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Original Article

Effect of Citrus Flavonoid on Storage Time and Meat Quality of Pharaoh Quail (Coturnix Pharaoh)

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■Keywords

Hesperidin, lipid peroxidation, meat quality, storage times, quail.

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ABSTRACT

This study investigates the effects of Hesperidin added to quail ration at different rates on some microbiological and physicochemical, lipid peroxidation, and lipid profiles in thigh meat. The current study had a duration of 35 days and used Pharaoh quails (Coturnix Pharaoh). The grouping was done in three treatment groups: Control, HES500, and HES1000 (each group was divided into five subgroups), and 0, 500, and 1000 mg/kg of Hesperidin was added to the basal diet of the groups, respectively. Adding Hesperidin and storage time affected the pH parameter in meat. It affected colour parameters depending upon the added Hesperidin (p<0.05). There was a significant difference in the number of total mesophilic aerobic bacteria (TMAB) in comparison with the control group according to the storage time (p<0.05). Palmitic, α-linolenic, oleic acid, eicosapentaenoic, and docosahexaenoic acids, which are among the individual fatty acids, differed between the control, HES500 and HES1000 groups (p<0.05). Hesperidin addition reduced lipid peroxidation on the 3rd, 5th, and 7th days of storage (p<0.05). Consequently, in direct proportion to the hypothesis at the beginning of the study, it was specified that adding Hesperidin reduced its concentration on lipid oxidation and had a positive effect on meat quality in terms of colour parameters.

INTRODUCTION

Poultry meat has a high concentration of polyunsaturated fatty acids (PUFA) at low intramuscular fat content (Simopoulos, 2000). However, the high degree of unsaturation of fatty tissue triggers the susceptibility of poultry meat to oxidative peroxidation. It leads to a decrease in meat colour, odour, flavour, and nutritional value (Engberg *et al.*, 1996). Oxidation in foods and especially meat products is one of the primary reasons for quality degradation (Kanner, 1994). The susceptibility of meat tissue to lipid oxidation can be reduced through antioxidants, although the process is dependent on various factors. Flavonoids are powerful antioxidant agents and inhibit the production of free radicals, superoxide anions and lipid peroxy radicals (Kumar & Pandey, 2013). The use of natural antioxidants can extend the shelf life of meat and boost its saleability by retail (Fellenberg & Speisky, 2006).

Green tea, rosemary, grape pulp and orange pulp which are rich in phenolic substances with antioxidant activity, have been added to the poultry rations as raw materials and extracts. In recent years, attempts to add by-products obtained especially from citrus fruits to the rations for evaluation have been developing in the sector. Citrus pulp is obtained after removing the juice from the fruit, and is therefore a mixture of citrus peels, their insides, and part of the peel (Kara *et al.*, 2021; Özbilgin *et al.*, 2021a).

Citrus pulp and residues are widely used in animal feeding. They have a positive impact in preventing the need for expensive waste management programs. Fibers obtained from citrus have an additional advantage compared to fibers from other sources due to the presence of bioactive compounds (flavonoids), and these fibers can be used as functional components for providing a health benefit. Bioflavonoids such as hesperidin and naringenin are abundant as an inexpensive by-product of citrus cultivation (Goliomytis *et al.*, 2015).

In previous studies, Chen *et al.* (2016) reported that the use of flavonoids in goose feeding did not have a negative effect on fattening performance, carcass and blood parameters. In addition, Dabbbou *et al.* (2018) reported that it had a positive effect on physical parameters and lipid oxidation in meat in their study on the use of flavonoids in rabbits. Goliomytis *et al.*, 2015 reported that naringin and hesperidin supplementation in broilers had no effect on color parameters, but decreased lipid oxidation parameter dose-dependently. There are many studies on the inclusion of flavonoids in the diet of pigs, turkeys and broilers (Grela *et al.*, 2009; Karwowska *et al.*, 2007, 2010).

In this study, it was hypothesized that the addition of hesperidin to the ration in different dosages would have positive effects on meat color, fatty acid and microbial oxidation in meat. In this context, meat quality, microbial and fatty acid profile analyses were carried out.

MATERIAL AND METHODS

Animal, trial experiment order, and rations

The study was conducted with the permission of Tokat Gaziosmanpaşa University Animal Experiments Local Ethics Committee (Tokat Province, Turkey), dated 2021 and numbered 51879863-37. Two hundred twenty-five mixed-sex quails (*Coturnix Pharaoh*) were sheltered in cages measuring 20x45x100 cm in a closed area at Sivas Cumhuriyet University Faculty of Veterinary Medicine five weeks for the trial period in the study. Quails were distributed between the control and experimental groups regarding average body weight values without inducing a statistical difference. The animals were divided into three groups, 75 animals in each group, and five subgroups in each group, with 15 quails in each group in the experiment. The grouping was done in the form of three groups: the control (K) group fed only with basal ration, the HES500 group fed with basal ration + 500 mg/kg ration hesperidin, and the HES1000 group fed with basal ration + 1000 mg/kg ration hesperidin. Hesperidin (Molecular formula $C_{28}H_{34}O_{15}$, cas no: 520-26-13, purity grade 91%, Chem-Impex International Company, USA) was obtained from the market as purified from orange fruit. The selection of 500 mg/kg and 1000 mg/kg levels in the study referenced previous poultry study (Goliomytis, 2015). Feed and water were given to the animals ad libitum. The animals were provided with a comfortable temperature (22-24 °C), 23 hours of light, and one hour of darkness daily during the study. Animal rations were formulated according to the recommendations of NRC (1994), and their chemical analysis was conducted according to AOAC (2000) (Table 1).

Table 1 – Basal ration and nutrient content used in the study.

	Groups		
Feed raw materials, %	C*	HES500*	HES1000*
Maize	28.83	28.83	28.83
Wheat	20.24	20.24	20.24
Barley	4.96	4.96	4.96
Soybean meal, %48	33.35	33.35	33.35
Sunflower meal, %28	10.00	10.00	10.00
Limestone**	1.37	1.37	1.36
Dicalcium Phosphate	0.65	0.65	0.65
Vitamin-Mineral mix***	0.25	0.25	0.25
Salt	0.24	0.24	0.24
L-Lysine,hydrocloride	0.12	0.12	0.12
Hesperidin****	0	0.05	0.10
Nutrient content			
Dry matter, %	90	90	90
Crude protein, %	23	23	23
Metabolic energy, Mj/kg	12.55	12.55	12.55
Calcium,%	0.80	0.80	0.80
Usable phosphorus,%	0.30	0.30	0.30

*C group (basal ration), HES500 group (500mg/kg hesperidin added basal ration), HES1000 group (1000mg/kg Hesperidin added basal ration).

** Hesperidin replaced limestone in the same amount in the groups, including Hesperidin.

***Vitamin-Mineral mix: 3 mg of retinol (vitamin A), 62.5 µg of cholecalciferol (vitamin D3), 30 mg of tocopherol (vitamin E), 5 mg of menadione (vitamin K3), one milligram of thiamine (vitamin B1), 5 mg of riboflavin (vitamin B2), 3 mg of pyridoxine (vitamin B6), 20 µg of cobalamin (vitamin B12), 30 mg of nicotinic acid, 10 mg of pantothenic acid, 0.8 mg of folic acid, 100 µg of biotin, 10 mg of ascorbic acid (Vitamin C), 450 mg of choline chloride, 0.2 mg of Co, 0.5 mg of I, 0.3 mg of Se, 25 mg of Fe, 120 mg of Mn, 10 mg of Cu, and 100 mg of Zn.

**** The additive used in the study, Hesperidin, was obtained from the Chem-Impex International company, and its molecular formula is (C28H34015), cas no is (520-26-13), and it has a purity grade of 91%.

Slaughtering and Meat Sampling

At the end of the experiment, eight animals from each group were slaughtered in a commercial slaughterhouse after a total of twenty-four animals

were not fed for 6 hours. The blood of the slaughtered quails was taken. The carcasses were placed in plastic bags one by one after the internal organs were evacuated and kept at +4 C for 24 hours. Meat measurements were performed on breast and thigh muscles.

Meat Quality Characteristics

Twenty-four quails from groups were slaughtered in a hygienic environment after being given no food for 10 hours at the end of the study's fattening period. Breast meat and thigh meat from the slaughtered animals was conserved in polyethylene plates, covered with cling film, and stored at +4 °C during the analysis of the meat (9 days). Water activity (a_w), pH, and color parameters ([L*, a*, b*) were determined on the 1st, 3rd, 5th, 7th, and 9th days of sampling. Water activity (a_w) value was determined with Aqualab 4TE (USA) device. Some meat was placed in the container of the device, and the a_w value was read.

The pH values of the samples were identified by Gökalp *et al.* (2001) following the method reported. Accordingly, 10 g of homogenized samples were weighed correspondingly, and 100 ml of purified water was added. pH values were specified by reading with a pH-meter (WTW Inolab, Germany) in pursuit of homogenizing with Ultra–Turrax (IKA Werk T 25, Germany) for one minute.

The samples' cross-section surface colour densities (L*, a*, b*) were determined using a Minolta (CR-200, Minolta Co, Osaka, Japan) colorimeter device.

Lipid Peroxidation Analysis

Two g was taken from the homogenized samples, and 12 ml of TCA solution (7.5% TCA, 0.1% EDTA, 0.1% Propyl gallate (dissolved in 3 ml ethanol) was added. After being homogenized in Ultra-Turrax for 15-20 seconds, it was filtered through Whatman 1 paper filter to analyse TBARS values. Three ml of the filtrate was received and transferred to the test tube, and 3 ml of TBA (0.02M) solution was placed on it and homogenized. The test tubes were kept in a water bath at 100°C for 40 minutes and then simmered down in cold water for five minutes. The absorbance values were read at 530 nm in a spectrophotometer (Aquamate Thermo electron corporation, England) following the centrifuge procedure (5 min at 2000 g). The results are given as µmol malonaldehyde/kg (Lemon, 1975).

TBARS = ((absorbance / k (0.06) x 2 / 1000) x 6.8) x 1000 / sample weight

Microbiological Analysis

Microbiological analyses of the samples were carried out according to the method set forth by Baumgart *et al.* (1993). 25 g of a thigh and breast sample flesh mixture were homogenized with 225 mL of sterilized Ringer solution. After that, the other solutions were prepared. The spread plate technique was utilized while performing inoculation. The total aerobic mesophilic bacteria (TMAB) was determined on Plate Count Agar (PCA, Merck, Germany) growth medium. Plates were incubated at 30 ± 1 °C for 72+1 hours under aerobic conditions. The total number of psychrophilic aerobic bacteria (TPAB) was determined on Plate Count Agar (PCA, Merck, Germany) growth medium. Plates were incubated at 7 ± 1 °C for ten days under aerobic conditions. The dilutions whose coliform count was appropriate were inoculated in VRBA (Violet Red Bile Agar, Merck, Germany) growth medium in the volume of 0.1 ml. Petri plates were incubated at 30 °C for two days under anaerobic conditions. The number of *Micrococcus/Staphylococcus* was determined on the Mannitol Salt Agar (MSA, Merck, Germany) growth medium. Plates were incubated at 30±1 °C for 48±1 hours under aerobic conditions. The number of *Pseudomonas spp.* was determined in Pseudomonas Agar Base (Oxoid, UK) growth medium supplemented by CFC (Cephalothin, Fucidin, Cetrimide). Plates were incubated at 30 ± 1 °C for 48±1 hours under aerobic conditions. The determined bacterial counts were expressed as log cfu g^{-1}.

Analysis of fatty acid profile

Fatty acid analysis was performed according to the three-stage modified procedure of Wang *et al.* (2015). This procedure was mixed and vortexed with 40 µl of oil, 0.7 ml of potassium hydroxide (10 M), and 5.3 ml of methanol in falcon tubes with volumes of 15 ml. The mixture was incubated at 55 °C for 45 minutes in the incubator (Core, Turkey) and cooled down to 21 °C. H_2SO_4 (10M) of 0.58 ml had been added to the mixture and vortexed. After this mixture had been at 55 °C for 45 minutes, 3 ml of n-hexane was added. The tubes were centrifuged at 1600 g for five minutes. After that, the 1.5 ml supernatant was taken into vials with a Polytetrafluorethylene (PTFE)/ white silicone septa blue cap and analyzed in a gas chromatography (Thermo Scientific, USA) device with automatic sampling (Thermo AI 1310). While analyzing, a column of Fatty Acid Methyl Esters (FAME) with an injection compartment temperature of 255 °C (Length 60 m, I.D: 0.25 mm, film: 0.25 µm, and

Table 2 – The effects of storage time and groups on some chemical parameters in quail meat (n = 10).

		Storage Times (Day, at +4 °C)					
Analyze	Treatments	1	3	5	7	9	*p*
pH	C	6.13±0.10^{aB}	6.38±0.02^{aA}	5.96±0.06^{C}	6.42±0.03^{aA}	6.28±0.06^{A}	0.001***
	HES500	6.16±0.03^{aC}	6.24±0.02^{bBC}	6.02±0.06^{D}	6.33±0.02^{bB}	6.43±0.02^{A}	0.001***
	HES1000	5.94±0.04^{bC}	6.19±0.52^{bAB}	6.05±0.01^{C}	6.24±0.02^{cAB}	6.35±0.05^{A}	0.001***
	P	0.008**	0.002**	0.41	0.001**	0.12	
L*	C	39.08±1.59^{C}	41.62±0.99^{BC}	43.16±1.24^{ABC}	42.46±0.86^{AB}	45.26±0.88^{A}	0.01***
	HES500	44.72±1.85	41.27±1.10	42.82±0.56	45.92±1.42	42.72±0.61	0.09
	HES1000	42.08±1.13	42.77±1.06	44.33±1.17	44.52±0.65	44.25±1.63	0.49
	P	0.07	0.58	0.59	0.08	0.30	
a*	C	12.64±0.54	11.74±0.46	11.10±0.53	12.03±0.89	11.57±0.36	0.45
	HES500	10.56±0.95	12.70±0.93	11.24±0.70	10.29±1.07	11.89±0.43	0.28
	HES1000	12.36±1.10	11.37±0.46	11.70±0.97	12.21±0.38	11.29±0.61	0.79
	P	0.23	0.34	0.84	0.23	0.68	
b*	C	6.78±0.85	4.63±0.27^{b}	8.77±0.84^{a}	6.42±1.40	6.91±1.06	0.08
	HES500	5.69±0.33	6.15±0.72^{b}	5.30±1.03^{b}	7.26±0.98	6.42±0.54	0.45
	HES1000	7.05±1.04^{AB}	8.59±0.72^{aA}	5.44±0.54^{bB}	7.56±0.58^{AB}	8.36±0.75^{A}	0.05***
	P	0.46	0.001**	0.02**	0.73	0.25	
a_w*	C	0.9909±0.001	0.9891±0.001	0.9900±0.001	0.9908±0.002	0.9888±0.001	0.60
	HES500	0.9911±0.002	0.9910±0.001	0.9910±0.001	0.9909±0.002	0.9910±0.002	0.95
	HES1000	0.9932±0.001^{A}	0.9920±0.002^{A}	0.9928±0.001^{A}	0.9876±0.001^{B}	0.9901±0.001^{AB}	0.01***
	P	0.38	0.27	0.16	0.24	0.33	

L* brightness, a* redness, b* yellowness, a_w*: water activity.

**a, b, c According to the treatment, different superscripts in the same column represent significant variance (p<0.05).

***A, B, C Depending on the storage time, different symbols in the same line represent significant variance (p<0.05).

maximum temperature 250-260 °C), and a column of 140 °C, and a flow rate of 30 ml/min, was used for 42 minutes for the process. Fatty acid identification was performed by comparing and calculating the standard fatty acid peaks in the samples with the Xcalibur program according to their retention times (Kramer *et al.*, 1997). Saturated fatty acids (SFA), unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), and very-long-chain fatty acids (VLCFA) were detected.

Statistical Analysis

The obtained data were evaluated by utilizing the SPSS 20.0 statistical software. One-way analysis of variance (ANOVA) was used to detect whether there was a statistical difference between the data in all parameters, and Bonferroni's multiple comparison test was used for paired comparisons between treatments and storage times, groups (p<0.05).

RESULTS

It was determined that there was a statistically significant difference between the control, HES500 and HES1000 groups on the 1st, 3rd, and 7th days of the treatment (adding hesperidin) (p<0.05), and that the control, HES500 and HES1000 groups were similar (p<0.05) on the 5th and 9th days concerning the pH value parameter. In terms of storage time parameter, it was specified that control, HES500, and HES1000 groups were different from each other on the 1st, 3rd, 5th, 7th, and 9th days (p=0.001).

In terms of lightness (L) value among the color parameters, the experimental groups were similar (p>0.05) on the 1st, 3rd, 5th, 7th, and 9th days of treatment. However, the control group was different on all days (p<0.05). The control, HES500, and HES1000 groups were similar on all days regarding redness (a) value among color parameters (p>0.05) from the point of treatment and storage time. The control, HES500, and HES1000 groups were similar (p>0.05) on the 1st, 7th, and 9th days regarding the yellowness (b) value among color parameters concerning the treatment and storage time. On the other hand, in terms of storage time, every day had a statistically different yellowness (p<0.05) value only in the HES1000 group.

In terms of treatment, it was determined that the control, HES500, and HES1000 groups were similar on all days regarding water activity (a_w). On the other hand, in terms of storage time, every day had a statistically different water activity value (p<0.05) only in the HES1000 group.

Table 3 – Effect of storage time and treatment on some bacterial counts in quail meat (log cfu g^{-1}), (n = 8).

		Storage Times (Day, at +4°C)					
Analyze	Treatments	1	3	5	7	9	*p*
TMAB*	C	3.00±0.001^{cB}	4.20±0.20^{A}	4.43±0.30^{A}	4.32±0.12^{A}	4.78±0.24^{A}	0.001***
	HES500	3.64±0.15^{bC}	453±0.12^{B}	4.96±0.03^{A}	4.94±0.23^{A}	5.26±0.01^{A}	0.001***
	HES1000	4.24±0.18^{a}	4.27±0.24	4.63±0.15	4.98±0.57	5.16±0.14	0.22
	P	0.002**	0.35	0.39	0.38	0.17	
Enterobacteriacea	C	2.00±0.001^{bD}	2.97±0.01^{aC}	3.60±0.31^{AB}	3.91±0.07^{A}	3.20±0.09^{bBC}	0.001***
	HES500	2.79±0.16^{a}	2.84±0.18^{a}	3.27±0.34	3.42±0.36	3.52±0.08^{a}	0.20
	HES1000	2.13±0.15^{bC}	2.16±0.17^{bC}	2.94±0.12^{B}	3.31±0.17^{AB}	3.58±0.06a^{A}	0.001***
	P	0.004**	0.02**	0.29	0.23	0.03**	
Lactobacillus spp.	C	3.80±0.10^{C}	4.00±0.001^{BC}	4.16±0.16^{AB}	4.30±0.17^{bAB}	4.45±0.12^{bA}	0.03***
	HES500	4.41±0.24	4.41±0.19	4.45±0.24	4.60±0.18^{b}	5.13±0.02^{a}	0.11
	HES1000	3.53±0.41^{B}	4.52±0.27^{A}	4.63±0.15^{A}	5.18±0.13^{aA}	5.06±0.15^{aA}	0.01***
	P	0.16	0.20	0.28	0.02**	0.009**	
Lactococcus spp	C	2.20±0.20^{bC}	3.43±0.22^{bB}	4.00±0.001^{AB}	4.28±0.28^{A}	4.47±0.09^{bA}	0.001***
	HES500	4.45±0.19^{a}	4.40±0.24^{a}	4.58±0.27	4.58±0.19	5.00±0.07^{b}	0.33
	HES1000	3.53±0.41^{aC}	3.94±0.09^{abBC}	4.41±0.13^{AB}	4.59±0.07^{AB}	4.68±0.04^{aA}	0.02***
	P	0.004**	0.03**	0.19	0.50	0.006**	
Micrococcus/ Staphylococcus	C	2.50±0.10^{cD}	3.75±0.03^{bB}	3.08±0.08^{bC}	3.49±0.25^{bB}	4.73±0.07^{A}	0.001***
	HES500	4.30±0.14^{aB}	4.50±0.13^{aB}	4.39±0.10^{aB}	4.46±0.13^{aB}	4.93±0.13^{A}	0.04***
	HES1000	3.95±0.001^{bB}	4.56±0.15^{aA}	4.57±0.16^{aA}	4.63±0.19^{aA}	4.78±0.17^{A}	0.03***
	P	0,001**	0.004**	0.001**	0.01**	0.56	
TPAB*	C	1.98±0.27^{cC}	4.23±0.35^{B}	4.00±0.001^{cB}	4.26±0.14^{B}	4.98±0.03^{bA}	0.001***
	HES500	4.52±0.02^{aB}	4.49±0.16^{B}	4.74±0.09^{aB}	4.80±0.11^{B}	5.13±0.04^{abA}	0.01***
	HES1000	3.76±0.25^{bB}	4.29±0.04^{B}	4.35±0.15^{bB}	4.36±0.23^{B}	5.54±0.20^{aA}	0.001***
	P	0,001**	0.70	0.006**	0.14	0.04**	

*TMAB, Total mesophilic aerobic bacteria count, TPAB, Total psychrophilic bacteria count.

**a, b, c According to the treatment, different superscripts in the same column represent significant variance (p<0.05).

***A, B, C Depending on the storage time, different symbols in the same line represent significant variance (p<0.05).

There was a statistical difference (p<0.05) between the control, HES500, and HES1000 groups only on the 1st day of treatment, and the experimental groups were similar on the other days (p>0.05) concerning the total aerobic mesophilic bacteria (TMAB) parameter. Furthermore, it was stated that there was a statistical difference between the days in the control and HES500 groups and that the total aerobic mesophilic bacteria count was similar for the HES1000 group on all days (p>0.05) in terms of storage time.

Regarding the Enterobacteriaceae parameter, there was a difference between the experimental groups on the 1st, 3rd and 9th days only in the control, HES500, and HES1000 groups (p<0.05), while the experimental groups were similar on the other days (p>0.05). In terms of storage time, on the other hand, it was determined that there was a statistical difference (p<0.05) between the days in the control and HES1000 groups.

In terms of treatment, there was a difference between the experimental groups only on the 9th day in the control, HES500 and HES1000 groups (p<0.05), while they had similar values on other days (p>0.05) regarding Lactobacillus spp. In terms of storage time, it was determined that each day had a different value in the control and HES1000 groups (p<0.05).

In terms of treatment, there was a difference between the experimental groups on the 1st, 3rd, and 9th days in the control, HES500, and HES1000 groups (p<0.05). At the same time, they had similar values on other days (p>0.05) regarding Lactococcus spp. parameter. On the other hand, in terms of storage time, it was determined that every day had a different value in the control and HES1000 groups (p<0.05).

In terms of treatment, there was a difference between the experimental groups on the 1st, 3rd, 5th, and 7th days in the control, HES500 and HES1000 groups (p<0.05), while they had similar values on other days (p>0.05) regarding Micrococcus/Staphylococcus parameter. In terms of storage time, on the other hand, it was determined that every day had a different value in the control, HES500, and HES1000 groups (p<0.05).

In terms of treatment, there was a difference between the experimental groups on the 1st, 5th, and 9th days in the control, HES500 and HES1000 groups (p<0.05), but they had similar values on the 3rd and 7th days (p>0.05) regarding total psychrophilic aerobic

bacteria (TPAB) parameter. In terms of storage time, it was determined that every day had a different value in the control, HES500 and HES1000 groups (p<0.05).

There was a difference between the control, HES500, and HES1000 groups on the 3rd, 5th, and 7th days (p<0.05), while they had similar values on other days (p>0.05) regarding the TBARS parameter regarding the treatment. In terms of storage time, on the other hand, every day had a different value in the control, HES500, and HES1000 groups (p<0.05).

In terms of fatty acid profile, it was determined that there were differences between control, HES500 and HES1000 groups in terms of individual fatty acids, namely, palmitic, oleic, α-linolenic, eicosapentaenoic, and docosahexaenoic acids (p<0.05) and that other individual fatty acids were similar in all groups (p>0.05). In terms of total fatty acids, MUFA, PUFA, MCFA, and n-3 fatty acids were different (p<0.05) in the control, HES500, and HES1000 groups regarding treatment, but other total fatty acids were similar (p>0.05).

DISCUSSION

Flavonoids are polyphenolic compounds that have positive effects on growth performance and meat and egg quality parameters in farm animals when added to the ration. In previous studies on this subject, there are studies examining the effects of flavonoids on growth performance in animals under heat stress (Tuzcu *et al.*, 2008; Kamboh & Zhu, 2013; Özbilgin *et al.*, 2021b). However, in the current study, the effects of hesperidin flavonoid supplementation in the diet under normal conditions on the physical, microbial quality and fatty acid profile of the carcass were investigated.

pH

Ph is important to preserve meat during storage. Meat quality is based on pH and water holding capacity. Riegel *et al.* (2003) reported that the pH of meat in most domesticated poultry species varies between 6.02 and 6.41. The pectoral muscle, particularly in Japanese quails, has pH values of 6.2-6.3 at the 20th minute after slaughter. Drbohlav & Drbohlavova (1987), who investigated the timespan of the pH values of the pectoral muscle in broilers, concluded that the glucose was depleted in the pectoral muscle at the 45th minute after slaughter. The pH values were almost unchanged after that. It has a low pH bacteriostatic effect, however, a pH above the normal range can trigger the growth of proteolytic microorganisms (Toldra, 2017). The present study obtained the lowest pH value on the 1st day after slaughter in the HES1000 group. Moreover, it was seen that the meat pH increased if the timespan extended, notwithstanding the treatment. Genchev *et al.* (2008) stated that, like the present study, the pH was 6.17 at the 24th hour after slaughter, and again identical to the results of the present study, it increased to 6.47 on the 7th day. Contrary to other bird species (for instance, chicken and turkey, where pectoral muscles are the glycolytic type entirely), dark muscle fibers of oxidative type predominate (Afanasiev *et al.*, 2000; Riegel *et al.*, 2003). Correspondingly, rapid pH declining after slaughter does not accrue in quails, and thus low pH does not occur.

Color Parameters

Extracts from plants have various colors and flavors that can affect meat quality characteristics (Jin *et al.*, 2015). Sensorial parameters such as food color and general look are fundamental criteria in consumers' purchases (Hernandez *et al.* 2016). The sensory quality of meat increases thanks to flavonoids or flavonoid-rich plant extracts (Kim *et al.*, 2009; Ouyang *et al.*, 2016). Nevertheless, Kamboh & Zhu (2013) stated that flavonoids added to the ration did not affect the sensory properties of broiler meat. The L* (brightness) value is the color coordinate related to lightness, distinguishing white objects from dark objects (Hunter & Harold 1987). In the present study, it was seen that there was a very rapid color lightening by increasing the brightness value in the control group, to which Hesperidin was not added from the 1st to the 9th

Table 4 – Effect of Hesperidin and storage time on TBARS in quail meat (μmol malonaldehyde/kg).

		Storage Times (Day)					
Analyze	Treatments	1	3	5	7	9	*p*
TBARS*	C	6.52±0.19B	7.02±0.22^{a}B	9.29±0.72^{a}A	9.96±0.89^{a}A	9.47±0.52A	0.001***
	HES500	6.28±0.20CD	5.94±0.10^{b}D	6.76±0.17^{b}BC	7.26±0.18^{b}B	8.76±0.35A	0.001***
	HES1000	6.09±0.21C	6.66±0.14^{a}C	6.01±0.08^{b}C	8.81±0.52^{ab}B	10.58±0.90A	0.001***
	p	0.33	0.001**	0.001**	0.01**	0.14	

*TBARS: Thiobarbituric acid reactive substances.

**a, b, c According to the treatment, different superscripts in the same column represent significant variance (p<0.05).

***A, B, C Depending on the storage time, different symbols in the same line represent significant variance (p<0.05).

day. However, an irregular increase was observed from the 1st to the 7th day in the HES500 group. A lower increase was observed in the HES1000 group from the 1st to the 9th day (Table 2). In previous studies, higher values were reported in many studies than the present study with values 47.60-53.33 L in the pectoral muscle and 44.24-45.42 L in the iliotibial muscle (Lukanov, 2019) at the 24th hour after slaughter (Genchev *et al.*, 2008; Wilkanowska & Kokoszynski, 2011; Narinc *et al.*, 2013). However, Tarasewicz *et al.* (2007) noticed lower L values than the present study. Hernandez *et al.* (2016) stated the L value as 37-40 in beef. The brightness value of 39.08, considered low in the Control group, has darker color formation due to oxidation and a lower L value (Hernandez *et al.*, 2016) in quail meat in the present study. HES500 and HES1000, other experimental groups, are not exposed to oxidation effects owing to the added Hesperidin.

The fact that oxymyoglobin gives a red color to meat is known (Hernandez *et al.*, 2016). In the present study, the control group contained 12.64-11.5 from Day 1 to Day 9,7; the HES500 group was 10.56-11.89, andthe HES1000 group a* (redness) value of 12.36-11.29. Lukanov *et al.* (2019) suggested higher redness values than the present study, with 13.92-16.98 in the pectoral muscle and 4.78-6.59 in the iliotibial muscle. Also, many studies noticed a lower a* value like the present study (Ribarski *et al.*, 1995; Narinc *et al.*, 2013).

Values between 6.1 and 6.4 in terms of b* value from the color parameters with the addition of clover extract flavonoids to rabbit rations were reported by Dabbou *et al.* (2018). In the present study, a value between 6.8-6.9 was found in the control group depending on the storage time. However, in the HES500 group, values similar to 5.69-6.42, and in the HES1000 group, higher b* values were obtained with the values of 7.05-8.36. It is thought that the reason for the high b* value in the present study is related to added Hesperidin and storage time.

Microbial Load

Oxidative stability is another essential property for meat quality. Oxidation of unsaturated fatty acids causes unwished flavors in the meat. Unsaturated fatty acids concentrations are higher in meat from monogastric animals and generally have faster lipid oxidation than in red meat from ruminant animals (Faustman *et al.*, 2010). It was reported that it increases the shelf life of meat by reducing the microorganisms that cause spoilage and lipid oxidation via flavonoids added to the ration increase (Simitzis *et al.*, 2011; Goliomytis *et al.*, 2015). However, a positive correlation could not be made between adding Hesperidin and the microorganism concentration in the present study. However, it was reported in the previous studies that supplementation of Genistein (5 mg/kg feed) and Hesperidin (20 mg/kg feed) or in combination (20% at 5, 10, and 20 mg/kg feed: 80%) reduces the number of psychrophilic bacteria, lactobacilli, and Enterobacteriaceae, as well as total bacterial count in meat, cooled for up to 15 days (Kamboh *et al.*, 2017). He *et al.* (2014) reported that Hesperidin had an antimicrobial effect on E. coli bacteria concentration. In the present study, the reason why the bacterial concentration did not decrease with Hesperidin and storage time is unknown.

Lipid Oxidation

The susceptibility of muscle tissue to lipid oxidation depends on several factors, but antioxidants can reduce it. Flavonoids are powerful antioxidant agents, and they protect cells by inhibiting the production of free radicals, superoxide anions, and lipid peroxyl radicals or by activating enzymes (Dabbou *et al.*, 2018). Due to the concentration of malondialdehyde (MDA), a secondary lipid peroxidation product, meat frozen for a long time may not be safe for human consumption (Reitznerová *et al.*, 2017). In vitro and in vivo studies have also revealed that flavonoid supplementation to ration significantly reduces MDA concentration in meat. It was reported that genistein supplementation (5 mg/kg diet) to ration reduces MDA production in meat by 41.4% on day 0 and 31.5% on day 15 of cooling (Kamboh *et al.*, 2017). A dose-related decrease in MDA content of meat was stated with the addition of naringin and Hesperidin (0.75 and 1.5 g/kg feed) to the diet (Goliomytis *et al.*, 2015). In another study, it was noticed that the addition of quercetin (1 g/kg feed) to the ration positively affected the oxidative stability of meat during three and nine days of storage (Goliomytis *et al.*, 2014). As expected, the present study corresponds to previous studies. Especially in the 1st, 3rd, 5th and 7th days, MDA concentration reduced the oxidation in Hesperidin added groups.

Fatty acid concentration

The effects of adding Hesperidin to ration on the fatty acid profile in meat are shown in Table 5. In general, saturated fatty acids are essential for heart health due to their hypercholesterolemic properties (Ahmed *et al.*, 2015). An increase was observed in the saturated fatty acids in terms of heneicosanoic and palmitic acid in thigh meat ($p<0.05$), but a decrease was observed in

Table 5 – Effect on individual and total fatty acid profile in thigh meat of Hesperidin added to quail rations (g/100g), (n = 8).

Fatty acids (g/100g concentration)	C*	HES500*	HES1000*	*p*
	$\bar{x}\pm S\bar{x}$**	$\bar{x}\pm S\bar{x}$**	$\bar{x}\pm S\bar{x}$**	
Caprylic acid (C8:0)	0.01±0.00	0.03±0.02	0.02±0.01	0.47
Capric acid (C10:0)	0.04±0.01	0.03±0.01	0.03±0.00	0.22
Undecanoic acid(C11:0)	0.02±0.01	0.06±0.04	0.04±0.02	0.59
Lauric acid (C12:0)	0.14±0.02	0.10±0.03	0.13±0.04	0.66
Myristic acid (C14:0)	0.78±0.10	0.77±0.10	0.81±0.06	0.96
Pentadecanoic acid (C15:0)	0.09±0.03	0.17±0.09	0.17±0.09	0.65
Palmitic acid (C16:0)	17.35±0.45^{b}	19.70±0.53^{a}	19.47±0.65^{a}	0.02***
Heptadecanoic acid (C17:0)	0.17±0.02	0.12±0.01	0.12±0.01	0.12
Stearic acid (C18:0)	5.08±0.67	6.75±0.57	5.79±0.51	0.16
Arachidic acid (C20:0)	0.04±0.01	0.06±0.00	0.18±0.11	0.29
Heneicosanoic acid (C21:0)	0.03±0.01^{b}	0.07±0.01^{a}	0.08±0.01^{a}	0.005***
Tricosanoic acid (C23:0)	0.15±0.09	0.02±0.00	0.04±0.00	0.24
Lignoceric acid (C24:0)	0.71±0.25	0.29±0.14	0.19±0.14	0.13
Myristoleic acid (C14:1)	0.28±0.06	0.23±0.07	0.27±0.06	0.87
Palmitoleic acid (C16:1)	7.23±0.81	5.35±1.09	7.49±1.00	0.26
Heptadecanoic acid (C17:1)	0.08±0.02	0.06±0.01	0.05±0.01	0.28
Oleic acid (C18:1n9c)	32.46±0.95^{b}	36.33±0.92^{a}	33.47±1.26^{ab}	0.03***
Eicosenoic acid (C20:1)	0.06±0.03	0.19±0.13	0.14±0.11	0.62
Nervonic acid (C24:1)	0.07±0.02	0.08±0.08	0.10±0.07	0.92
Linoleic acid (C18:2n6c)	25.98±1.05	26.57±0.79	28.34±1,10	0.26
Eicosadienoic acid (C20:2)	0.03±0.01	0.01±0.00	0.03±0.01	0.23
Beheric acid (C22:2)	0.04±0.00	0.12±0.04	0.24±0.12	0.17
α-linolenic acid (C18:3n3)	0.09±0.03^{b}	0.30±0.08^{a}	0.17±0.01^{ab}	0.02***
Eicosatrienoic acid (C20:3n3)	0.01±0.01	0.14±0.10	0.29±0.14	0.22
γ-linolenic acid (C18:3n6)	1.19±0.12	1.42±0.23	1.32±0.13	0.65
Arachidonic acid (C20:4n6)	1.48±0.33	1.24±0.36	2.17±0.42	0.22
Eicosapentaenoic acid (C20:5n3)	0.06±.0.01^{b}	0.12±0.03^{ab}	0.26±0.06^{a}	0.008***
Docosahexaenoic acid (C22:6n3)	0.23±0.07^{c}	1.10±0.33^{a}	0.99±0.17^{ab}	0.01***
ΣSFA	24.91±0.90	27.43±1.02	26.44±0.79	0.17
ΣUFA	71.49±1.54	72.87±1.17	73.51±1.51	0.61
ΣMUFA	40.23±0.58^{b}	42.36±0.57^{ab}	44.17±1.38^{a}	0.02***
ΣPUFA	28.17±0.66b	29.20±0.40b	34.44±1.56a	0.01***
Σw-3	0.56±0.18^{b}	0.78±0.15^{ab}	1.37±0.25^{a}	0.03***
Σw-6	28.65±1.10	29.11±0.80	30.47±1.75	0.59
w-9	41.49±1.00	42.38±1.64	40.58±1.90	0.51
w-3/w-6	0.04±0.02	0.03±0.01	0.10±0.06	0.30
MCFA	0.22±0.04^{a}	0.10±0.01^{b}	0.12±0.01^{ab}	0.02***
LCFA	96.32±1.24	99.08±0.10	97,56±1,25	0.25
VLCFA	1.24±0.26	0.89±0.12	1.36±0.22	0.34

*C group (basal ration), HES1 group (1g/kg hesperidin added basal ration), HES2 group (2g/kg Hesperidin added basal ration)

** $\bar{x}\pm S\bar{x}$: Mean± Standard error.

***a, b, c According to the treatment, different superscripts in the same column represent significant variance (p<0.05).

stearic acid, especially in the HES1000 group (p 0.05) in the present study. The level of stearic acid decreased significantly, especially at HES1000 concentration, with the adding of Hesperidin to the ration and the MUFA ratio increased inversely to the decrease. Like the present study, Bruce & Salter (1996) expressed the increase in oleic acid with the decrease in the ratio of stearic acid. Omega 3 fatty acids have an essential role in health by regulating the cardiovascular and immune systems (Grashorn, 2007). Adding Hesperidin to the ration caused significant increases in the concentration of omega 3 fatty acids α-linolenic, eicosapentaenoic, and docosahexaenoic acids in the present study. It was also observed that oleic acid, called omega 9 fatty acid, increased due to the addition of hesperidin. Concentrations of UFA, MUFA, and PUFA increased significantly with the addition of Hesperidin. The fact that there was no significant increase in omega 6 fatty acids (p>0.05) despite the increase in omega 3 fatty acids due to adding Hesperidin (p<0.05) was attributed

to their competition for the same enzymes (Nuernberg *et al.*, 2005).

As a result, the addition of Hesperidin positively affected lipid oxidation, significantly reducing the concentration. It positively affected meat quality in terms of L and a* value from color parameters. As expected, a difference in pH and b value could not be determined with the control group. As expected, there was no decrease in microorganism concentration. It did not affect saturated fatty acids in terms of fatty acid concentration; however, adding Hesperidin on alpha-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) from omega 3 fatty acids positively increased the total concentration of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. This study was not supported with a project.

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ERRATUM

In the article Effect of Citrus Flavonoid on Storage Time and Meat Quality of Pharaoh Quail (Coturnix Pharaoh), Doi: 10.1590/1806-9061-2022-1645, published in the Revista Brasileira de Ciência Avícolas/Brazilian Journal of Poultry Science, v24 (4):001-010.

In page 08 table 5 where it was written:
ΣSFA
ΣUFA
ΣMUFA
Σw-3
Σw-6

The correct form is:
SFA
UFA
MUFA
w-3
w-6

CC BY