Journal MVZ Cordoba
2021; January-April. 26(1):e1940.
https://doi.org/10.21897/rmvz.1940

Original

The effect of dried white mulberry (*Morus alba*) pulp supplementation in diets of laying quail

Ahmet Yusuf ŞENGUL[1*] Ph.D; Turgay ŞENGUL[1] Ph.D; Şenol CELİK[1] Ph.D; Gülüzar ŞENGÜL[2] Ph.D; Aydın DAŞ[3] Ph.D; Hakan İNCİ[1] Ph.D; Aydın Şukru BENGU[4] Ph.D.

[1]Universidad de Bingol, Facultad de Agricultura, Departamento de Ciencias Animales, Bingol-Turquía.
[2]Universidad de Bingol, Facultad de Ciencias Veterinarias, Departamento de Ciencias Animales, Bingol-Turquía.
[3]Universidad de Harran, Facultad de Ciencias Veterinarias, Departamento de Ciencias Animales, S.Urfa-Turquía.
[4]Universidad de Bingol, Escuela Vocacional de Servicios de Salud, Departamento de Servicios y Técnicas Médicas, Programa de Laboratorio Médico, Bingol-Turquía.
*Correspondencia yusufsengul24@hotmail.com

Received: March 2019; Accepted: October 2020; Published: November 2020.

ABSTRACT

Objective. This study was conducted to research the effects of different levels of dried white mulberry *(Morus alba*) pulp supplementation in diets of laying quail on yield performances, egg quality, blood parameters, yolk fatty acid profiles and cholesterol concentrations. **Materials and Methods.** A completely randomized experimental design, with four treatments and four replicates, was applied. The experimental treatments were M0: control diet; M4: dietary inclusion of 4% mulberry pulp; M8: dietary inclusion of 8% mulberry pulp; M12: dietary inclusion of 12% mulberry pulp. This experiment was carried out for 4 weeks, and 128 7-week-old female quail were used. **Results.** Addition of dried mulberry pulp to the diet significantly affected weekly feed intake, egg yield, albumin index, yolk weight, triglyceride, LDL, serum cholesterol and yolk cholesterol levels ($p<0.05$, $p<0.01$). The feed conversion ratio, egg weight, and egg yolk fatty acid profile were not significantly affected by the dried mulberry pulp in the diet. **Conclusions.** As a result, it may be stated that adding dried mulberry pulp up to 8% of the diets of laying quail does not cause any adverse effects and may be used without any problems.

Keywords: Blood analysis; egg quality; egg production; fatty acid; feed intake; yolk cholesterol (*Source: CAB Thesaurus*).

RESUMEN

Objetivo. El presente estudio tiene por objetivo investigar los efectos de los diferentes niveles de suplemento de la pulpa de mora blanca seca (Morus alba) en las dietas de la codorniz ponedora, el rendimiento, la calidad del huevo, los parámetros sanguíneos, perfiles de los ácidos grasos de la yema y las concentraciones de colesterol. **Materiales y Métodos.** Fue aplicado un diseño experimental totalmente aleatorio, con cuatro tratamientos y cuatro réplicas. Los tratamientos experimentales fueron M0: dieta de control; M4: introducción de 4% de pulpa de mora en la dieta; M8: introducción

How to cite (Vancouver).
Sengul Ay, Sengul T, Celik S, Sengul G, Das A, Inci H, Bengu AS. The effect of dried white mulberry (*Morus alba*) pulp supplementation in diets of laying quail. Rev MVZ Cordoba. 2021; 26(1):e1940. https://doi.org/10.21897/rmvz.1940

ISSNL: 0122-0268

de 8% de pulpa de mora en la dieta; M12: introducción de 12% de pulpa de mora en la dieta. Este experimento se llevó a cabo durante 4 semanas, y se utilizaron 128 codornices hembras de 7 semanas de edad. **Resultados.** La agregación de pulpa de morera seca a la dieta influyó notablemente en la ingesta semanal de piensos, la producción de huevos, el índice de albúmina, el peso de la yema, el triglicérido, LDL, los niveles de colesterol en el suero y el colesterol en la yema ($p<0.05$, $p<0.01$). La tasa de proporción de conversión del pienso, el peso del huevo y el perfil de ácidos grasos de la yema de huevo no se vieron afectados significativamente por la pulpa de mora seca en la dieta. **Conclusiones.** Como resultado, se puede señalar que la agregación de pulpa de more seca hasta el 8% de las dietas de codorniz ponedora no causa ningún efecto adverso y se puede utilizar sin ningún problema.

Palabras clave: Análisis sanguíneo; calidad del huevo; producción de huevos; ácidos grasos; ingesta de pienso; colesterol de la yema (*Fuente: CAB Thesaurus*).

INTRODUCTION

Mulberry is a kind of fruit grown in many parts of the world due to its high adaptability to different climatic and soil conditions (1). Mulberry originates from four main species, the white mulberry (*Morus alba*), M. multicaulis, M. bombycis and M. atropurpurea. The varieties of mulberry which are adapted to different climatic conditions can be grown in areas up to 4,000 m above sea level and in damp and semi-arid areas. Although mulberry (*Morus sp.*) cultivation is generally carried out for silkworm breeding in the world, in some countries, the plant is cultivated for its leaves and fruits which are used as animal feed (2).

Fresh mulberry (*Morus alba*) is reported to contain 7.8-9.0% carbohydrates, 0.5-1.4% protein, 0.3-0.5% fatty acids (linoleic, stearic and oleic acids in seeds), 1.1-1.8% free acid (mainly malic acid), 0.9-1.3% fiber, 0.8-1.0% ash and 85-88% moisture (2).

Mulberry is a fruit that has been produced in Turkey for thousands of years and is consumed in different ways including fresh and dried. Turkey's total annual production of mulberry is 69,334 tons (3), and 97% of this production is white mulberry (4). While the mulberry fruit is consumed as fresh and dried, it is also used for production of goods such as molasses, jelly, dried rollup, mulberry paste, ice cream, churchkhela, vinegar, concentrated fruit juice and methylated spirit (5). Pekmez is a Turkish food, produced in Turkey's rural areas traditionally and enjoyed in the winter season. Mulberries harvested in the summer season are boiled for a few hours in large cauldrons while they are fresh, and then juices and pulp are separated by pressing. (6). The remaining pulp after pressing the boiled mulberry to make molasses is dried by spreading under the sun. The dried mulberry pulp is generally milled and fed to ruminants. When Turkey's annual production of mulberry is considered, it may be guessed how high the pulp volume released after processing will be. The chemical composition of sun-dried mulberry pulp is reported as 855.9 g/kg (fresh sample) of dry matter, 876.0 g/kg DM of organic matter, 218.6 g/kg DM of crude protein, 208.5 g/kg DM of WSC, 490.6 g/kg DM of NDF and 379.6 g/kg DM of ADF (7).

It is not common practice to feed mulberry pulp to poultry. The high price of feed raw materials is one of the most important problems in animal production which increase costs and reduce profitability. This raises the use of waste products of the agricultural production and food processing industry in animal nutrition. Mulberry pulp is one of the pulps that can be used in animal breeding. It can be used for feeding animals, and it is usually preserved by sun-drying or storage in silos.

Supplementation of mulberry meal has a potential for reduction of blood cholesterol, it could modulate the antioxidative status of laying hens and improve their production performance and egg quality, and without adverse effects, it could used in the diets of laying hens to a certain level (8,9,10). Liu et al (11) reported that dried and ground mulberry promotes beneficial cholesterol and helps regulate carbohydrate digestion. Some researchers suggested that mulberry leaf meal addition may be utilized by up to 10% without any adverse effects (8,12).

In particular, there is a need for studies on the effects of mulberry pulp use in poultry diets. Therefore, a study was planned for the first time on poultry, and the effects of mulberry pulp in the diets of quail during laying were investigated.

MATERIALS AND METHODS

Housing. The animal material of the study consisted of 128 female laying Japanese quail (*Coturnix coturnix japonica*) aged 7 weeks. The study was conducted in a windowed coop of Bingol University, Faculty of Agriculture, Department of Animal Science, eastern part of Turkey in Bingol (38.8855 latitude and 40.4966 longitude). The experiment was designed in four dietary treatments with four replications of each. A total of 128 laying female quail were used in the experiment, 8 per repetition. The study was conducted in February-March of 2019 and completed in 4 weeks. The quail were housed in 96 x 42 x 30 cm compartments in 6-story cages during the experiment. The lighting program was applied as 16 hours light - 8 hours dark.

Diets. The mulberry pulps that were used in the study were obtained by collecting the remaining wet pulps after molasses production. The collected mulberry pulps were dried under the sun for 4 days and then ground in the laboratory. Table 1 shows the chemical composition analysis results of the dried mulberry.

Table 1. Chemical composition of dried mulberry pulp.

DM (%)	CA (%)	OM (%)	EE (%)	CP (%)	Sugar (%)	Starch (%)	ME (kcal/kg)
96.83	6.18	90.65	10.05	12.36	45.34	0	2692.63

DM: Dry matter, CA: Crude Ash, OM: Organic matter, EE: Ether extract, CP: Crude protein, ME: Metabolizable energy

Nutrient analyses of all raw materials to be used in the diets were conducted, and the experimental diets were prepared according to the results of these analyses. In the experiment, 4 different diets were used, one as control (without mulberry pulp) and 3 as treatment groups (containing 4%, 8% and 12% mulberry pulp). The diets of quail belonging to the experimental groups were prepared as isocaloric and isonitrogenic. All groups were fed *ad libitum* with feed containing 20% HP and 3000 kcal/kg ME during the experiment (Table 2).

Metabolizable energy (ME) is calculated using the following formula (13):

ME (kcal/kg) = 37.07*Protein+ 82*Oil + 39.89*Starch
+ 31.1*Sugar

Table 2. Diets used in the experiment and their analyzed and calculated nutrient values.

	Diets			
Ingredients	**M0**	**M4**	**M8**	**M12**
Corn, %	53.39	49.47	45.55	41.63
Soybean meal, %	32.32	31.96	31.59	31.23
Dried mulberry pulp, %	0	4	8	12
Sunflower oil, %	6.74	7	7.26	7.52
Limestone, %	5.44	5.43	5.42	5.42
DCP, %	1.59	1.62	1.64	1.66
Methionine, %	0.06	0.07	0.08	0.09
Salt, %	0.20	0.20	0.20	0.20
Vit-Min., %	0.25	0.25	0.25	0.25
Total	100	100	100	100
Analyzed values				
ME, kcal/kg	3000	3000	3000	3000
Crude protein, %	20	20	20	20
Ether extract, %	8.65	9.18	9.71	10.23
Crude fiber, %	2.43	2.33	2.23	2.13
Crude Ash, %	10.04	10.23	10.41	10.60
Ca, %	2.5	2.5	2.5	2.5
P, %	0.64	0.64	0.63	0.62
K, %	0.81	0.80	0.78	0.77
Mg, %	0.19	0.18	0.18	0.17

M0: Control, M4: 4% mulberry pulp, M8: 8% mulberry pulp, M12: 12% mulberry pulp, Vit- min. = Vitamin A 12000000 IU, Vitamin D_3 2000000 IU, Vitamin E 35000 mg, Vitamin K_3 5000 IU, Vitamin B_1 3000 mg, Vitamin B_2 6000 mg, Vitamin B_6 5000 mg, Vitamin B_{12} 15 mg, Vitamin C 50000 mg, D-Biotin 45 mg, Niacin 20000 mg, Ca D Pantothenate 6000 mg, Folic acid 750 mg, Choline chloride 125000 mg, Mangan 80000 mg, Iron 60000 mg, Zinc 60000 mg, Copper 5000 mg, Iodine 1000 mg, Cobalt 200 mg, Selenium 150 mg, Canthaxanthin 15.000 mg, ß-apo-8'- Carotenoic acid ethyl ester 5.000 mg.

Feed conversion ratio (FCR) is calculated using the following formula (14):

FCR = (FI (g of feed/bird/period))/(EM (g of egg/bird/period))

FI: Feed intake
EM: Egg mass

Parameters. In this study, the feed intake, feed efficiency, egg yield, egg weight, egg quality characteristics, some blood parameters, yolk fatty acid profile and cholesterol levels of quail were determined. The feed consumption, egg yield and egg weight of the groups were measured daily. Egg quality characteristics were measured twice, in the 2nd and 4th weeks. Blood parameters, yolk fatty acid profiles and yolk cholesterol levels were determined by the samples taken at 4 weeks.

Egg quality measurement. An electronic scale was used for weighing the eggs with

0.01 g accuracy, a digital caliper was used for egg width and length measurements, a tripod micrometer was used for yellow and white heights, and a digital micrometer was used for measuring shell thickness. The eggs being examined were collected for 3 days and stored at room temperature for 24 hours before the measurements. The eggs were first numbered then weighed. The width and length of the eggs were then measured. After these procedures, 10 minutes after the eggs were broken on a glass on the prepared table, measurements related to internal quality were made. The weights of the egg yolks were determined by separating the yolks from the egg whites. On the other hand, the eggshells were washed in water and dried at room temperature for 24 hours. The dried shells were first weighed together with the shell membranes, and their weights were determined. Then, the thickness of the shell was measured from three different places, namely the pointed, blunt and middle parts of the egg, and the shell thickness was determined by averaging these three measurements. The yolk color of the eggs was determined using the DSM Yolk Color Fan.

The shape index is calculated using the following formula:

Shape Index=(Egg width (mm))/(Egg length (mm)) x 100

Albumen index. Albumen height of the broken egg was measured with a three-legged micrometer, and albumen length and width were

measured with a digital caliper. The albumen index is calculated using the following formula.

Albumen Index=(Albumen height (mm))/([Albumen length (mm) + Albumen width (mm)]/2) x 100

Yolk index. Egg yolk was measured with a three-leg micrometer, yolk diameter was measured with a digital caliper. The yolk index is calculated using the following formula.

Yolk Index=(Yolk height (mm))/(Yolk diameter (mm)) x 100

Haugh Unit. Haugh unit was calculated using the formula.

HU = 100 log (H + 7.57 - 1.7 * W0.37)
H = Albumen height (mm)
W = Egg weight (g)

Blood analysis. At the end of the experiment, 3 animals from each group (1 from each repetition group) were slaughtered, and their samples were placed into yellow-cap blood collection tubes with BD Vacutainer gel. After the tubes were centrifuged at 3000 rpm for 10 minutes, the samples obtained were stored frozen at -80°C until analysis. Glucose (Beckman Coulter Glucose OSR 6121), triglyceride (TG), LDL-cholesterol, total cholesterol, Ca, P, Mg, K and Na analyses of the serum samples were carried out with an auto-analyzer (Olympus AU400 Chemistry Analyzer-OLY-AU400), using commercial-use kits (Beckman Coulter OSR) and by the photometric method.

Lipid extraction. The method described by Folch (15) was used to determine the fatty acid profiles in egg yolks. For this purpose, an Optima brand delta-6-0.25 µm (100 m × 0.25 mm ID) column, an Agilent 7890A / 5970C brand Gas Chromatography / Mass Spectrometry (GC-MS) device and an FID detector was used. Chromatographic conditions were as follows: Oven temperature started at 120°C, reached 250°C (5°C/min) and was kept at this temperature for 3 minutes. Then it reached 270°C (2°C/min), it was kept at this temperature for 16 minutes, and this process lasted 55 minutes in total. The injection volume was 1 µl. Before and after each injection, the syringe was washed 5 times with hexane. The main sample was withdrawn after 2 samples were withdrawn and dropped into the waste bottle. Thus, contamination from the previous sample was prevented.

Determination of cholesterol. For the cholesterol analysis of the egg yolk samples, 0.3 g of sample was weighed and homogenized with 2 ml of an acetonitrile/methanol (70/30, v/v) mixture for 1 min. The homogenate was transferred into 2-ml Eppendorf tubes, centrifuged at 6000xg for 10 minutes at 4°C, and the supernatant was taken into 1 ml vials and analyzed by HPLC. A mixture of 70% acetonitrile and 30% isopropanol was used as the mobile phase; the mobile phase flow rate was 1 ml/min, and the temperature of the analysis column was set to 40°C. Supelcosil LC 18 DB (250 x 4.6 mm, 5 µm, Sigma, USA) column was used for analysis, and 202 nm was used as the detection wavelength.

Statistical analysis. The data obtained from the study were analyzed using the SAS 9.1.3 program. Analysis of variance was performed by using the PROC GLM command, and Duncan's

test was used to determine the differences between the means which were considered significant.

RESULTS

Feed consumption and feed efficiency. There were no significant differences between the control and treatment groups in the first 2 weeks of the experiment in terms of their daily feed consumption values, while the differences in the 3rd and 4th weeks were statistically significant ($p<0.05$, $p<0.01$). Table 3 shows the daily feed consumption values of the groups.

Table 3. Daily feed consumption means (g) and standard errors of the groups.

Levels	1st wk	2nd wk	3rd wk	4th wk	Overall
0	27.09 ±1.59	31.63 ±1.56	28.90 ±1.11a	29.35 ±1.23ab	29.24 ±1.23
4	30.32 ±0.78	34.66 ±1.23	28.74 ±1.30a	27.43 ±0.46bc	30.28 ±0.89
8	29.63 ±0.78	33.74 ±2.49	29.95 ±0.82a	30.90 ±1.30a	31.05 ±1.29
12	26.50 ±0.95	31.30 ±2.99	24.55 ±0.73b	24.56 ±0.41c	26.72 ±1.08
P	NS	NS	*	**	NS

a,b,c: Differences between means in the same column with different letters are significant. NS: Not significant. *: $p<0.05$, **: $p<0.01$.

Daily feed consumption of the groups started to show differences starting from the 3rd week. In the 3rd week, the control group, 4% and 8% mulberry-supplemented groups consumed more feed than the 12% group. A similar situation was observed in the 4th week, and the 12% supplemented group consumed less feed than the other groups (except the 4% group). The results showed that addition of 12% mulberry pulp to the diet caused a significant ($p<0.05$ and $p<0.01$) reduction in daily feed consumption in the 3rd and 4th weeks (Table 4).

Table 4. Means and standard errors of feed conversion ratios (g:g) of the groups.

Levels	1st wk	2nd wk	3rd wk	4th wk	Overall
0	4.20 ±0.51	3.66 ±0.19	3.81 ±0.19	4.33 ±0.26	4 ±0.24
4	3.72 ±0.22	4.03 ±0.29	4.45 ±0.42	4.46 ±0.54	4.16 ±0.33
8	3.57 ±0.06	3.58 ±0.18	4.02 ±0.40	4.69 ±0.49	4.03 ±0.24
12	3.40 ±0.10	4.18 ±0.24	4.87 ±0.19	4.88 ±0.37	4.65 ±0.29
P	NS	NS	NS	NS	NS

Differences between means in the same column are insignificant. NS: Not significant.

Although the feed conversion ratio of the control group was partially better than the treatment groups in the third week of the experiment, the difference was not statistically significant. The same situation was observed in the 4th week of the experiment, but the mean values of the feed conversion ratios of the groups were found to be closer to each other. It may be stated that addition of dried mulberry pulp up to 12% in the diets of laying quail does not cause a significant reduction in feed efficiency.

Egg production and egg weight. When the control and treatment groups were compared in terms of egg production, no significant differences were found between the groups in the 1st, 2nd and 4th weeks. In the third week, the differences between the mean egg production values of the groups were statistically significant ($p<0.01$). Table 5 shows the weekly egg production values of the groups during the 4-week period.

Table 5. Means and standard errors of egg production (%) of the experimental groups.

Levels	1st wk	2nd wk	3rd wk	4th wk	Overall
0	61.73 ±7.28	79.75 ±3.68	80.30 ±5.52a	72.52 ±1.99	73.57 ±3.05
4	74.49 ±4.45	70.41 ±4.12	65.31 ±6.06ab	64.80 ±6.47	68.75 ±4.85
8	76.53 ±5.03	80.24 ±2.77	77.38 ±5.25a	66.44 ±7.60	74.15 ±3.99
12	69.90 ±1.93	67.08 ±5.62	52.99 ±3.00b	61.72 ±1.08	59.39 ±3.96
P	NS	NS	**	NS	NS

a,b: Differences between means in the same column with different letters are significant. NS: Not significant.**: $p<0.01$.

In the third week of the experiment, the 12% mulberry-supplemented group yielded significantly ($p<0.01$) fewer eggs than the control and other treatment groups. The egg yields of the other 2 treatment groups (4%, 8% supplemented groups) were also partially reduced, but this difference was not significant. In the 4th week, the differences between the egg yields of the groups disappeared, and all groups provided similar values. It may be stated that the egg production difference between the groups in the 3rd week and the disappearance of this difference in the 4th week was because it was taking time for the quail to get used to the mulberry pulp.

When the control and treatment groups were compared in terms of egg weight, no significant differences were found between the groups.

Table 6 shows the values of the egg weights of the groups for the 4-week period. In the 3rd and 4th weeks, the quail belonging to the 12% supplemented group had lower egg weights in comparison to the other groups.

Table 6. Means and standard errors of the egg weights (g) of the groups.

Levels	1st wk	2nd wk	3rd wk	4th wk	Overall
0	10.82 ±0.17	10.68 ±0.08	10.31 ±0.29	10.15 ±0.25	10.49 ±0.15
4	11.06 ±0.30	10.59 ±0.25	10.15 ±0.22	9.86 ±0.13	10.41 ±0.22
8	11.16 ±0.28	10.62 ±0.14	10.20 ±0.23	10.19 ±0.20	10.54 ±0.16
12	11.17 ±0.22	10.35 ±0.23	9.94 ±0.14	9.65 ±0.13	10.27 ±0.15
P	NS	NS	NS	NS	NS

Differences between means in the same column are insignificant. NS: Not significant.

However, these differences were not statistically significant. It was determined that mulberry pulp added to the diet was not effective on egg weight for the first 2 weeks and caused a partial decrease in the 12% supplemented group in the 3rd and 4th weeks.

Egg Quality. In the 2nd and 4th weeks of the experiment, some quality characteristics of the eggs collected from the control and treatment groups were measured. Table 7 shows the obtained results. Significant differences were observed between the control and treatment groups in terms of the albumen index, yolk index and Haugh unit ($p<0.05$). The differences between the mean values in terms of egg weight, yolk weight, albumen weight, shell weight, shell thickness, yolk color and shape index were not significant. The values of the treatment groups in terms of the albumen index and yolk index were generally higher than the control group. However, the highest values for these two properties were obtained from the 8% supplemented group. The differences between the groups in terms of yolk weight were found to be significant ($p<0.05$), while the differences between other quality characteristics were not significant. Addition of mulberry pulp (in the 4th week) significantly affected yolk weight ($p<0.05$).

Table 7. Means and standard errors related to external and internal quality characteristics of eggs of the groups.

Characteristics	Mulberry pulp levels (%)									
	2nd week					4th week				
	0	4	8	12	P	0	4	8	12	P
Egg weight, g	10.52 ±0.14	10.47 ±0.16	10.52 ±0.12	10.51 ±0.11	NS	10.48 ±0.12	10.39 ±0.16	10.51 ±0.16	10.19 ±0.11	NS
Yolk weight, g	3.31 ±0.06	3.25 ±0.08	3.38 ±0.07	3.22 ±0.07	NS	3.19 ±0.06b	3.21 ±0.08b	3.47 ±3.47a	3.16 ±0.06b	*
Albumen weight, mm	6.36 ±0.10	6.31 ±0.11	6.27 ±0.08	6.44 ±0.06	NS	6.37 ±0.09	6.32 ±0.10	6.12 ±6.12	6.12 ±0.09	NS
Albumen index	11.07 ±0.36b	12.00 ±0.36ab	12.89 ±0.35a	11.85 ±0.45ab	*	12.44 ±0.44	13.73 ±0.66	12.59 ±12.58	13.18 ±0.59	NS
Shell weight, g	0.85 ±0.01	0.91 ±0.02	0.86 ±0.02	0.85 ±0.02	NS	0.91 ±0.02	0.86 ±0.02	0.92 ±0.03	0.91 ±0.02	NS
Shell thickness, mm	0.22 ±0.00	0.22 ±0.00	0.22 ±0.00	0.21 ±0.00	NS	0.22 ±0.00	0.22 ±0.00	0.22 ±0.00	0.21 ±0.00	NS
Yolk color	10.62 ±0.57	10.81 ±0.49	9.62 ±0.51	11.25 ±0.53	NS	10.68 ±0.60	11.37 ±0.44	10.87 ±0.44	11.56 ±0.59	NS
Yolk index	43.11 ±0.87b	44.96 ±0.77ab	46.03 ±0.57a	44.76 ±0.58ab	*	48.05 ±0.66	47.80 ±0.97	45.68 ±0.80	46.61 ±1.04	NS
Shape index	77.36 ±0.58	79.12 ±0.68	78.70 ±0.42	78.58 ±0.51	NS	77.08 ±0.57	78.46 ±0.52	76.88 ±0.75	78.78 ±0.45	NS
Haugh unit	88.34 ±0.55b	89.48 ±0.77ab	91.48 ±0.81a	89.79 ±0.74ab	*	93.03 ±0.65	93.92 ±1.08	91.77 ±0.84	93.06 ±0.99	NS

a,b: Differences between means in the same row with different letters are significant. NS: Not significant. *: $p<0.05$

Yolk fatty acid. Table 8 shows the fatty acid profiles of the egg yolks collected from all groups in the 4th week of the experiment. The differences between the mean values of the fatty acid levels of the control and treatment groups were not significant. It may be stated that addition of different amounts of mulberry pulp to the diet has no negative effect on the egg yolk fatty acid profiles.

Table 8. Yolk fatty acid profiles (%) and standard errors of the groups.

Fatty acids	Mulberry pulp levels (%)				
	0	4	8	12	P
C14:0 (Myristic acid)	0.23±0.08	0.46±0.06	0.39±0.01	0.44±0.03	NS
C16:0 (Palmitic acid)	23.99±0.94	25.51±0.28	23.71±0.16	22.08±1.10	NS
C16:1 (Palmitoleic acid)	1.86±0.27	1.02±0.58	1.73±0.06	1.98±0.06	NS
C17:0 (Margaric acid)	0.06±0.06	0.34±0.23	0.27±0.13	0.50±0.14	NS
C18:0 (Stearic acid)	16.76±.1.01	18.15±3.09	16.94±0.52	16.01±0.16	NS
C18:1 (Oleic acid)	25.48±1.48	22.71±3.51	24.06±1.07	22.80±0.26	NS
C18:2 (Linoleic acid)	24.74±0.87	25.20±0.64	25.20±0.60	26.15±2.01	NS
C18:3 (Linolenic acid)	0.86±0.09	1.39±0.45	1.19±0.20	1.50±0.27	NS
C20:2 (Eicosadienoic acid)	0	0.04±0.04	0.04±0.04	0.09±0.05	NS
C20:3 (Gamma-linolenic acid)	0	0.11±0.05	0.12±0.01	0.16±0.10	NS
C20:4 (Arachidonic acid)	4.73±0.59	4.41±0.51	4.37±0.32	4.95±0.28	NS
C20:5 (Eicosapentaenoic acid)	0.07±0.07	0.02±0.02	0.24±0.24	0	NS
C22:6 (Docosahexaenoic acid)	1.41±0.47	0.58±0.58	1.86±0.84	3.29±0.75	NS

Differences between means in the same row are insignificant. NS: Not significant.

Yolk cholesterol. Table 9 shows the cholesterol analysis results of the egg yolks collected from the control and treatment groups in the 4th week. The differences between the cholesterol values of the groups were significant ($p<0.05$). The lowest cholesterol level was obtained from the 8% supplemented group, while the results of the other groups were similar. Addition of 8% mulberry pulp to the diet may be stated to have a positive effect on egg yolk cholesterol levels.

Table 9. Means and standard errors of yolk cholesterol levels of the groups.

	Mulberry pulp levels (%)				
	0	4	8	12	P
Cholesterol (mg/yolk)	58.71 ±4.80a	58.43 ±3.77a	45.16 ±0.77b	57.56 ±2.23a	*

a,b: Differences between means in the same row with different letters are significant. NS: Not significant. *: $p<0.05$.

Blood parameters. Table 10 shows the results of the analysis performed for some parameters with the blood samples taken from the quail in the 4th week of the experiment. Addition of mulberry pulp to the diet significantly affected blood triglyceride, total cholesterol and LDL (Low-density lipoprotein) levels ($p<0.05$, $p<0.01$). Other blood parameters (Glucose, Ca, P, Mg, Na and K) of the groups were not affected by mulberry pulp addition. The triglyceride levels were lower in the 12% supplemented group than those in the other groups ($p<0.05$). The triglyceride levels in the 8% supplemented group showed a partial decrease, although not statistically significant, compared to the control group. Addition of mulberry pulp to the diet may be stated to cause a decrease in blood triglyceride levels starting from 8%. When the total cholesterol levels of the groups were examined, it was seen that the differences between the means were significant ($p<0.05$). The results on the cholesterol levels were similar in the control group, 8% supplemented group and 12% supplemented group, but higher in the 4% supplemented group. Addition of mulberry pulp to the diet does not have a significant effect on the 8% and 12% groups.

Table 10. Means and standard errors of some blood parameters of the groups.

	Mulberry pulp levels (%)				
Parameters	**0**	**4**	**8**	**12**	**P**
Glucose, mg/dL	201.02 ±12.83	199.20 ±10.38	203.60 ±18.31	205.25 ±13.22	NS
Triglycerides, mg/dL	465.00 ±86.92ab	596.00 ±57.14a	399.25 ±72.61ab	279.42 ±20.18b	*
Total cholesterol, mg/dL	123.25 ±20.21b	192.50 ±25.79a	116.75 ±7.79b	166.50 ±18.25ab	*
LDL, mg/dL	64.10 ±11.62b	146.45 ±15.30a	79.37 ±10.89b	131.50 ±22.03a	**
Ca, mg/dL	8.27 ±0.83	7.45 ±0.42	7.59 ±1.03	7.23 ±2.01	NS
P, mg/dL	6.42 ±1.23	6.20 ±1.26	5.36 ±0.90	7.20 ±1.59	NS
Mg, mg/dL	3.64 ±0.30	3.73 ±0.36	3.98 ±0.37	3.03 ±0.19	NS
Na, mmol/L	137.00 ±2.73	136.50 ±2.60	140.00 ±0.91	137.75 ±6.52	NS
K, mmol/L	5.08 ±0.03	5.26 ±0.09	5.33 ±0.22	5.66 ±0.30	NS

a,b: Differences between means in the same column with different letters are significant. NS: Not significant.
*: $p<0.05$, **: $p<0.01$.

In terms of serum LDL levels, the differences between the means of the control and treatment groups were significant. The LDL levels were higher in the 4% and 12% supplemented groups than the control and 8% supplemented groups ($p<0.01$). The results on the LDL levels were similar in the control and 8% supplemented groups. It may be stated that the effect of mulberry supplementation on serum LDL levels in quail varies for different levels.

DISCUSSION

While the differences in the daily feed consumptions of the quail in the control and experiment groups on whose diets mulberry pulp was added were not significant at the 1st and 2nd weeks, they were significant at the 3rd and 4th weeks ($p<0.05$, $p<0.01$). The daily feed consumptions were found to be the lowest in the group that was fed with feed containing 12% mulberry pulp. While adding mulberry pulp into the diets by up to 8% did not affect the feed consumption of the experiment groups, significant decreases were observed in feed consumption at increased ratios (12%). The quail in the group fed with the feed containing 8% mulberry pulp had the highest feed consumption values.

While the findings obtained regarding feed consumption were in agreement with another study which reported that addition of mulberry flour into the rations of broiler quail increased their feed consumption (16), they were different to the results of another study where mulberry leaf flour was added to the rations of laying quail (17). Values regarding feed consumption obtained in studies where mulberry leaf flour was added to the rations of laying hens were different to the findings in this study (8,9,10,18) and partially similar to Panja's findings (19).

The differences between the feed conversion ratios of the quail in the control and experiment groups were not significant. The results showed that, at all weeks, adding mulberry pulp into the rations by up to 12% did not affect feed efficiency negatively. The results were different to those reported by a study where mulberry flour was added to the diets of broiler quail (16). On the other hand, in comparison to those in other studies where mulberry leaf flour was added to the diets of laying hens, the results were similar to a great extent (8,9,10,18).

When the results on the egg yields of the groups were examined, it was seen that the egg yields were similar at the 1st, 2nd and 4th weeks. However, at the 3rd week, significant reductions were observed in the case of increasing the mulberry pulp level in the diets above 8% ($p<0.01$). The results obtained on egg yield were similar to those reported by Al-Kirshi et al. (8) and Hermana et al (17) and different to those reported by Lin et al (9), Kamruzzaman et al (10) and Kamruzzaman (18).

Considering the values on egg weight, the results of all groups were similar. Addition of mulberry pulp to the diets of the quail by different ratios did not significantly affect their egg weight values. The results on the egg weights were similar to those reported by a previous study (17) where mulberry leaf flour was added to the rations of laying quail by up to 10% and other studies (10, 18) where mulberry leaf flour was added to the rations of laying hens. On the other hand, the results were different to those in another study where mulberry leaf flour was added to the diets of laying hens (8).

When the eggs of the control and experiment groups were evaluated in terms of their internal and external quality characteristics, there were significant differences in the albumen index, yolk index and Haugh unit values at the 2nd week and yolk weight at the 4th week ($p<0.05$). The results were similar in terms of the other quality characteristics. It was determined that addition of mulberry pulp to the diets of quail

by up to 12% did not have a negative effect on the internal and external quality characteristics of eggs. Panja (19) reported that mulberry leaves do not affect yolk color because diets have enough corn gluten. The yolk finding in this study was similar to the study by Panja (19), but it differred with the results of Olteanu (20). Other results were in agreement with those of Al-Kirshi et al (8) and Panja (19). Addition of different ratios of mulberry to the rations did not significantly affect the yolk fatty acid profile, and the mean values of the control and experiment groups were similar.

The differences in the mean values of the groups were found significant in terms of the yolk cholesterol levels ($p<0.05$). Panja (19) reported that inclusion of mulberry leaves decreased blood cholesterol same as the yolk cholesterol content but had no effects on thigh meat cholesterol levels. According to ration content, yolk cholesterol levels may change between 5% and 30% (21). While the cholesterol levels were similar in the control, 4% and 12% groups, they were lower in the 8% group. The findings that were obtained were similar to those reported by Kamruzzaman et al (10).

Considering the results of the groups regarding the blood parameters, it was determined that the differences in the triglyceride, total cholesterol and LDL levels were significant ($p<0.05$, $p<0.01$), while those in the other parameters were insignificant. Zhang (12) reported that the blood triglyceride levels in their 15% dietary mulberry leaves group were significantly lower than those in the control group. Their findings were similar to the results of this study. However, they reported that inclusion of 15% mulberry leaf flour also provided significantly lower values than those in the control group. The findings differed for these parameters. The lowest values were found in the 8% group in terms of the blood cholesterol levels and the 12% group in terms of the blood triglyceride levels. The results were similar to those reported by Kamruzzaman et al (10).

In conclusion, in this study, it was found that adding different levels (0, 4%, 8% and 12%) of dried mulberry pulp to the diets of laying quail was effective on daily and weekly feed intake, egg yield, albumen index, yolk index, Haugh unit, yolk weight, yolk cholesterol, triglyceride, total cholesterol and LDL levels. Addition of mulberry pulp to the diet did not affect the egg yolk fatty acid levels significantly. As a result, when the effects of 4%, 8% and 12% dried mulberry pulp added to the diets of laying quail were examined, it was observed that mulberry pulp had no negative effect up to the ratio of 8%. When the mulberry pulp supplement reaches 12%, some negative effects (especially feed intake and egg yield) may be observed. Therefore, it may be stated that it is beneficial to add an 8% ratio of dried mulberry pulp to the diet.

Conflict of interests

The authors declare no conflict of interest with publication of this manuscript.

Acknowledgement

The authors would like to express their deepest gratitude to Bingol University Research Fund for supporting this research (BAP-Project number BAP-ZF.2018.00.010).

REFERENCES

1. Liu Y, Willison JHM. Prospects for cultivating white mulberry (*Morus alba*) in the drawdown zone of the hree orges eservoir, China. Environ Sci Pollut Res. 2013; 20(10):7142–7151. https://doi.org/10.1007/s11356-013-1896-2

2. Singhal BK, Khan MA, Dhar A, Baqual FM, Bindroo BB. Approaches to industrial exploitation of mulberry (Mulberry sp.) fruits. J Fruit Ornam. Plant Res. 2010; 18(1):83-99 http://www.inhort.pl/files/journal_pdf/journal_2010_1/full8%202010_1_.pdf

3. TUIK - Turkish Statistical Institute. Mulberry production [On line]. 2015. [accessed 20 December 2019] URL Available in: http://www.tuik.gov.tr/PreIstatistikMeta.do?istab_id=68

4. Ukav I. Mulberry production economy in district of Adiyaman. AJAEES. 2018; 23(1):1-10. https://doi.org/10.9734/AJAEES/2018/39462

5. Ekin I, Celikezen F. Bitlis İlinde Geleneksel Olarak Üretilen Gezo Pekmezinin Bazı Kimyasal Özelliklerinin İncelenmesi. BEU Fen Bilimleri Dergisi. 2015; 4(2):138-149. https://doi.org/10.17798/beufen.03575

6. Akbulut M, Ozcan MM. Comparison of mineral contents of mulberry (*Morus* spp.) fruits and their pekmez (boiled mulberry juice) samples. Int J Food Sci Nutr. 2009; 60(3):231-239. https://doi.org/10.1080/09637480701695609

7. Zhou B, Meng QX, Ren LP, Shi FH, Wei Z, Zhou ZM. Evaluation of chemical composition, in situ degradability and in vitro gas production of ensiled and sun-dried mulberry pomace. J Anim Feed Sci. 2012; 21(1):188–197. https://doi.org/10.22358/jafs/66063/2012

8. Al-Kirshi RA, Alimon AR, Zulkifli I, Sazili AQ, Zahari WM, Ivan M. Utilization of mulberry leaf meal (Morus alba) as protein supplement in diets for laying hens. Ital J Anim. 2010; 9(3):e51. https://doi.org/10.4081/ijas.2010.e51

9. Lin WC, Lee MT, Chang YL, Shih CH, Chang SC, Yu B, Lee TT. Effects of mulberry leaves on production performance and the potential modulation of antioxidative status in laying hens. Poult Sci. 2017; 96:1191–1203. http://dx.doi.org/10.3382/ps/pew350

10. Kamruzzaman M, Islam MS, Ferdous KA, Haque MA, Afroz F, Rahman MK. Dietary effect of mulberry leaf (Morus alba) meal in the reduction of blood cholesterol of laying hens. Int J Bus Soc Sci Res. 2018; 6(4):32-36. http://www.ijbssr.com/user/images/journalpaper/jppdf-14013291.pdf

11. Liu LK, Chou FP, Chen YC, Chyau CC, Ho HH, Wang CJ. Effects of mulberry (*Morus alba L.)* extracts on lipid homeostasis in vitro and in vivo. J Agr Food Chem, 2009; 57(16):7605-7611. https://doi.org/10.1021/jf9014697

12. Zhang X, Li Y, Zhang L, FAN J**,** Zhong S, Li Q, Lou L. Effect of dietary mulberry leaves on productive performance, egg quality and serum biochemical indices of laying hens. China Poulty. 2014; 34(16):25-28. http://en.cnki.com.cn/Article_en/CJFDTotal-ZGJQ201216008.htm

13. Karabulut A, Canbolat O. Yem Degerlendirme ve Analiz Yontemleri. Bursa; Turkey; 2005. ISBN:975-6149-07-8.

14. Olgun O, Yildiz AO. Effect of Dietary Alfalfa Meal on Performance, Egg Quality, Egg Yolk Cholesterol and Hatchability Parameters of Quail Breeders. TURJAF, 2015; 3(3):103-106. https://doi.org/10.24925/turjaf.v3i3.103-106.208

15. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957; 226:497–509. https://www.ncbi.nlm.nih.gov/pubmed/13428781

16. Perdomo CDA, Briceño A, Díaz CD, González D, González L, Moratinos LPA, Núñez GEK, Perea GFP. Effect of dietary supplementation with mulberry (*Morus alba*) meal on the productive performance of growing quails (*Coturnix coturnix japonica*). Rev Inv Vet Perú. 2019; (30):2 634-644. http://dx.doi.org/10.15381/rivep.v30i2.15088

17. Hermana W, Toharmat T, Sumiati, Manalu W. Performances and egg quality of quail offered feed containing sterol fromkatuk (Sauropus androgynus) and mulberry (Morus alba) leaf meal. Int J Poult Sci. 2014; 13(3):168-172. http://dx.doi.org/10.3923/ijps.2014.168.172

18. Kamruzzaman M, Khatun MA, Islam MS, Rahman MZ, Yeasmin T. Effect of dietary mulberry leaf meal on egg quality of laying hens. JST. 2014; 12:12-17. https://jst.hstu.ac.bd/assets_vcc/files/vol_12/3_jst%2013-21.pdf

19. Panja P. The effects of dietary mulberry leaves (morus alba l.) on chicken performance, carcass, egg quality and cholesterol content of meat and egg. WJST. 2013; 10(2): 121-129. http://wjst.wu.ac.th/index.php/wjst/article/view/306/286

20. Olteanu M, Panaite T, Ciurescu G, Criste RD. Effect of dietary mulberry leaves on performance parameters and nutrient digestibility of laying hens. Indian J Anim Sci. 2012; 82(8):914-917. https://www.cabdirect.org/cabdirect/abstract/20123298484

21. Calislar S, Kustimur H. The effects of safflower meal on the performance, egg quality traits, yolk fatty acids and cholesterol levels in laying hens. Anadolu J Agr Sci. 2017; 32(2):269-278. http://dx.doi.org/10.7161/omuanajas.321121