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Performance and biochemical parameters of Japanese quail blood to different levels of hydroalcoholic extract of *Malva sylvestris*

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Abstract

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This experiment was performed to investigate the effect of different levels of *Malva sylvestris* extract on growth performance, carcass characteristics, and some blood parameters of Japanese quail, in a completely randomized design with 16 experimental units including 4 treatments, 4 replicates, and 10 birds in each replication. Experimental treatments included basal diet (control) and different levels of *Malva sylvestris* extract (1, 2, and 3% of diet). Feed intake and overweight were recorded weekly. The results revealed that during the experimental period, *Malva sylvestris* extract was not significant effect on weight gain, feed intake, feed conversion ratio, and carcass characteristics ($P > 0.05$), but there was a significant difference between the experimental treatments related to blood parameters such as cholesterol, triglyceride (TG), HDL, LDL and total antioxidant properties (TAP) ($P < 0.05$). The addition of *Malva sylvestris* extract significantly reduced the concentration of cholesterol, TG, HDL, and LDL in the blood and increased the TAP ($P < 0.05$). According to the results of this study, it seems that the use of 2% *Malva sylvestris* extract can influence the bird's health status by changing blood factors.

Keywords: blood parameters; performance; *Malva sylvestris*; quail

Introduction

The use of antibiotics causes the emergence of antibiotic resistance and leaves irreversible side effects. According to existing reports, the increasing number of congenital anomalies, the occurrence of chronic diseases, the ineffectiveness of antibiotics, the increase in the phenomenon of microbial resistance, and hundreds of other small and large complications are known as current health problems in human societies. These problems have been attributed to the overuse of antibiotics (Mehdi Youcef et al., 2018; Roth et al., 2019; Montoro-Dasi, 2020). For these reasons, the use of those additives that while maintaining the desired properties, do not have adverse health and environmental consequences has been considered by researchers around the world for years. Among the additives accepted by researchers, medicinal plants and their products have gained special importance (Cross et al., 2007). Medicinal plants are those plants that are used for medical, therapeutic, clinical, and pharmaceutical purposes in humans and livestock (Talebi et al., 2022), and selection of herbs as an alternative to safer treatment or in some cases as the only treatment is considered. Many of these plants and their compounds show beneficial therapeutic effects such as antioxidants, anti-inflammatory, anti-cancer, antimicrobial and immune system effects (Huffman, 2003)

Research has revealed that the addition of herbal medicinal compounds to the diet of birds modulates their intestinal microflora (Oviedo-Rondón, 2019; Rubens et al., 2023). In addition to the role that medicinal plants and their derivatives play in reducing pathogens and boosting the level of immunity in poultry, they also enhance the color, aroma, quality, and shelf life of products (Talebi et al., 2021). The researchers demonstrated that the use of medicinal plants and their essential oils involves the composition of the digestive flora of broiler chickens, sustains the immune system, and declines blood cholesterol, thereby enhancing the performance of broiler chickens (Cross et al., 2007; Mashayekhi et al., 2018; Talebi et al., 2022).

One of the most important and influential issues in the poultry industry is oxidative stress. Oxidative stresses are caused by the imbalance between the production and elimination of free radicals caused by factors such as environmental pollutants, which cause more than one hundred diseases. One of the most important ways to deal with the oxidation process is to use antioxidants. Fortunately, all oxygen-consuming organisms in the body have a comprehensive antioxidant system, including enzymatic and non-enzymatic compounds (Khosravinezhad et al., 2017; Talebi et al., 2022). But this system cannot support the bird's physiological condition Although today most of the antioxidants employed in industry are synthetic, many of these compounds have harmful effects on the health of the consumer. Since herbal plants are one of the sources of antioxidants, and researchers have concentrated on finding antioxidants of plant origin. The antioxidant effects of plant materials are partly attributed to the presence of their phenolic and flavonoid compounds, which are present in all different parts of the plant such as roots, leaves, skin, fruits, and seeds (Mathew & Abraham, 2006).

One of the plants used in traditional medicine is *Malva sylvestris*. The biological activity of *Malva sylvestris* leaves is dependent on antioxidants, polyphenols, vitamin C, vitamin E, beta-carotene, and other chemical components (Tabaraki, 2012). This plant is prescribed for the treatment of cough, respiratory problems, and the digestive system (Baytop, 1984). The *Malva sylvestris* can be used as a food preservative as well as a preventive substance against various diseases. This study aimed to investigate the effect of different levels of *Malva sylvestris* extract on the economic and biochemical traits of Japanese quail blood.

Materials and Methods

Preparation of Malva sylvestris plant extract

To prepare the *Malva sylvestris* extract, first, the aerial parts of the *Malva sylvestris* were purchased and after the approval of the botany expert of the Darab Branch, Islamic Azad University, the possible impurities were removed. The dried plant was well ground and completely powdered. The powder was placed in a 50:50 solution of water and ethanol for 48 hours to dissolve all substances that were soluble in water and alcohol. After 48 hours, strain the mixture, and place the resulting liquid in the laboratory for a week until the water and alcohol evaporate and the material is a completely concentrated solution. After one week, the concentrated solution was placed in the refrigerator and the required amounts were removed and added to the test diets every week.

Birds and management

A total number of one hundred and sixty, one-day-old Japanese quail chicks were purchased from a farm located in Darab, Iran, and after weighing randomly distributed in cages. The layout of the test units, heating, and ventilation facilities in the housing hall was designed to be standard and the same for all test units. Cages with dimensions of 60×80×80 cm^3 were used to apply the experimental groups. For each pen, drip drinking (nipple) and feeding were considered. To prepare the rearing house, first, feed and other movable items were taken out of the rearing house. The house and its equipment were washed using hot water under pressure and then disinfected using the mass disinfectant solution. Quail was exposed to 16 h light/day during the experiment using 60-watt lamps. The hall's temperature in the first week was 33°C, which decreased with the growth of the chickens so that the hall's temperature decreased by 0.5°C every day, and finally, after 28 days, the hall's temperature fixed at 24°C. The feed was monitored several times during the day and provided to the chickens properly with appropriate drinking water (*ad libitum*).

Diets and experimental groups

Japanese quail chicks were allocated in a completely randomized design (CRD) into four experimental groups: 1 – Basal ration (control), 2 – Basal diet+1% *Malva sylvestris* extract, 3 – Basal diet+2% *Malva sylvestris* extract, 4 – Basal diet+3% Malva sylvestris extract, and each experimental group had four replications (each replication consisted of 10 birds). The basal diet was formulated to meet the quail requirements according to NRC (2001) using UFFDA (user-friendly feed formulation program) software (Table 1).

Performance indicators

At the beginning of the rearing period, the quail chicks were weighted and their mean weight was calculated. At the end of each week, all chickens in each experimental unit starved for about 3 to 4 h, and body weights were recorded. The feed intake of the experimental units was determined by the difference between the amount of feed allocated at the beginning of the week and the remaining feed at the end of

Table 1. Nutritional diet combinations for the experimental groups

Nutrient	%
Corn grain	49.00
Soybean meal	45.30
Limestone	1.45
Dicalcium phosphate	0.87
Vegetable oil	2.60
Vitamin premix[1]	0.125
Mineral premix[2]	0.125
D,L-Methionine	0.13
Sodium chloride	0.30
Threonine	0.10
Total	100
Nutrient levels	
Nonphytate phosphorus, %	0.30
Calcium, %	0.80
Sodium, %	0.15
Cation + anion balance, meq/kg	250.00
Methionine, %	0.50
Lysine, %	1.30
Methionine + cysteine, %	0.75
ME (MJ/kg)	12.13
Crude proteinm, %	24.00
Threonine, %	1.02
Tryptophan, %	0.23

[1]Composition/kg of vitamin premix: Vit. A: 12.000.000 U.I., Vit D_3:3.600.000 U.I., Vit. E: 3.500 U.I., Vit B_1:2.500 mg, Vit B_2: 8.000 mg, Vit B_6:5.000 mg, Pantothenic acid: 12.000 mg, Biotin: 200 mg, Vit. K: 3.000 mg, Folic acid: 1.500 mg, Nicotinic acid: 40.000 mg, Vit. B_{12}: 20.000 mg, Selenium: 150 mg, inert to achieve 1.000 g. [2]Composition/kg of mineral premix: Manganese: 160g, Iron: 100 g, Zinc: 100 g, Copper: 20 g, Cobalt: 2 g, Iodine: 2 g, inert to achieve 1000 g

the week. The calculation of the feed conversion ratio was also obtained by dividing feed intake by weight gain in the same period. During the test period, if there were any losses, the number, weight, and date of losses were accurately recorded to make the necessary corrections in the calculation of feed intake and feed conversion ratio.

Sampling of internal organs

On day 35 of the rearing period, two birds from each pen, weighing the average weight of each cage, were randomly selected, weighed, and then slaughtered. The weight of the carcass was recorded after filling. The carcass was then separated. Evaluated factors were: carcass weight, empty abdominal carcass weight (without amputation), liver, heart, small intestine, gizzard, cecum, abdominal fat, pancreas, spleen, and bursa Fabricius, measured by digital scales.

Blood sampling and laboratory operations

To determine some blood metabolites, blood samples were taken from the jugular vein (two samples per replicate) at the time of slaughter (35 days). Blood samples were placed in test tubes without anticoagulant and coagulation; the samples were centrifuged at 3000 rpm for 7 minutes to extract their serum and transferred to special micro tubes until the main tests were stored at -20°C in the freezer. Serum samples were sent to a laboratory located in Shiraz to determine intended blood metabolites such as glucose, triglyceride, cholesterol, HDL, LDL, and total antioxidant properties and were analyzed by colorimetric method using commercial kits of Spanish Biosystems Company.

Statistical model

The experimental design was a completely randomized design (CRD) and the statistical model of the design was as follows:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij}: The observed value, μ: Average, T_i: treatment effect, e_{ij}: Effect of experimental error

All statistical analyses were performed using SAS statistical software (version 9.1) with GLM proc and post-hoc Tukey's range test was used to compare the means at the significance level of 0.05 ($P < 0.05$).

Results

Feed intake

The effect of experimental treatments on the feed intake of Japanese quail in different weeks of rearing is shown in Table 2. The results revealed that there was no significant difference between different groups ($P > 0.05$), but numerically the highest feed intake in the whole weeks, was observed for 2% of the *Malva sylvestris* extract (538.52) and the lowest feed intake was observed for the control treatment (525.37). In Figure 1, the feed intake ratio of 1% *Malva sylvestris* extract exhibited an increase (1.72) and the 2% extract had a decreasing trend (1.66).

Weight gain

The results (Table 3) demonstrated that the experimental treatments did not differ significantly in terms of weight gain in the first to fifth weeks of rearing ($P > 0.05$), but the highest and lowest mean weight gain in these five weeks were numerically related to the 2% of *Malva sylvestris* extract and control treatments, respectively. In the entire rearing period (1–35 days), the effect of rising levels of *Malva sylvestris* extract on the weight gain of Japanese quails was meaningful

Table 2. The effect of *Malva sylvestris* extract on the weekly feed intake of Japanese quail

Treatment	Feed intake, g/bird/period					
Period, day	1–7	7–14	14–21	21–28	28–35	1–35
Control	67.82	75.87	102	122.50	157.87	525.37
Malva sylvestris 1%	67.12	76.00	102.25	125.12	158.87	530.07
Malva sylvestris 2%	70.32	79.20	104.25	125.50	159.25	538.52
Malva sylvestris 3%	67.97	78.50	102.50	124.75	159.75	533.47
SEM	0.4265	0.1031	0.875	1.066	1.344	5.474
P-value	0.4343	0.3761	0.4907	0.5801	0.9029	0.1352

SEM: Standard error of the mean

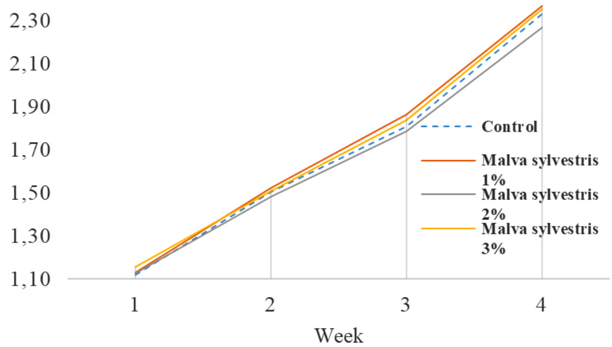

Fig. 1. The effect of *Malva sylvestris* extract on the weekly feed intake ratio to the first feed intake

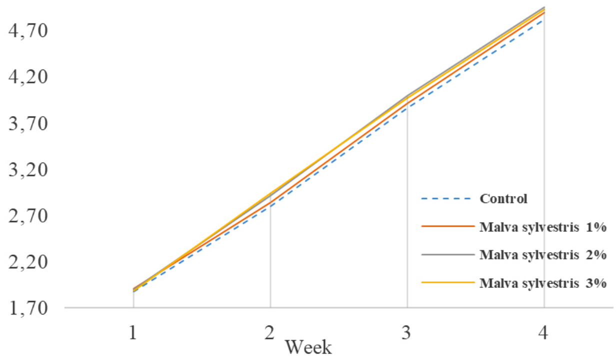

Fig. 2. The effect of *Malva sylvestris* extract on the weekly weight gain ratio to the first week weight gain

(P < 0.05), so the level of 2% *Malva sylvestris* extract illustrated the highest weight gain (201.85 g) and control treatment compared with other groups had the lowest weight gain (192.62 g). In Figure 2, the weight gain ratio of 2% *Malva sylvestris* extract displayed an increase (3.44) and the control group had a decreasing trend (3.34).

Feed conversion ratio

The results in Table 4 indicated that the mean feed conversion ratio was not affected by experimental treatments (P > 0.05). The mean feed conversion ratio of 1% *Malva sylvestris* extract was calculated lowest (2.33), and the 3% *Malva sylvestris* had the highest ratio (2.38).

Relative weight of carcasses and internal organs

According to Table 5, the effect of different levels of *Malva sylvestris* extract on carcass parameters including carcass weight, empty stomach carcass weight (without destruction and viscera), liver, heart, pancreas, abdominal fat, spleen, gizzard, small intestine, and bursa fabricius had no significant effect (P > 0.05).

Blood parameters

According to the results in Table 6, the effect of *Malva sylvestris* extract on the measured blood parameters including TG, cholesterol, LDL, HDL, and TAP was meaningful (P < 0.05), while blood glucose was not influenced by ex-

Table 3. The effect of *Malva sylvestris* extract on the weekly weight gain of Japanese quail

Treatment	Weight gain (g/bird/period)					
Period, day	1–7	7–14	14–21	21–28	28–35	1–35
Control	39.75	34.87	36.62	42.37	38.00	192.62 [b]
Malva sylvestris 1%	40.13	36.25	37.62	43.15	39.12	197.37 [ab]
Malva sylvestris 2%	41.00	36.87	41.50	44.35	39.37	201.87 [a]
Malva sylvestris 3%	40.81	36.12	42.87	42.17	39.25	198.75 [ab]
SEM	0.0520	1.096	0.5109	0.7399	1.135	2.874
P-value	0.3000	0.4514	0.3636	0.7414	0.5424	0.0234

SEM: Standard error of the mean

[a-b]: Dissimilar letters in each column indicate a significant difference (P < 0.05)

Table 4. The effect of *Malva sylvestris* extract on the feed conversion ratio of Japanese quail

Treatment	Feed conversion ratio					
Period, day	1–7	7–14	14–21	21–28	28–35	1–35
Control	1.69	2.17	2.72	2.89	4.15	2.72
Malva sylvestris 1%	1.69	2.09	2.68	2.86	4.06	2.68
Malva sylvestris 2%	1.71	2.17	2.59	2.93	4.01	2.68
Malva sylvestris 3%	1.66	2.32	2.59	2.93	4.01	2.68
SEM	0.0069	0.0172	0.0255	0.0441	0.0274	0.0023
P-value	0.8547	0.7926	0.5927	0.9101	0.7814	0.3827

SEM: Standard error of the mean

perimental treatments (P > 0.05). The use of different levels of *Malva sylvestris* extract in the diet greatly declined the cholesterol and TG levels compared to the control group (P < 0.05), so that the lowest TG level (183.00 mg/dl) was observed in the treatment of 3% *Malva sylvestris* extract. The lowest amount of blood cholesterol was observed in the treatment of 2% *Malva sylvestris* extract (199.75 mg/dl). Concerning HDL, the control treatment had the highest quantity (89.75 mg/dl), and treatments of 2% and 1% of *Malva sylvestris* extract retained the lowest amount (68.75 mg/dl). The lowest level of LDL (47.00 mg/dl) was observed in the treatment of 2% *Malva sylvestris* extract, which was remarkably different from the level of 1% (56.50 mg/dl) *Malva sylvestris* extract (P < 0.05). Serum glucose level was not affected by experimental treatments. According to the results, the TAP was affected by increasing levels of *Malva sylvestris* extract (P < 0.05), so the 3% *Malva sylvestris* extract treatment had the highest amount of antioxidant properties (42.50 mg/dl).

Table 5. Effect of *Malva sylvestris* extract on the relative weight of carcasses and internal organs of Japanese quail

Treatment	Weight, %					
	carcass	Empty carcass[1]	Liver	Heart	Small intestine	
Control	73.92	59.92	2.07	0.95	3.28	
*Malva sylvestris*1%	75.24	59.69	2.09	0.96	3.41	
*Malva sylvestris*2%	75.47	59.75	2.08	0.98	3.35	
*Malva sylvestris*3%	74.80	60.07	2.10	0.97	3.43	
SEM	13.91	12.60	0.2965	0.0476	0.8395	
P-value	0.9512	0.9984	0.9993	0.9925	0.9883	
Treatment	Weight, %					
	Gizzard	Cecum	Ab. Fat[2]	Pancreas	Spleen	B.F[3]
Control	1.58	0.63	0.60	0.23	0.07	0.10
Malva sylvestris 1%	1.68	0.66	0.53	0.25	0.08	0.11
Malva sylvestris 2%	1.83	0.69	0.52	0.30	0.09	0.13
Malva sylvestris 3%	1.85	0.68	0.47	0.28	0.08	0.12
SEM	0.1592	0.0488	0.1111	0.0143	0.0008	0.0033
P-value	0.4958	0.9673	0.8798	0.6857	0.7212	0.7428

SEM: Standard error of the mean, [1]Carcass without organs and viscera, [2]Abdominal fat [3], Bursa of Fabricius

Table 6. The effect of *Malva sylvestris* extract on blood parameters of Japanese quail

Treatment	mg/dL					
	Glucose	Triglyceride	Cholesterol	HDL	LDL	TAP[1]
Control	261.00	230.50^{a}	229.50^{a}	89.75^{a}	52.25^{ab}	23^{b}
Malva sylvestris 1%	265.50	227.25^{a}	216.75^{ab}	70.00^{b}	56.50^{a}	30.52^{ab}
Malva sylvestris 2%	266.50	222.00^{a}	199.75^{b}	68.75^{b}	47.00^{b}	35.90^{a}
*Malva sylvestris*3 %	263.75	183.00^{b}	204.75^{b}	80.75^{ab}	51.00^{ab}	42.50^{a}
SEM	3.89	8.97	8.43	5.18	8.31	7.62
P-value	0.9029	< 0.0001	0.0041	0.0007	0.0537	0.0040

SEM: Standard error of the mean, [1]Total antioxidant properties

$^{a\text{-}b}$: Dissimilar letters in each column indicate a significant difference (P < 0.05).

Discussion

The effect of experimental treatments on feed intake was not a significant difference (P > 0.05) so the highest feed intake was related to the treatment of 2% *Malva sylvestris* extract and the minimum feed intake was related to the control group. The findings of this study were consistent with the results of other studies (Nobakht & Aghdam, 2010; Soltani & Nobakht, 2016). Failure to observe the difference between the treatments used on feed intake of medicinal plant extracts can be due to differences in research methods, amount and type of plants consumed, time studied, type of bird, sex of chickens used, and the amount of contamination. It should be mentioned that 7 species of *Malva sylvestris* have been reported in Iran, 3 of which are native to Iran (Nasiri et al., 2015).

Feed consumption can be controlled by several mechanisms. The most common regulators of feed intake include glucostatic theory, thermostatic theory, gastrointestinal dilation, the transformation of the amino acids entered into the body, and lipostatic theory (Harris, 2017). The glucostatic hypothesis explains the regulation of blood glucose and the amount of glucose entering the liver after eating. Decreased blood glucose (hypoglycemia) stimulates the central nerves and stimulates the appetite, while increased blood glucose (hyperglycemia) stimulates the satiety center. Forbes (1985) exhibited that injecting glucose into the hepatic veins of hungry chickens reduces their feed intake. While injecting glucose into the vein, no such reaction was observed. According to the results obtained in this study, the use of different treatments did not affect blood glucose levels, and therefore it is expected that due to the same experimental conditions and the same level of ME and CP in diets, the only reason was lack of changes in blood glucose levels.

Experimental treatments did not differ significantly in terms of weight gain in different experimental weeks (P > 0.05) but in the whole period (1–35 days), the effect of increasing levels of *Malva sylvestris* extract on weight gain of Japanese quails was significant (P < 0.05). The level of 2% *Malva sylvestris* extract had the highest weight gain and the control treatment had the lowest weight gain, which was also consistent with the results of Mansourzadeh et al. (2015). Differences in the size of the bird's eggs and, of course, the size of the chicks when they hatch, can result in reduced growth at the time of sale. Because no effective research has been done on this bird, intra-breed differences can change the size of quail eggs. On the other hand, medicinal and aromatic plants contain compounds of flavonoids, carotenoids, and vitamin C, which generally have beneficial effects on the immune system (Hernandez et al., 2004; Talebi et al., 2022). By producing secondary metabolites, these plants prevent the occurrence of physiological and environmental stresses caused by pathogenic microorganisms (Windisch et al., 2008; Grashorn, 2010) improves the immune system against environmental stresses, and improve the parameters related to the blood safety of chickens. Therefore, the increase in mean weight over the entire period is due to the consumption of plant extracts and genetic changes in chickens. The mean feed conversion ratio was not affected by experimental treatments. Medicinal and aromatic plants accelerate digestion and shorten the passage of digestive material through the gastrointestinal tract (Windisch et al., 2008). Failure to improve the above traits is also attributed to factors such as insufficient plant active ingredients used, insufficient duration or incorrect method of use of materials, improper density or concentration of materials used, special conditions, and different responses of the tested animals (Grashorn, 2010).

On the other hand, the effects of improvement appear better when the chickens are not in favorable breeding conditions, for example, birds are fed a diet with low digestibility or are in polluted environmental conditions (Shariatmadari & Mohiti Asl, 2008).

The effect of different treatments on carcass weight, carcass weight without amputation, liver weight, heart, gizzard, small intestine, cecum, abdominal fat, pancreas, and Fabricius bursa was not significant (P > 0.05). The results of this research were consistent with the results of the study of Mansourzadeh et al. (2015). Nobakht & Aghdam (2010) did not observe a significant effect on carcass characteristics using a mixture of medicinal plants such as *Malva sylvestris*, sage, and mint. Due to the lack of observation of differences in the average feed intake, weight gain, and feed conversion ratio, not observing the difference in parts of the carcass in completely standard environmental conditions is not far from expectation.

Polyphenolic compounds inhibit the activity of the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-coA). As a result, cholesterol synthesis is inhibited. This action increases LDL receptors at the level of liver cells and accelerates LDL catabolism. HMG-coA reductase inhibitors decrease LDL and, to a lesser extent, plasma TG concentrations and slightly increase HDL concentrations (Barreto et al., 2008). Improvements in blood biochemical parameters in this study are probably related to polyphenolic compounds in the *Malva sylvestris* (Dehkordi, 2003). The amount of phenolic compounds in the *Malva sylvestris* has been reported to be 317 mg/g of the extract (Barros et al., 2010). Cholesterol-lowering using herbs is due to the presence of polyphenols and flavonoids that have antioxidant activity (Ajith et al., 2007). Nobakht & Aghdam (2010) observed a decrease in blood cholesterol and TG levels numerically using a mixture of herbs, *Malva sylvestris*, sage and mint.

The results of studies demonstrate that high phenolic compounds are the main reason for the high antioxidant activity of some extracts, including methanolic and ethanolic extracts, according to the available evidence, there is a positive relationship between phenolic compounds and plant antioxidant power. On the other hand, it seems that phenolic compounds, which are widely found in plants and have high antioxidant power, can be extracted more through plant extracts compared to their essential oils. It should be noted that phenolic compounds act effectively as hydrogen donors and therefore act as an effective antioxidant. The results of this study showed that phenolic compounds may be responsible for the antioxidant activity of the extracts. These compounds increase the activity of the catalase enzyme, which in turn neutralizes hydrogen peroxide and converts lipid hydro peroxides into non-toxic substances. The use of antioxidants in poultry diets reduces lipid peroxidation in feed, and body tissues and ultimately increases the quality and shelf life of poultry products.

Conclusion

The addition of 1–3% Malva sylvestris extract has a significant positive effect on total weight gain (1–35 days of age) (Table 3).

The highest positive statistical effect of the herbal preparation is reported for the blood indicators of Triglyceride and Cholesterol. The use of *Malva sylvestris* at more than 1% reduced Triglyceride and Cholesterol by more than 20 and 12%, respectively, compared to the control group. Reducing these two blood factors has a positive effect on enhancing the health condition of the birds.

We recommend using a concentration of 2% *Malva sylvestris* in poultry feed to employ the benefits of *Malva sylvestris* extract.

Conflict of interest statement

We declared that no conflict of interest exists.

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