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Research Article

Effects of grape (*Vitis vinifera* L.) seed oil and St John's wort (*Hypericum perforatum* L.) extract supplementation into laying hens diets on performance, egg quality, and some blood parameters

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Abstract

This study set out to determine the effects of dietary supplementation of grape seed oil and Hypericum perforatum L. extract to the laying hens (Lohmann White, 40 weeks of age) on performance, egg quality and some blood parameters. For this purpose, a total of 336 hens were randomly distributed to the control and other treatment groups, 12 replicates/group with 4 hens/replication. Birds were fed with basal diet only (control group) and the basal diet supplemented with different levels (100, 200, 300 mg/kg) of grape seed oil (GSO-1, GSO-2, GSO-3) and *Hypericum perforatum* L. extract (HPE-1, HPE-2, HPE-3). Dietary treatments had no significant effect on performance parameters. GSO-3 and HPE-1 supplemental groups presented with increased shell weight ($p<0.01$) while only GSO-1 group showed increased egg albumen index ($p<0.01$) and Haugh unit ($p<0.05$). Among blood parameters, except for serum HDL and total protein, the other blood parameters (serum triglyceride, total cholesterol, LDL, AST, Ca and P) were not affected by the treatments. In conclusion, results showed a partial improvement in some egg quality traits, however, further studies are needed to fully investigate the beneficial effects of these additives in laying hens reared under different stresses.

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1. Introduction

Various feed additives have been used in the poultry diets for different purposes especially for the improvement in feed efficiency, and quantity and quality of animal products. In animal feeding, with the ban on antibiotics as growth promoters due to bacterial resistance and residues in animal products (Brenes and Roura, 2010), a great number of efforts have been made searching for new additives that could be used as an alternative to the antibiotics (Alagawany et al., 2018). Various organic acids (Soltan, 2008; Swiatkiewicz et al., 2010), probiotics (Deng et al., 2012; Wijayanti et al., 2019), prebiotics (Sarangi et al., 2016; Abdel-Hafeez et al., 2017), synbiotics (Radu-Rusu et al., 2010; Sarangi et al., 2016) and herbal extracts (Murugesan et al., 2015; Ahsan et al., 2018) are some notable examples of such alternative feed additives. Recently, herbal extracts owing to their bioactive components have received a lot of attention (Wallace et al., 2010) and showed beneficial effects such as antimicrobial, antioxidant, anti-inflammatory, and antiparasitic (Cheng et al., 2014). In poultry nutrition, many studies have evaluated the use of herbs as additives, for example, black cumin (El-Bagir et al., 2006; Yalçın et al., 2009), thyme (Al-Kassie, 2009; Ghasemi et al., 2010), sage (Demir et al., 2008; Rasouli et al., 2019), rosemary (Yeşilbağ et al., 2011), olive leaf (Parsaei et al., 2014), *Hypericum perforatum* L. extract (Landy et al., 2012; Banisharif et al., 2016) and grape seed oil (Tekeli et al., 2014; Salman, 2019).

The fruit grape (*Vitis vinifera*) is widely cultivated in the world, and the oil obtained from its seeds is highly valuable in terms of unsaturated fatty acids (Salman, 2019) particularly oleic and linoleic acids compared to other oil seeds (Tekeli et al., 2014). Grape seed oil is reported to possess various phenolic compounds with powerful antioxidant effects (Salman, 2019), capable of increasing HDL cholesterol while lowering LDL cholesterol (Tekeli et al., 2014). In a study investigating the effects of various aromatic oils (grape seed oil, coriander oil, laurel leaf oil) and vitamin E in broiler diets on intestinal microbiology and oxidative stability, Okur (2010) observed lowest coliform while highest lactic acid bacteria counts in ileum of birds supplemented with grape seed oil (200 mg/kg). In another study with broilers (Erkan, 2013), no significant differences were observed between groups supplemented with grape seed oil (300 mg/kg), vitamin E and selenium in terms of body weight, body weight gain, feed consumption and feed conversion ratio. Similar results were obtained in terms of body weight gain, feed consumption, blood parameters (plasma glucose, cholesterol, triglyceride, VLDL, acid phosphatase) in broilers when fed diets supplemented with grape

seed oil (5, 10, 15 g/kg), however, 15g/kg supplementation of grape seed oil improved the feed efficiency (Tekeli et al., 2014). Salman (2019) also investigated the use of grape seed oil (1%, 2%) and black seed oil (1%, 2%) in broiler diets and found significant effects on some blood parameters (total protein, globulin, cholesterol, HDL, LDL) except albumin and VLDL. An experiment with laying hens showed no effects of grape seed oil supplementation (1%, 2%, 4%) on feed consumption, egg weight, egg production, feed conversion ratio, and body weight but grape seed oil at 2% level increased the egg albumen index and lowered blood plasma glucose and cholesterol levels (Ozgan, 2008).

The *Hypericum perforatum* L. plant, also known as St John's Wort, belonging to Hypericeae family (Etemad et al., 2011) contains many bioactive components (hypericin, hyperforin, flavonoids) with antioxidant, antimicrobial and antidepressant properties (Landy et al., 2012). Supplementation of dried Hypericum perforatum L. (5 and 10 g/kg) in broiler diets as a substitute for antibiotic growth promoter had no effects on serum total protein, albumin, LDL-cholesterol and triglyceride but it provided with the highest feed conversion ratio compared to other groups (Landy et al., 2012). Landy et al. (2012) concluded that *Hypericum perforatum* L. supplementation to broilers had no positive effects on growth performance. In another study with broilers, Hosseini et al (2015) also found no effect of supplemental *Hypericum perforatum* L. (aqueous extract, 150, 300, 450 mg/kg) on performance parameters except feed consumption. They also observed higher HDL and lower glucose levels at the end of 24 days with 150 mg/kg of *Hypericum perforatum* L. supplementation while the group having 300 mg/kg of the additive had higher AST and ALT enzyme activities, however, the differences in these enzyme activities were non-significant at the end of 42 days. Addition of different herbal extracts (*Matricaria chamomilla* L., *Melissa officinalis* L., and *Hypericum perforatum* L.) to the drinking water (2ml/L) of broilers resulted in reduced cholesterol and increased immunoglobulin level (Skomorucha and Sosnowka-Czajka, 2013). *Hypericum perforatum* addition also resulted in increased body weight. Similarly, Davoodi et al. (2014) also reported that Hypericum perforatum extract (150, 200 and 250 mg/L) added to the drinking water of broilers lowered plasma triglyceride, cholesterol, and LDL levels while increasing HDL levels. The present study was proposed to determine the effects of different doses (0, 100, 200 and 300 mg/kg) of grape seed oil and *Hypericum perforatum* L. extract supplementation into the diets of laying hens on performance, egg quality, and some blood parameters.

2. Materials and Methods

2.1. Animals and Experimental Design

Three hundred and thirty-six Lohmann white commercial laying hens 40 weeks of age were used in the present experiment. The birds were raised in the Poultry Research and Application Unit of the Agricultural Management at Atatürk University Agricultural Faculty. The experiment consisted of seven groups (one control and six treatment groups as GSO-1, GSO-2, GSO-3, HPE-1, HPE-2, and HPE-3) and birds were distributed to 3-layer cages with 12 replicates per group and four chickens per replicate. Before hens were placed in cages, they were weighed and the SPSS package software was used to test the homogeneity of the groups in terms of body weight (Table 1).

Table 1. Experimental design

Group	Feed Additive (mg/kg)	No of replicates	Hens per replicate	Total hens per treatment group
Control	0*	12	4	48
GSO-1	100	12	4	48
GSO-2	200	12	4	48
GSO-3	300	12	4	48
HPE-1	100	12	4	48
HPE-2	200	12	4	48
HPE-3	300	12	4	48

GSO: Grape seed oil; HPE: Hypericum perforatum L. extract
*Basal diet

2.2. Basal Diet Composition and Nutritive Value

In the present study, first-period laying hen feed was used as a basal diet and was obtained from a feed factory. A basal diet with no additive served as control while for treatment groups basal diet was supplemented with different doses (100, 200, 300 mg/kg) of grape seed oil (GSO) and *Hypericum perforatum* L. extract (HPE). Grape seed oil and *Hypericum perforatum* L. extract used in the trial were obtained from private companies. The nutrient composition of basal diet used in the experiment was determined by the methods described in AOAC (1990) (Table 2).

Table 2. Ingredients and chemical composition of the basal diet

Ingredients	%	Chemical composition	
Corn	52.73	Dry matter, %	88.2
Soybean meal (46% CP)	18.72	Crude protein, %	16.6
Sunflower seed meal (36% CP)	5.35	Crude fiber, %	4.18
Bonkalite	7.50	Crude fat, %	2.74
Fish meal	2.16	Crude ash, %	13.6
Vegetable oil	0.50	ME (kcal/kg)**	2653
Limestone	9.80		
Dicalcium phosphate (DCP)	2.04		
Salt	0.25		
DL-Methionine	0.40		
L-Lysine	0.25		
Vitamin+mineral premix*	0.30		

*****Vitamin-mineral content of each kilogram of premix: 15.000.000 IU Vitamin A, 1.500.000 IU Vitamin D_3, 30.000 mg Vitamin E, 5.000 mg K_3, 3.000 mg Vitamin B_1, 6.000 mg Vitamin B_2, 20.000 mg Nicotinamide, 8.000 mg Calcium D Pantothenate, 5.000 mg Vitamin B_6, 15 mg Vitamin B_{12}, 1.000 mg Folic acid, 80.000 mg Manganese, 60.000 mg Zinc, 30.000 mg Iron, 5.000 mg Copper, 2.000 mg Iodine, 150 mg Selenium.

******ME **(**Metabolizable energy) was calculated according to the equation of Carpenter and Clegg (1956).

2.3. Determination of Performance Parameters

To determine the change in body weight, the hens were weighed at the beginning and end of the experiment. Egg weight, egg production, feed consumption, feed conversion ratio and cracked egg ratio were determined every 15 days of the experiment (Kaya, 2009).

Feed conversion ratio (FCR) = kg feed intake/kg egg

2.4. Determination of Egg Quality Traits

Egg quality parameters such as shape index (%), shell strength (kg/cm^2), shell weight (g), shell thickness (mm), albumen index (%), yolk index (%), and Haugh units were determined every 14 days. Egg width and length, yolk diameter, albumen width and length were measured using a caliper; albumen and yolk heights were measured using a three-leg micrometer while shape index (%), albumen index (%), yolk index (%) and Haugh units were calculated using the following formulas (Sarica and Erensayin, 2009).

Shape index (%) = egg width (cm)/egg length (cm) x 100

Albumen index (%) = albumen height (mm)/avarage of albumen length (mm) and albumen width (mm) x 100

Yolk index (%) = yolk height (mm)/yolk diameter (mm) x 100

Haugh unit = 100 Log ($H+7.57-1.7 \times W^{0.37}$) [H = Albumen height (mm), W = Egg weight (g)]

Shell strength was measured as kg/cm^2 using a breaking strength measuring tool. Eggshells free from albumen residues and membranes were weighed using precision scales. Later on eggshells were sampled from 3 different points and the average shell thickness was determined using a micrometer.

2.5. Blood Sampling and Analyses

At the end of the experiment, a total of 35 blood samples (5 hens/group) were taken from vena cutanea ulnaris into the coagulation activator tubes to determine the levels of serum triglyceride, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein, aspartate aminotransferase (AST), calcium (Ca) and phosphorus (P). The tubes were centrifuged for 5 minutes at 3000 *g* to remove the blood serum samples which were then stored at -80 °C until analyzed. Measurements of serum parameters were made using the autoanalyzer in the Laboratory of Biochemistry Department of Atatürk University Faculty of Medicine.

2.6. Statistical Analysis

All the data were analyzed by variance analysis with Duncan test for comparisons between groups using GLM (General Linear Model procedure). The effects of increased doses of grape seed oil and *Hypericum perforatum* L. extract were determined through polynomial analysis using SPSS 10.01 package software. The effects (significance) of the treatments were evaluated at $p<0.05$ level.

3. Results and Discussion

3.1. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Performance Parameters*

The results of different performance parameters (live weight, feed consumption, egg production, FCR, egg weight and cracked egg ratio) are presented in Table 3.

Table 3. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on performance parameters

Group	Level (mg/kg)	Feed consumption (g)	Egg production (%)	FCR	Egg weight (g)	Cracked egg ratio (%)	Live weight (g)		
							Initial	Final	Gain
Control	0	127.71	87.35	2.29	65.34	0.65	1602.56	1649.76	47.20
GSO-1	100	133.55	86.81	2.45	63.99	1.09	1602.06	1654.29	52.23
GSO-2	200	132.35	87.44	2.38	64.42	0.49	1591.38	1615.88	24.51
GSO-3	300	128.90	86.52	2.32	65.40	0.28	1595.00	1655.19	30.19
HPE-1	100	132.15	85.54	2.38	67.06	0.55	1585.31	1637.23	51.98
HPE-2	200	128.71	86.91	2.40	65.53	0.26	1586.29	1623.42	37.13
HPE-3	300	130.37	84.57	2.46	65.56	1.21	1591.71	1671.20	79.49
Pooled SEM		2.88	2.66	0.08	0.94	0.30	7.19	21.27	21.30
P value		0.743	0.998	0.746	0.384	0.256	0.489	0.522	0.627
Polynomial Contrasts									
GSO	Linear	0.846	0.858	0.980	0.813	0.278	0.294	0.210	0.368
	Quadratic	0.094	0.933	0.153	0.049	0.361	0.770	0.904	0.986
	Cubic	0.694	0.792	0.440	0.633	0.365	0.439	0.309	0.449
HPE	Linear	0.729	0.607	0.195	0.861	0.246	0.331	0.612	0.416
	Quadratic	0.635	0.929	0.851	0.461	0.029	0.122	0.180	0.404
	Cubic	0.326	0.610	0.779	0.348	0.163	0.670	0.528	0.447

GSO: Grape seed oil, HPE: Hypericum perforatum L. extract, FCR: Feed conversion ratio (kg feed intake/kg egg)

The present study showed that grape seed oil and *Hypericum perforatum* L. extract supplementations to laying hen diets had no significant effect on final body weight, body weight change, feed consumption, egg production, FCR, egg weight and cracked egg ratio. These results were consistent with the previously published studies using grape seed oil at 300 mg/kg (Erkan, 2013) and at 1, 2 and 4% (Ozgan, 2008) in broilers and laying hens, respectively. They also reported no significant effect of grape seed oil addition on body weight, body weight gain, feed consumption, and feed conversion ratio. On the contrary, Tekeli et al. (2014) reported an improvement in feed conversion ratio in broilers fed diets supplemented with 15 g/kg grape seed oil. In another study with Japanese quails, Banisharif et al. (2016) reported an increase in feed consumption with *Hypericum perforatum* L. (0.2, 0.4 and 0.6%) supplementation. Although the present study showed no difference among groups for body weights, Skomorucha and Sosnowka-Czajka (2013) reported an increase in the body weight of broilers provided drinking water with added *Hypericum perforatum* L. extract.

3.2. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Egg Quality Traits*

Results obtained for the egg quality characteristics (shape index, shell strength, shell thickness, shell weight, yolk index, albumen index and Haugh unit) are presented in Table 4.

Table 4. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on egg quality

Group	Level (mg/kg)	Shape index (%)	Shell strength (kg/cm^2)	Shell thickness (mm)	Shell weight (g)	Yolk index (%)	Albumen index (%)	Haugh unit
Control	0	76.62	2.79	0.46	8.21^{c}	39.78	9.39^{b}	84.49^{b}
GSO-1	100	76.54	3.02	0.45	8.35^{bc}	40.24	10.22^{a}	87.20^{a}
GSO-2	200	76.96	2.70	0.44	8.17^{c}	40.27	9.65^{ab}	85.19^{ab}
GSO-3	300	76.18	2.84	0.46	8.75^{a}	39.83	9.52^{b}	84.59^{ab}
HPE-1	100	75.59	2.58	0.45	8.73^{a}	40.12	9.27^{b}	83.86^{b}
HPE-2	200	76.30	2.66	0.47	8.64^{ab}	40.27	9.15^{b}	83.16^{b}
HPE-3	300	76.07	2.69	0.45	8.41^{abc}	40.18	9.11^{b}	83.39^{b}
Pooled SEM		0.35	0.14	0.01	0.12	0.34	0.21	0.88
P value		0.171	0.361	0.071	**0.002**	0.884	**0.007**	**0.036**
Polynomial Contrasts								
GSO	Linear	0.544	0.808	0.491	0.020	0.921	0.858	0.684
	Quadratic	0.284	0.735	0.019	0.105	0.210	0.048	0.046
	Cubic	0.253	0.109	0.609	0.078	0.991	0.092	0.153
HPE	Linear	0.553	0.736	0.827	0.301	0.336	0.245	0.269
	Quadratic	0.267	0.386	0.744	0.001	0.491	0.813	0.593
	Cubic	0.096	0.574	0.045	0.359	0.970	0.903	0.785

a, b, c: The averages with different superscripts in the same column differ significantly ($p<0.05$). **GSO:** Grape seed oil, **HPE:** *Hypericum perforatum* L. extract

The findings observed in the present study suggested no significant effects of supplemental grape seed oil and *Hypericum perforatum* L. extract on egg shape index, shell strength, shell thickness, and yolk index ($p> 0.05$). However, significant differences were observed between the groups in terms of shell weight, albumen index and Haugh unit. Eggshells from GSO-3 and HPE-1 groups were the heaviest compared to other groups ($p<0.01$) while only GSO-1 group presented with the highest values for the albumen index ($p<0.01$) and Haugh unit ($p <0.05$). Scientific data on the effects of grape seed oil and *Hypericum perforatum* L. extract on egg quality is scarce. The present finding in this study regarding albumin index was in agreement with Ozgan (2008) who also reported an increase in the egg albumin index when diets of laying hens were supplemented with grape seed oil (2 %). Ozgan (2008) attributed this improvement in the albumen index to the antioxidant properties of grape seed which might have preserved the β-ovomucin responsible for the gelatinousness of albumin resulting

in the increase in the albumin height. Also, for Haugh Units, there was a similarity between the results of the present study and that described by Kaya et al. (2014) who stated a significant effect on Haugh units when laying hens' diets were supplemented with grape seeds and grape seed extract. Egg shape index, shell strength and shell thickness remained unaffected in their experiment. In a study conducted by Kara et al. (2016), it was shown that grape pomace added to the laying hen diets had no effect on the egg albumen index and Haugh unit.

3.3. Effects of Grape Seed Oil and Hypericum perforatum L. *Extract Supplementation on Some Blood Parameters*

The average values of some blood serum parameters evaluated in this study are presented in Table 5. The effects of both additives (GSO and HPE) on serum triglyceride, cholesterol, LDL, AST, Ca and P levels were non-significant, whereas significant variations among groups were observed for the levels of serum HDL and total protein. Although no difference was observed among groups for HDL when compared to the control group, the GSO-2 and GSO-3 presented with numerically higher values. Compared to the control group a reduction in total protein ($p<0.05$) in HPE-2 was observed. In an experiment with broilers supplemented with dried *Hypericum perforatum* L. (5, 10 g/kg), Landy et al. (2012) also found no effects on serum triglyceride and LDL levels. On the contrary, Davoodi et al. (2014) with supplemental *Hypericum perforatum* L. (150, 200 and 250 mg/L) in broiler drinking water and Salman (2019) with supplemental grape seed oil (1 and 2%) in broiler diets showed lowering effects of these additives on LDL levels. Additionally, the 2% grape seed oil in diets of laying hens (Ozgan, 2008), 1 and 2% grape seed oil in broiler diets (Salman, 2019), 2 ml/l *Hypericum perforatum* L. in broiler drinking waters (Skomorucha and Sosnowka-Czajka, 2013) resulted in lower cholesterol levels.

Table 5. Effects of grape seed oil and *Hypericum perforatum* L. extract supplementation on some blood parameters

Group	Level (mg/kg)	TG	TC	HDL	LDL	TP	AST	Ca	P
Control	0	1399.20	152.60	37.00^{ab}	52.60	6.66^{a}	235.60	31.30	6.00
GSO-1	100	1045.00	122.68	26.68^{b}	47.64	6.00^{abc}	192.32	30.77	6.78
GSO-2	200	1281.40	180.00	45.20^{a}	40.00	6.14^{abc}	195.00	33.56	6.58
GSO-3	300	913.40	162.60	44.00^{a}	67.80	6.24^{ab}	175.40	24.00	4.50
HPE-1	100	1244.00	127.76	25.20^{b}	35.76	6.08^{abc}	206.00	27.88	5.33
HPE-2	200	1277.00	150.50	39.00^{ab}	43.26	5.53^{c}	202.00	30.76	6.23
HPE-3	300	1243.00	137.80	35.00^{ab}	43.80	5.92^{bc}	189.60	31.64	5.32
Pooled SEM		47.15	21.13	4.36	6.95	0.21	13.75	2.45	0.53
P value		0.090	0.507	**0.016**	0.063	**0.031**	0.125	0.174	0.056
Polynomial Contrasts									
GSO	Linear	0.045	0.288	0.064	0.334	0.332	0.030	0.060	0.078
	Quadratic	0.957	0.729	0.319	0.073	0.149	0.488	0.073	0.021
	Cubic	0.051	0.059	0.026	0.032	0.250	0.374	0.117	0.073
HPE	Linear	0.291	0.830	0.665	0.240	0.001	0.041	0.732	0.599
	Quadratic	0.507	0.788	0.339	0.023	0.004	0.555	0.403	0.810
	Cubic	0.532	0.416	0.026	0.061	0.171	0.601	0.469	0.131

a, b, c: The averages with different superscripts in the same column differ significantly ($p<0.05$). **GSO:** Grape seed oil, **HPE:** *Hypericum perforatum* L. extract, **TG:** Triglyceride (mg/dL), **TC:** Total cholesterol (mg/dL), **HDL:** High-density lipoprotein (mg/dL), **LDL:** Low-density lipoprotein (mg/dL), **TP:** Total protein (g/dL), **AST:**Aspartate aminotransferase (unit/L), **Ca:** Calcium (mg/dL), **P:** Phosphorus (mg/dL).

4. Conclusion

In conclusion, the present study showed that grape seed oil and *Hypericum perforatum* L. extract can be used in the diets of laying hens without any negative effects on birds' performance. The results also showed partial improvement in some egg quality parameters including egg shell weight, albumin index and Haugh units with grape seed oil and *Hypericum perforatum* L. extract supplementation. Further studies are needed to determine the effects of these supplements in laying hens reared under different stresses.

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