Effects of Lemon Juice on Performance, Egg Quality Trait, and Some Blood Parameters of Laying Hens in the Late Phase of Production

Eyüp Eren GÜLTEPE, Aamir IQBAL, İbrahim Sadi ÇETİNGÜL, Cangir UYARLAR, Ümit ÖZÇINAR, İsmail BAYRAM

Department of Animal Nutrition and Nutritional Diseases, Afyon Kocatepe University Faculty of Veterinary Medicine, Afyonkarahisar, Turkey

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ORCID IDs of the authors: E.E.G. 0000-0002-2404-1232; A.I. 0000-0003-4473-2329; İ.S.Ç. 0000-0002-7608-6176; C.U. 0000-0002-7803-2454; Ü.Ö. 0000-0002-1143-1215; İ.B. 0000-0002-9993-7092.

Abstract

A total of 120 laying hens (57 weeks old) were randomly assigned to 5 groups and lemon juice (LJ) was daily added to drinking water (0%, 0.5%, 1%, 2.5%,5%) during 4 weeks. Egg production increased ($p<0.05$) in 1% and 2.5% LJ groups. The LJ had no effect on feed and water intake, live weight, feed conversion ratio, egg mass and weight. Haugh unit increased in 1% LJ group and egg yolk color was lighter in 5% LJ group ($p<0.05$). The LJ had no effect on eggshell thickness, albumin and yolk index. Serum HDL levels increased and Total Antioxidant Status decreased in 0.5% LJ group ($p<0.05$). The LJ juice had no effect on serum AST, ALT, LDL, Cholesterol, Glucose, Total Protein, IgG levels and Oxidant Status. Blood lymphocyte decreased ($p<0.05$) in 2.5% LJ over control, however, red blood cell numbers ($p<0.01$) increased in 1% LJ group. The LJ had no effect on other hematology parameters. In conclusion, the water supplementation of LJ showed positive effects on production without adverse effects on egg quality traits and health of late-phase laying hens. However, the positive responses may be more relevant to acidity of water.

Keywords: Aged laying hens, *Citrus lemon,* water supplement

Introduction

The production of good quality eggs with maintaining health of laying hens is one of the most critical goals of the industry. Low egg quality, shorter egg production period and metabolic diseases relevant to age are big challenges in late-phase aged laying hens. Although earlier stage of growth and peak period have great importance for sustainable production, late-phase should also be well-managed before molting or culling period of layers. Gradual decrease of egg production and low egg quality with an increase of feed intake in late-phase of the production cycle of aged laying hens has given rise to high expenses and low profitability in the industry until culling the birds (Roberts, 2004). Although egg size increases with age, Elaroussi et al. (1994) reported that ratio of shell weight to egg weight gradually decreases. Due to the decreasing eggshell quality with age, egg loss may increase in late phase of laying cycle (Roberts, 2004). In last decade, some researchers have focused to improve production and quality of eggs from aged laying hens via dietary manipulations and supplements (Catli et al., 2012; Mabe et al., 2003; Min et al., 2018; Molnar et al., 2018; Zhang et al., 2017).

The different supply route of same supplement or nutrient may cause different responses in poultry. For instance, Noy and Sklan (1999) concluded that providing nutrients via drinking water to earlier stage of life improved weight gain in poultry rather than supply of same nutrients via diet. Some evidence suggests that supplementation of probiotics via drinking water may have more beneficial rather than in-feed route on broilers (Karimi Torshizi et al., 2010; Ritzi et al., 2014). Although Virden et al. (2009) concluded that feeding supplemental water-sucrose to stressed broilers resulted no beneficial response under stress condition, but it had a

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Address for Correspondence: Eyüp Eren GÜLTEPE • **E-mail:** eegultepe@gmail.com

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good response on feed conversion ratio (FCR) during post-stress recovery period. Recently, Karadağoğlu et al. (2018) concluded that supplementing drinking water with different concentrations of an essential oil blend (peppermint, oregano, anise) improved egg production and weight, Haugh Unit, eggshell thickness, and yolk height of laying hens from week 20 to week 36 of age. Even though some evidence has been reported on broilers and laying hens, research data on effects of nutrient supplementation via drinking water on late-phase laying hens is still limited.

Lemon (*Citrus limon* L.) is an evergreen native plant of Asia and belongs to family Rutaceae. The juice of lemons are commonly used for cleaning and culinary purposes throughout the world. The lemon juice (LJ) is sour in taste and contains 5% to 6% citric acid (Yapo, 2009). Lemon fruits are rich in Vitamin C and contain different phytochemicals such as tannins, polyphenols, terpenes and flavonoids. The concentration of citric acid in LJ is twice than grapefruit juice and about five times higher than orange juice (Penniston et al., 2008). Although citrus fruits have different flavonoids such as narirutin, hesperidin, naringin and neohesperidin, only hesperidin transmission could be detected from fruit to juice (Xu et al., 2008). Hesperidin had significant benefits on poultry, such as anti-inflammatory, anti-stress, antioxidant, growth promoting, anticancer and immunological properties (Yatao et al., 2018). LJ in drinking water increased the immunity of broiler chickens (Behboudi et al., 2016; Kadam et al., 2009) and eggshell quality of layers (Tavakkoli et al., 2014) under heat stress. Furthermore, Farghly et al. (2018) concluded that supplying with water containing 10% LJ during the growing period of turkey chicks improved growth performance, immune response, antioxidant status and economic efficiency.

Keeping in view the above points, LJ may be used as potent water supplement to improve health and extend performance of late-phase layer hens. Therefore, the present study was aimed to explore the effects of different level of LJ on performance, egg quality and serological parameters in late-phase laying hens.

Materials and Methods

Experimental design and management

A total of 120 Babcock white laying hens (57 weeks old) were divided into 5 groups (n=24) with 4 replication groups containing 6 hens in each subgroup. LJ was added to the drinking water of the experimental groups with 0%, 0.5%, 1%, 2.5%, and 5% respectively during 4 weeks. The LJ levels were determined according to reported positive effects of LJ in literature (Kadam et al., 2009). Sixteen hours light and eight hours dark were applied and also feed and water were supplied *ad libitum*. In this study, all treatment groups were fed a basal diet, which was prepared according to requirement of the birds (Table 1) (NRC, 1994). In 0.5% LJ group, one bird has been moved out of the study due to sudden death. No mortality was recorded during the study except for the mentioned animal. LJ was poured on daily bases in fresh drinking water. Lemon fruits were provided from a commercial local trader.

Automatic nipple drinking system was used and each group have separated water tank where different concentration of LJ was added in their water tank. Graduated cylinder glass was used for scaling of LJ. Then, the LJ was mixed with water at the mentioned ratio in 20 liter water box between 1 p.m. to 2 p.m.

Table 1. Ingredient composition and chemical composition of basal diet

Ingredients	**%, as fed basis**	
Corn	54.90	
Vegetable oil	0.34	
Sunflower meal (32% CP)	16.92	
Full fat soya	10.00	
Soybean meal (44% CP)	7.39	
Limestone	7.87	
Dicalcium phosphate	1.73	
Common salt	0.40	
Vitamin-mineral premix[1]	0.25	
L-Lysine HCl	0.10	
DL-Methionine	0.10	
Calculated values[2]		
CP, %	17.00	
ME, kcal/kg	2750	
Ca	3.71	
Av.P	0.38	
Na, %	0.20	
Met+Sis	0.71	
Lysine, %	0.83	
Treonin, %	0.61	
Triptophane, %	0.20	
Linoleic acid, %	2.36	
pH levels of drinking water	$\bar{X}$	SEM
0% Lemon	7.07^{a}	0.23
0.5% Lemon	3.22^{b}	0.07
1% Lemon	3.13^{bc}	0.10
2.5% Lemon	3.02^{cd}	0.01
5% Lemon	2.89^{d}	0.03
p	0.0001	

[1]Provided per kg of diet: Vitamin A:12.000.000 IU, Vitamin D3:3.000.000IU, Vitamin E:35.000 IU, Vitamin K3:3.500 IU,Vitamin B1:2.750IU, Vitamin B2:5.500IU, Nicotinamid: 30.000IU,Ca-D-Panthotenate:10.000IU,Vitamin B6: 4.000IU, Vitamin B12: 15IU, Folic acid:1.000IU, D-Biotin: 50IU,Cholin clorid:150.000IU, Manganese: 80.000mg, Iron: 60.000 mg, Zinc:60.000 mg, Copper:5.000 mg, Iodine:2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant:15.000 mg.
[2]NRC (1994)
Values with different superscripts differ significantly ($p<0.05$)
HCl: Hydrochloride; DL: Dextrorotation & Levorotation; CP: Crude protein; ME: Metabolisable energy; Ca: Calcium; Av.P: Available phosphorus; Na: Sodium; Met: Methionine; Sis: Sistein; The different superscripts (a, b, c, d) represent significant differences between the values ($p<0.05$).

every day. The LJ could be solved easily in the water and homogenity was confirmed visually. During the study, water consumption (L) was measured by total water consumption per each groups after 24 hours interval.

Data collection and analyses

Hens were weighted at the beginning and at the end of the study to determine their live weights. Egg production was recorded daily and was expressed percent of hen-day egg production (HDEP). Feed intake and egg weight of hens was recorded weekly. Mortality was recorded daily while eggs were weighed once a week. Egg mass was calculated as follows: Egg Mass = Percent of HDEP x average egg weight in grams. FCR values were calculated as follows: FCR = feed intake (g) / egg mass (g).

Eggs were delivered to the laboratory at the end of the 4th week as three egg samples from each subgroup to determine egg quality parameters. Eggs were kept for 24 hours at room temperature before the egg trait analyses. Egg weight, breaking strength, and eggshell thickness were determined in these eggs. Egg breaking strength was measured by using ORKA Egg Force Reader (EF 0468-2011; Orka Food Tech. Ltd., Hong Kong, China) and Haugh Unit were calculated by measuring albumen height (Digital micrometer). Egg yolk color was determined by using Yolk Color Fan (DSM; Basel, Switzerland) and comparing the color of yolks with 15 bands of the color fan.

At the end of the trial, 3 hens were randomly selected from each replication group and blood was collected from the heart, then, the samples were transferred into two separate tubes (vacutainer tubes without anticoagulant and with ethylenediaminetetraacetic acid-EDTA, Becton Dickinson; Franklin Lakes, NJ, USA). Blood samples were immediately arrived in the laboratory under a cold chain. For serum biochemical analyses, the samples in vacutainer tubes were centrifuged at 5000 rpm for 10 minutes. Supernatants were transferred to Eppendorf tubes and stored at -20°C till biochemical analyses. Serum glucose, total cholesterol (CHO), high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Gamma-Glutamyl Transpeptidase (GGT), total protein (TPRO), phosphorus, calcium and Immunoglobulin G (IgG) concentrations were determined by automated ELISA analyzer (Elisys Uno; Human mbH, Wiesbaden, Germany). The effect of LJ on pH levels of drinking water was determined with a portable bench-top digital pH meter at 0 h and 23rd h.

Statistical analysis

The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively. The statistical analysis was performed with MedCalc (MedCalc Software bvba, Oostend, Belgium, v.18). General Linear Model was used for group comparison followed by Tukey-Kramer for post-hoc (Neter et al., 1996). The statistical model used to test the effects of treatment on variables was:

$$Yij = \mu + \alpha i + eij$$

where Yij = the response variable, μ = the general mean, αi = the effect of dietary treatments and eij = the random error. The significance level was considered as $p<0.05$ and all data were expressed as $\bar{X} \pm SEM$.

Results

In this study, pH levels of drinking water in treatment groups were significantly decreased according to control group ($p<0.05$). Low pH was observed in 2.5% and 5% LJ supplemented groups. However, the control group had a neutral pH (7.07±0.23, Table 1).

The result of recent study indicates that HDEP significantly ($p<0.05$) increased in 1% and 2.5% LJ supplemented groups; however, re-

Table 2. Effect of lemon juice given in drinking water on performance parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Feed intake, g/b/d	115.0	1.58	113.8	2.72	117.5	2.5	117.5	3.48	115.0	2.89	0.819
Water intake, L/b/d	0.40	0.008	0.39	0.007	0.41	0.007	0.38	0.008	0.40	0.008	0.072
HDEP[1], %	83.17^{b}	1.61	86.07^{ab}	1.77	88.54^{a}	1.51	89.88^{a}	1.25	86.45^{ab}	1.67	0.032
Egg weight, g	64.81	0.56	65.36	0.77	66.02	0.68	66.82	0.50	66.13	0.67	0.240
Egg mass, g	54.31	1.91	56.26	1.52	58.36	1.31	60.08	1.29	57.19	1.45	0.096
FCR[2]	2.15	0.08	2.03	0.05	2.02	0.05	1.97	0.08	2.04	0.07	0.504
Initial BW, g	1587.5	19.3	1602.0	33.3	1624.3	35.1	1630.8	33.4	1623.1	35.2	0.861
Final BW, g	1535.5	26.6	1448.2	97.1	1626.1	31.0	1616.5	28.9	1571.2	35.6	0.104

[1]Hen day egg production

[2]Feed conversion ratio, feed intake/egg mass

Values with different superscripts (a, b) in same row differ significantly ($p<0.05$)

HDEP: hen day egg production; FCR: feed conversion ratio; BW: body weight; SEM: standart error of mean

sults from feed intake, water intake, egg mass, FCR and egg weight were not significantly different (p>0.05) among all LJ supplemented groups as compared with control group. Similarly, hens live weight of all LJ supplemented groups were not significantly different (p>0.05) during the whole period of the trial (Table 2).

Regarding egg quality parameters, Haugh unit significantly (p<0.05) increased in 1% LJ supplemented group. In contrast egg yolk color significantly (p<0.05) decreased in 5% LJ supplemented group. Moreover, differences in eggshell thickness, albumin index, and yolk index were not significant (p>0.05) in all treatment groups as compared with control group (Table 3).

For serological parameters, HDL level significantly increased (p<0.05) however, TAS level significantly decreased (p<0.05) in 0.5% group as compared with other treatment and control group. Moreover, glucose, AST, LDL, CHO, ALT, TPRO, TOS, and IgG level remained unaffected (p>0.05) in all LJ supplemented group over control (Table 4).

Regarding blood parameters, lymphocyte count decreased significantly (p<0.05) in 2.5% LJ treatment group over control and red blood cell (RBC) significantly (p<0.01) increased in 1% supplemented group compared to 5% LJ group. Moreover, total leukocyte, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet, lymphocyte, neutrophil and monocyte counts were unaffected by LJ supplemention (p>0.05) (Table 5).

Discussion

In our knowledge, this is the first report on effects of drinking water mixed with LJ on late-phase aged layer hens. Phenolic compounds of citrus species are flavonoids and phenolic acids. Dominant flavonoids of citrus fruits are flavanone glycosides such as narirutin, hesperidin, naringin, and neohesperidin. Although Xu et al. (2008) reported no presence of naruritin, naringin, and neohesperidin in LJ, they just determined trans-

Table 3. Effect of lemon juice given in drinking water on egg trait parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Eggshell thickness, mm	40.01	0.01	38.89	0.02	40.04	0.06	37.43	0.03	38.88	0.01	0.513
Haugh unit	92.49^{b}	1.37	94.77^{ab}	1.06	97.30^{a}	1.40	92.64^{b}	1.27	95.46^{ab}	1.00	0.036
Yolk color score	11.3^{a}	0.15	10.9^{ab}	0.10	11.3^{a}	0.12	11.2^{a}	0.13	10.7^{b}	0.15	0.018
Albumen index, %	10.99	0.41	11.10	0.30	12.06	0.49	10.77	0.38	11.69	0.31	0.142
Yolk index, %	54.31	1.91	56.26	1.52	58.36	1.31	60.08	1.29	57.19	1.45	0.195

Values with different superscripts (a, b) in same row differ significantly (p<0.05)
SEM: standart error of mean

Table 4. Effect of lemon juice given in drinking water on serum biochemical parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
Glucose, mg/dL	163.31	9.23	175.08	6.17	169.93	8.04	172.25	8.28	179.47	8.64	0.753
CHO[1], mg/dL	88.94	10.36	128.91	14.56	98.27	10.77	109.18	9.35	92.60	7.80	0.116
HDL[2], mg/dL	15.38^{b}	0.51	17.36^{a}	0.51	16.83^{ab}	0.53	15.31^{b}	0.38	16.93^{ab}	0.41	0.004
LDL[3], mg/dL	27.69	4.52	39.50	6.37	30.20	3.68	34.44	3.38	30.73	3.10	0.782
AST[4], U/L	155.53	11.05	161.50	24.79	183.47	8.77	191.31	13.84	193.21	15.43	0.113
ALT[5], U/L	2.39	0.30	3.31	0.66	3.04	0.45	3.64	0.47	3.43	0.43	0.410
TPRO[6], g/dL	5.28	0.30	6.78	0.63	6.49	0.39	6.39	0.38	5.82	0.47	0.157
TOS[7], µmol/dL	58.60	10.89	101.90	10.39	94.56	18.99	76.24	12.83	66.06	12.03	0.146
TAS[8], mmol/L	1.20^{a}	0.05	0.87^{b}	0.02	1.28^{a}	0.11	1.00^{ab}	0.10	1.28^{a}	0.05	0.002
IgG, mg/dL	166.80	6.23	145.54	12.38	143.14	6.42	150.76	6.37	149.60	9.23	0.296

[1] CHO: total cholesterol; [2] HDL: high-density lipoprotein; [3] LDL: low-density lipoprotein; [4] AST: aspartate aminotransferase; [5] ALT: alanine aminotransferase; [6]TPRO: total protein; [7] TOS: total oxidant status; [8] TAS: total antioxidant status
Values with different superscripts (a, b) in same row differ significantly (p<0.05)
SEM: standart error of mean

Table 5. Effect of lemon juice given in drinking water on hematological parameters of laying hens from week 57 to week 61 of age (Mean ± SEM; n=24)

	0% Lemon		0.5% Lemon		1% Lemon		2.5% Lemon		5% Lemon		
Item	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	$\bar{X}$	**SEM**	**p**
TLC[1], 109/L	2.73	0.19	2.41	0.20	2.47	0.25	2.55	0.26	2.18	0.18	0.510
LC[2], 109/L	1.74[a]	0.03	1.75[a]	0.04	1.77[a]	0.03	1.62[b]	0.04	1.77[a]	0.04	0.031
NC[3], 109/L	0.70	0.04	0.76	0.05	0.77	0.03	0.77	0.04	0.72	0.04	0.690
MC[4], 109/L	0.043	0.001	0.045	0.001	0.044	0.002	0.044	0.001	0.044	0.003	0.100
RBC[5], 1012/L	2.70[ab]	0.03	2.66[ab]	0.04	2.74[a]	0.03	2.61[ab]	0.04	2.58[b]	0.04	0.009
Hemoglobin. g/L	10.75	0.23	10.42	0.22	10.44	0.17	10.20	0.18	10.81	0.20	0.212
Hematocrit. %	34.54	0.27	34.78	0.26	34.71	0.31	34.37	0.33	35.45	0.37	0.162
MCV[6]. fL	107.87	0.58	109.39	0.41	109.26	0.48	107.96	0.49	107.95	0.48	0.053
MCH[7]. pg	30.99	0.44	31.27	0.23	30.94	0.50	31.34	0.46	31.04	0.46	0.934
MCHC[8]. g/L	30.84	0.45	30.81	0.49	30.65	0.46	30.43	0.49	31.66	0.35	0.353
Platelet. 10^9/L	27.09	0.43	27.03	0.39	27.65	0.37	26.84	0.34	27.81	0.40	0.337
MPV[9]. pg	6.48	0.08	6.47	0.06	6.52	0.08	6.56	0.07	6.52	0.07	0.908

[1] TLC: Total leukocyte count; [2] LC: Lymphocyte count; [3] NC: Neutrophil count; [4] MC: Monocyte count; [5] RBC: Red Blood Cell count; [6] MCV: Mean Corpuscular Volume; [7] MCH: Mean Corpuscular Haemoglobin; [8] MCHC: Mean Corpuscular hemoglobin concentration; [9] MPV: Mean platelet
Values with different superscripts (a, b) in same row differ significantly ($p<0.05$)
SEM: Standart error of mean

mission of hesperidin from lemon fruit to juice (237.96±0.12 mg/L). Hesperidin supplementation improved immun response, antioxidant capacity, HDL percentage of total blood cholesterol pool and growth parameter in human and animal models (Yatao et al., 2018). Castillo et al. (2000) reported that freshly squeezed LJ had bactericidal activity *in vitro* against *Vibrio cholerae (V. cholerae)*. Also, supplementation of LJ has shown significantly better giardiacidal activity *in vitro* than unsupplemented group (Sadjjadi et al., 2006). The mentioned effects may relevant with hesperidin. Despite *in vitro* potential effects on pathogens, LJ had no constant effect on the immune response of bird's, except for a significant decrease of blood lymphocyte in this study. *In vivo* conditions such as a gut environment with a huge diversity of microorganism and nutrients in diet may have inhibited expected effects of LJ. On the other hands, the supplementation through drinking water may have led to inadequate intake of active ingredients with well-known positive effects on the immune response due to dilution effect (Del Toro-Arreola et al., 2005). Furthermore, the lack of expected antioxidant effect may also be due to the same reason.

Lemon juice had no significant effect on immune response in our study inconsistent with other studies which were determined an improved immune response with LJ supplementation via drinking water (Behboudi et al., 2016; Farghly et al., 2018; Kadam et al., 2009; Tavakkoli et al., 2014). Since Kadam et al. (2009), Behboudi et al. (2016) and Tavakkoli et al. (2014) studied under experimental heat stress conditions, their results may be differ from our results. Also, Farghly et al. (2018) used turkey chicks in earlier stage of their life as an experimental model. In our study, we had late-phase laying hens (57 week aged) as an experimental model and it is a first study on effects of LJ on late-phase laying hens in our knowledge. Due to different experimental animal model, the results may differ from the previous studies.

The pH of pure LJ was classified as strong acid (pH 2.39±0.05) characteristic. Citric acid is the predominant acid type (6% of the total juice weight) and providing approximately 95% of the overall acidity (Yapo, 2009). Due to the strong acidic capacity of LJ, the pH levels of drinking water significantly decreased as expected in our study. Recently, Shihab et al. (2019) have focused effects of using ionized water on performance of Japanese quails from week 6 to week 18 of age. They concluded that acidic drinking water (pH 5) has led to greater HDEP than water with neutral pH. Although there is no significant effect of treatment on immune response with insufficient active ingredients as a potential dilution effect, higher HDEP in treatment groups may be the result of acidic water caused by strong acidity effect of LJ in our study. Recently, Palamidi and Mountzouris (2018) concluded that dietary supplementation of an organic acids-based blend was increased expression of genes associated with gut barrier and health of broilers. Samanta et al. (2010) concluded that organic acids have led to a decreasing in pH of gizzard and selective promotion of beneficial bacteria species in the gut. Since dietary hesperidin has no effect on HDEP in laying hens (Goliomytis et al., 2014), improvement with LJ treatments in our study may be arise from acidity rather than direct effect of active ingredients of juice. Moreover, Ezzat et al. (2017) concluded that acidic water (pH 5) did not change egg traits (yolk diameter, yolk height, yolk index, albumen height)

of Japanese quails compared to control group and these results are inconsistent with our results. However, the researchers reported no effect of acidic water on Haugh Unit while we determined significant effects of 1% LJ supplementation on Haugh Unit compared to the control and other supplemented groups. Disagreement between studies probably due to a result of different water pH levels in both studies.

Goliomytis et al. (2014) concluded that dietary hesperidin supplementation improved egg yolk oxidative stability, however, the supplementation has shown no effect on egg quality parameters which is consisted with our results on egg quality parameters. In a recent study, the same researchers focused effects of dietary orange pulp on egg yolk traits (Goliomytis et al., 2018) and hesperidin was the most abundant in the orange pulp at a concentration of 8.52±0.78 mg/g among the flavonoids. Although dietary supplementation of a synthetic hesperidin+naringenin combination did not change egg yolk color properties (DSM Yolk Fan score, lightness – L, redness – a, yellowness – b), supplementation of orange pulp included abundant levels of Hesperidin significantly decreased all egg yolk color properties compared to control group. The observed results suggested that hesperidin led to lighter egg yolks even though synergetic effects with a combination with naringenin. In our study, lighter egg yolks in treatment groups than control group may be result of LJ supplementation which has just hesperidin among the other citrus flavonoids (Xu et al., 2008).

The inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase improves LDL receptors and increases plasma HDL concentration (Liang et al., 2005). Also, some evidence suggested that hesperetin acts as a cholesterol-lowering agent through decreasing activity of hepatic HMG-CoA reductase (Choi et al., 2004; Kim et al., 2003). In our study, we observed increase of serum HDL levels in 0.5% LJ supplemented group which can be explained by a potential HMG-CoA reductase inhibition of LJ via hesperidin. In contrast with the results of Kim et al. (2003) and Choi et al. (2004), we observed no effect of hesperetin on serum CHO and LDL levels. Differences may be caused by using hesperetin, which is an aglycone form of hesperidin, in mentioned studies. Goliomytis et al. (2014) concluded that dietary hesperidin did not affect plasma CHO levels in laying hens and this finding is consistent with our result. Moreover, hesperidin had a beneficial effect on human red blood cells (Allegra et al., 1995). Although there is no constant dose-dependent effect of treatment, significant changes in RBC levels in some treatment groups might be explained by mentioned effects of hesperidin. However, further evidence need to fully explain its potential mechanism.

In conclusion, LJ showed positive effects on HDEP without any adverse effects on the egg quality traits and health status of late-phase laying hens. However, the positive responses of LJ may be more relevant to acidity of water rather than active ingredients of juice, due to the dilution effect. Further side-by-side researches need the dose-controlled hesperidin and acidic water as well as higher concentration of LJs to explore accurate mechanism of their action and its positive effects on late-phase of laying hens.

Ethics Committee Approval: The current study was performed at the Animal Research Center of Afyon Kocatepe University Faculty of Veterinary Medicine after the approval of the Local Ethics Committee on the ethical use of animals under approval (Case No: 49533702/22, Date: 16/02/2017).

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