrient content of the leaves varies considerably from one plant to another, they are a good source of protein, vitamins, minerals, and essential amino acids. They also contain active ingredients such as flavonoids, glycosides, polyphenols, carotenoids, fats, alkaloids, saponins, and tannins with medicinal properties (Ashour *et al.,* 2020; Tokofai *et al.,* 2020). Therefore, various studies have been conducted with many plants regarding the usability of leaves as an alternative protein source for poultry. In the studies

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conducted with Japanese quail, it was observed that the growth performance (François *et* al., 2020; Talukdar *et al.,* 2020), egg production and egg quality (Ashour *et al.,* 2020; Garcia *et al.,* 2021) of Japanese quail increased with the addition of *Moringa oleifera*. Gadzirayi *et al*. (2012) concluded that *Moringa oleifera* leaf meal could be substituted for soybean meal up to 25% in broiler diets. Kamaruddin *et al.* (2021) reported that the addition of *Indigofera zollingeriana* leaf meal up to 10% increased the protein digestion of quail. It has also been reported that *Indigofera zollingeriana* can be included in a diet up to 20% without deleterious effects on performance and egg quality of quail (Purwanti *et al*., 2020; Has *et al*., 2021). Sarmad *et al.* (2020) reported that the addition of rosemary powder up to 2.5% increased the performance of quail. In another study conducted with olive leaves, it was reported that up to 3% olive leaves could be added to the ration of quail (Ait-Kaki *et al.,* 2021).

Mulberry is a deciduous perennial woody plant, belonging to *Morus* of Moraceae. Mulberry leaves have a great potential as an alternative protein source for the poultry industry due to rich protein, mineral, and metabolisable energy contents (Cai *et al*., 2019; Ding *et al.,* 2021). Besides the nutritive value, mulberry leaves are nontoxic natural therapeutic agents which are due to include the various phytochemicals (flavonoids, alkaloids, steroids and terpenoids), known to possess antidiabetic, antimicrobial, antimutagenic, antioxidant, anticancer, anxiolytic, anthelmintic, antistress, immunomodulatory, hypocholesterolaemic, nephroprotective, and hepatoprotective activities (Chen *et al.,* 2019; Dhiman *et al*., 2020). However, due to the high content of crude fiber in mulberry leaves and the presence of anti-nutritional factors such as tannin, the excessive addition of mulberry leaves can adversely affect the health of animals by reducing the digestion of nutrients (Cai *et al*., 2019; Ding *et al.,* 2021). In comparison to soybean meal, the main source of protein in commercial poultry feed, mulberry leaves have a higher amino acid content, which may compensate the adverse effect of the fibre on broilers, in particular (Shelke *et al.,* 2018).

Many studies have demonstrated that mulberry leaves have successfully been used as a dietary protein source in aquaculture and in poultry and livestock farming (Lin *et al.,* 2017; Cirne *et al.,* 2019; Kong *et al.,* 2019; Liu *et al.,* 2019; Ali *et al.,* 2020; Muthoni *et al.,* 2020; Neamat-Allah *et al.,* 2021). Shehata *et al.* (2021) reported that mulberry leaves can be included in quail diets up to 8% without adverse effects on growth parameters and economic measures, with no oxidative stress and no alteration in quail immunity. It has also been reported that egg production was increased by adding 10% mulberry leaf meal in layer diets (Muthoni *et al.,* 2020).

The current study was conducted to determine the effects of mulberry leaves on growth performance, carcass characteristics, and meat quality of Japanese quail.

Material and Methods

The experimental procedures described were approved by the Aydın Adnan Menderes University Animal Ethics Committee (64583101/2019/027).

Fresh mulberry leaves were collected from the mulberry trees on the Aydin Adnan Menderes University, Faculty of Agriculture campus and leaves were dried in the shade under room temperature. After drying, leaves were ground into a powder and the nutrients of mulberry leaf meal were determined (Table 1).

A total of 240 1-day-old Japanese quail chicks (*Coturnix coturnix japonica*) of mixed sex were randomly allocated to three experimental groups with four replicates of twenty chicks in each. The basal diet was based on corn and soybean meal and was balanced to meet the nutrient requirements for quail (NRC, 1994). The experimental diets consisted of the inclusion of mulberry leaf meal (MLM) at 0 g/kg (Control), 50 g/kg (MLM5), and 100 g/kg (MLM10).

Table 1 Chemical composition of mulberry leaves

Nutrient	Composition
Dry matter, g/kg	890
Crude protein, g/kg	200
Ether extract, g/kg	55
Crude fiber, g/kg	110
Ash, g/kg	118
Calcium, g/kg	27.3
Available Phosphorus, g/kg	2.8
Metabolizable energy, MJ/kg	7.44

The chemical composition of the experimental diets was determined based on the methods of the Association of Official Analytical Chemists (AOAC, 1997) (Table 2). The trial period lasted 42 days and feed and water were given *ad libitum* during the experimental period.

The body weight (BW) and feed intake (FI) of quail were recorded weekly during the experiment. Body weight gain (BWG) was determined by the taking body weight differences between weeks. Feed conversion ratio (FCR) was calculated as the ratio of feed intake over body weight gain.

At the end of the study, five male quail were randomly picked from each replication. Each bird was weighed, and pre-slaughter weights were determined. After slaughter, internal organs were removed and warm carcass, liver, heart, and gizzard weights were measured. To determine the cold carcass weight, the carcasses were kept in the refrigerator at 4 ºC for 24 h and weighed again. Carcass yield was calculated as a percentage of pre-slaughter weight. After slaughter, the right thighs and breasts of 60 birds were taken to determine the meat colour, water holding capacity (WHC), and shear force (SF).

Table 2 The ingredients and chemical composition of the experimental diets

Ingredients, (g/kg)	Control	MLM5	MLM10
Corn	470	426.5	390.1
Soybean meal	460	447.5	430
Vegetable oil	39.4	49	56.5
Mulberry leaf meal	0	50	100
Dicalcium phosphate	15.5	15.5	15
Calcium carbonate	8	4.5	1.5
Salt	3	3	3
DL-Methionine	0.6	0.5	0.4
Vitamin and mineral premix[1]	3.5	3.5	3.5
Nutrient Composition, (g/kg)			
Crude protein[2]	240.9	241.3	241.1
Calcium[3]	8.6	8.5	8.5
Available phosphorus[3]	4.5	4.6	4.6
Methionine[3]	4.2	4.2	4.2
Lysine[3]	13.4	13.9	14.3
Metabolizable energy, MJ/kg[3]	12.14	12.16	12.16

[1] Supplied per kg of diet: retinyl acetate 5.16 mg, cholecalciferol 0.07 mg, D-alpha-tocopherol 50 mg, menadione nicotinamide bisulfite 50 mg, thiamin 3 mg, riboflavin 6 mg, pyridoxine HCL 5 mg, cobalamin 0.03 mg, ascorbic acid 50 mg, niacin 55 mg, biotin 0.075 mg, folic acid 1 mg, pantothenic acid 12 mg, choline chloride 200 mg, manganese 80 mg, zinc 60 mg, iron 60 mg, copper 5 mg, iodine 1 mg, selenium 0.15 mg, cobalt 0.2 mg
[2] Determined according to AOAC (1997)
[3] The nutrient content of diets was calculated according to Dale and Batal (2003)

Meat colour was measured on surface area of breast and thigh meat samples (three measurements per sample). Colour determination of the samples was carried out using a colorimeter (Color Flex EZ, Hunterlab colorimeter, U.S.A.) equipped with standard D65 illuminant using a 2° position of the standard observer with a pulse xenon lamp and 8-mm reading surface area. CIE L*(lightness), a*(redness), and b* (yellowness) values were recorded. The instrument was calibrated with a white and black standard plate before measurements. Chroma (saturation index) and hue angle values were calculated from a* and b* values according to following formulae (AMSA, 2012):

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$
$$\text{Hue angle } (^{o}) = \arctan (b^{*}/a^{*})$$

Water holding capacity (WHC) of thigh and breast meat samples was determined based on release water (RW) and cooking loss (CL). Release water (RW) was determined using the method described by Joo (2018). Briefly, a 3-g meat sample was placed on a previously dried and weighed filter paper (Whatman No. 1 of 11 cm of diameter) with two thin plastic films and then the filter paper and plastic films with meat sample were placed between Plexiglas plates. A load of 2.5 kg was applied for 5 min. After precisely removing the compressed meat, the wet filter paper and plastic films were quickly weighed. The percentage of RW was calculated as follows:

$$\text{Release Water \%} = \frac{\text{damp filter paper and plastic film weights} - \text{filter paper and plastic film weights}}{\text{Meat sample weight}} \times 100$$

Cooking loss (CL) was determined as described by Honikel (1998). Breast and thigh meat samples were weighed, placed into a sealed polyethylene bag, and cooked in water bath at 80 °C for 30 min. The cooked meat samples were cooled down to room temperature under tap water and then reweighed. Cooking loss was calculated using the formula:

$$\text{Cooking Loss \%} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100$$

After the determination of cooking loss, cooked breast and thigh meat samples were used for shear force (SF) measurement. Two strips (2.5 × 1.0 × 1.0 cm^3) were cut parallel to the muscle fibres. Meat samples were shared perpendicular to the fibre direction using a Zwick Testing Machine Model Z2.5/TN1S (Zwick GmbH and Co, Germany) equipped with a Warner-Bratzler shear. Test speed was set at 2 mm/s. Maximum shear force values were obtained in Newton force (N).

The data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS 8 software (SAS, 1999). The differences between the means were determined using the LSD multiple comparison test at a significance level of P <0.05.

Results and Discussion

The effects of mulberry leaf meal addition on performance parameters of quail are given in Table 3. Body weight gain decreased by 7.80% in quail fed the 10% mulberry leaf meal diet compared to the control group in the starter phase (0–21 days). There was no difference between the control and 50 g/kg mulberry leaf meal group. The highest feed intake and feed conversion ratio values were observed in quail fed mulberry leaf meal. While there was no difference between the groups in terms of body weight gains between day 21 and day 42, feed consumption and feed conversion ratio values were higher in quail fed 50 g/kg and 100 g/kg mulberry leaf meal. During the entire trial, body weight gain decreased by 7.12% with an addition of 100 g/kg mulberry leaf meal compared to the control group. While feed intake increased by 25.92% and 24.79% respectively, with the addition of 50 g/kg and 100 g/kg mulberry leaf meal, the feed conversion ratio increased by 31.27% and 34.50%, respectively.

Table 3 Effects of mulberry leaf meal (MLM) on growth performance of quail

	Experimental Groups				
	Control	MLM5	MLM10	SEM	P-value
0–21 days					
BWG (g/bird)	107.09^{a}	105.60^{a}	98.74^{b}	0.957	0.013
FI (g/bird)	270.35^{b}	325.78^{a}	325.70^{a}	4.532	0.01
FCR	2.52^{c}	3.09^{b}	3.30^{a}	0.070	0.0003
22–42 days					
BWG (g/bird)	89.33	82.92	83.68	1.793	0.328
FI (g/bird)	458.10^{b}	591.46^{a}	583.33^{a}	4.532	0.0004
FCR	5.16^{b}	7.16^{a}	6.98^{a}	0.028	0.002
0–42 days					
BWG (g/bird)	196.41^{a}	188.52^{ab}	182.42^{b}	1.945	0.048
FI (g/bird)	728.44^{b}	917.24^{a}	909.03^{a}	13.676	0.0005
FCR	3.71^{b}	4.87^{a}	4.99^{a}	0.070	0.0001

Control: basal diet; MLM5: 50 g/kg mulberry leaf meal; MLM10: 100 g/kg mulberry leaf meal; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SEM: standard error of the mean; means within a row with no common superscript differ (P <0.05)

Nutrient digestion and absorption play an important role in improving growth performance. In the results of previous studies, it has been reported that the addition of increasing amounts of mulberry leaves to the diets leads to decreases in body weights and nutrient digestion of animals. Olmo *et al*. (2012) noted that inclusion of 10–30% mulberry leaf meal in the diets of broiler chicken markedly decreased performance. Similarly, Has *et al.* (2013) reported that the inclusion of 10% and 20% of mulberry leaf reduced performance in broilers. It was concluded that the addition of 7,5–22,5% mulberry leaf meal in diets decreased performance of broilers in the result of another study (Tilahun *et al.,* 2018). Wang *et al.*

(2017) reported that the addition of 4–10%mulberry leaf meal in goose diets adversely affected the performance of the geese. It has been reported that the high fiber content and some anti-nutritional factors in mulberry leaves can substantially shorten the retention of nutrients in the digestive tract, reducing digestion time and nutrient absorption (Has *et al*., 2013; Wang *et al.*, 2017; Ding *et al*., 2021; Jha & Mishra, 2021; Tejeda & Kim, 2021). Despite the decrease in live weights in our study, the highest feed consumption was observed in the MLM5 and MLM10 groups, indicating that animals were trying to meet their nutrient requirements due to decreased nutrient digestion.

However, in various studies, it has been reported that adding up to 10% MLM to laying hen (Panja, 2013; Lin *et al*., 2017; Kamruzzaman *et al*., 2018; Siddiqui *et al.,* 2022), broiler (Al-Kirshi *et al*., 2010; Olteanu *et al*., 2015; Ding *et al.,* 2021) and quail (Sengul *et al.,* 2021; Shehata *et al.,* 2021) diets did not affect performance. In contrast to these findings, Herrera *et al*. (2014) and Islam *et al*. (2014) reported that 3% and 4.5% MLM supplementation, respectively, affected broiler performance positively. It has been reported that performance of quail fed 10% and 20% MLM was substantially higher than the control group (Perdomo *et al.,* 2019). Likewise, Simol *et al*. (2012) reported that adding up to 30% MLM to broiler diets did not negatively affect bird performance and reduced feed costs.

Cutting and carcass parameters of birds are given in Table 4. Slaughter, liver, gizzard, and heart weights were not statistically different between groups. Carcass weights and carcass yields in the control group were higher than the experimental groups (P <0.05). Similarly, it was reported that the addition of MLM in the diets decreased the carcass weights of the chickens (Olmo *et al*., 2012; Herrera *et al*., 2014; Tilahun *et al*., 2018).

Table 4 Effects of mulberry leaf meal (MLM) on carcass parameters of quail

Parameters	Control	MLM5	MLM10	SEM	*P*-value
Slaughter weight, g	189.12	176.43	182.38	2.878	0.213
Warm carcass weight, g	137.52^{a}	123.34^{b}	127.74^{ab}	2.228	0.041
Warm carcass yield, %	72.75^{a}	70.08^{b}	70.00^{b}	0.003	0.001
Cold carcass weight, g	136.60^{a}	122.19^{b}	126.72^{ab}	2.208	0.035
Cold carcass yield, %	72.17^{a}	69.17^{b}	69.42^{b}	0.003	0.0002
Liver weight, g	3.62	2.92	3.10	0.150	0.162
Gizzard weight, g	3.48	3.68	4.03	0.103	0.101
Heart weight, g	1.42	1.38	1.54	0.027	0.073

Control: Basal diet; MLM5: 50 g/kg mulberry leaf meal; MLM10: 100 g/kg mulberry leaf meal; SEM: standard error of the mean; means within a row with no common superscript differ (P <0.05)

Meat quality is one of the most important economic features which mainly determines consumer preference (Rehman *et al*., 2018; Liu *et al*., 2019). When it comes to meat quality, the main considerations are water holding capacity, cooking yield, meat colour, and shear force (Liu *et al*., 2019). Water holding capacity, cooking loss, and shear force reflect the tenderness of meat (Rehman *et al*., 2018; Wu *et al*., 2019). Meat colour is an important quality that determines the visual appearance of the meat and can directly influence meat buying decisions (Zhang *et al*., 2016). L*, a* and b* are indicators of meat colour. The a* value expresses the quality and freshness of the meat and the L* value expresses the gloss of meat (Ding *et al*., 2021). Effects of MLM on breast and thigh meat quality parameters of quails are given in Table 5. In the present study, there was a difference in RW of the thigh meat between groups. The RW value of control group was higher than the experimental groups.

Shear force of breast meat of animals fed 100 g/kg supplemented MLM was higher than that of the other groups in this study. Meat tenderness (shear force) mainly depends the amount of connective tissue, muscle fibre type, diameter of intramuscular fat, and the extent of proteolysis in rigor muscle (Joo *et al.,* 2013; Kim *et al.,* 2013; Liu *et al.,* 2019). Muscle fibres are commonly classified as type I (slow-twitch, oxidative), IIA (fast-twitch, oxidative glycolytic), and IIB (fast-twitch, glycolytic). Kim *et al*. (2013) reported that muscles mainly composed of type IIB fibres were more susceptible to early post-mortem proteolytic degradation than muscles composed mainly of type I fibres. Therefore, type IIB fibres are closely related to toughness, paleness, protein denaturation, and a low water-holding capacity. Muscles with a larger size of type IIB fibres exhibit tougher meat or greater hardness. Our results are in line with this report. Similar results were also observed in a study investigating the effects of MLM supplementation on poultry meat quality (Ding *et al*., 2021). Similar results were observed in pigs (Liu *et al*., 2019; Zeng *et al*., 2019) and rabbits (Wu *et al*., 2019; Hou *et al*., 2020) with the addition of MLM. However, the mechanism underlying how mulberry leaf changed the muscle fibre types is unclear.

Table 5 Effects of mulberry leaf meal (MLM) on meat quality of quail

Parameters	Meat Part	Control	MLM5	MLM10	SEM	*P*-value
Release water, %	Breast	7.64	7.11	8.09	0.271	0.347
Cooking loss, %		21.86	22.54	23.15	0.236	0.096
Shear force, N		13.25[b]	13.00[b]	16.26[a]	0.552	0.036
L*		46.54[b]	49.70[a]	47.78[ab]	0.522	0.048
a*		9.18	8.97	9.72	0.240	0.428
b*		12.21	13.88	13.29	0.314	0.104
Chroma		15.29	16.59	16.48	0.350	0.256
Hue angle		53.05[b]	56.85[a]	53.95[b]	0.654	0.046
Parameters						
Release water, %	Thigh	5.97[a]	4.40[b]	5.13[b]	0.164	0.002
Cooking loss, %		13.72	11.44	11.11	0.594	0.166
Shear force, N		17.12	13.97	14.67	0.575	0.078
L*		47.06	46.90	47.71	0.623	0.855
a*		8.72	8.96	8.91	0.302	0.943
b*		11.61	12.19	13.09	0.342	0.220
Chroma		14.55	15.23	15.89	0.387	0.375
Hue angle		52.97	53.82	55.72	0.897	0.444

Control: Basal diet; MLM5: 50 g/kg mulberry leaf meal; MLM10: 100 g/kg mulberry leaf meal; SEM: standard error of the mean; means within a row with no common superscript differ (P <0.05)

It was found that supplementation of 50 g/kg MLM statistically increased the L* and hue angle values of breast meat. MLM supplementation also tended to increase a* and b* values of breast meat in this study. The colour of meat that depends on the pigments in the muscle, myoglobin and haemoglobin, is influenced by various factors such as genetics and nutrition (Liu *et al*., 2019; Wu *et al*., 2019). It has been reported that the improvement of meat colour with the addition of MLM may be related to the high beta carotene and iron content in the MLM (Monika, 2012; Dalle Zotte *et al*., 2016).

Conclusions

The results of this study indicate that the growth performance and carcass parameters of quail are negatively affected with the inclusion of MLM at 100 g/kg. Meat quality parameters were not adversely affected by the addition of MLM in this study. The results indicate that 50 g/kg MLM can be used easily in quail rations without any negative effects. It is also thought that more mulberry leaves can be used safely with the addition of enzymes to reduce the anti-nutritional factors. Different results have been obtained in studies investigating the effect of MLM on various animal species. Therefore, further research is needed to elucidate the underlying mechanism of the mode of action and find a suitable level of MLM that will not adversely affect the performance and meat quality parameters of poultry.

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Authors' Contributions

AOU designed and conducted the study, analysed the data, and wrote the manuscript; MO conducted the study, reviewed, edited, and prepared the original draft.

Conflict of Interest Declaration

The authors declare that they have no conflicts of interest relative to the content of this paper.

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