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The Effect of Phyto-chemical Compounds of Olive Leaf on Biochemical Indicators of Broiler Blood

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A B S T R A C T

Objective: Today, the possible side effects of chemical medicines in the treatment of various diseases, on the one hand and the positive effects of herbal medicines and reducing the negative effects of their use on the other hand, have led researchers to identify, analyze, purify and use beneficial compounds in such plants. Olive leaf extract, due to its beneficial chemical compounds, has been used as an effective herbal medicine in the treatment of various human and animal diseases.

Methods: In this study, the effect of olive leaf nutrition in different weeks of breeding period on performance and metabolic abnormalities was investigated; using 336 male Arian strain chickens in a completely randomized format with six treatments, four replications, and14 birds in each replication for of broiler blood, such as triglycerides, cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total protein, and blood glucose based on the AOAO 1990 method, were assessed.

Results: The mean difference of blood metabolites such as triglyceride and HDL among different treatments were not significant ($P>0.05$), however, for LDL and blood cholesterol levels this difference was significant ($P<0.05$). Regarding the anti-diabetic and hypoglycemic function of olive leaf extract in regulating blood glucose and increasing serum insulin, as well as the average total protein, the results showed no significant difference.

Conclusions: As a conclusion, the results of this research showed that the use of 1% olive leaves in broiler chickens' diet does not have a negative effect on functional traits and live weight. Therefore, present results emphasize that broiler chickens can be given olive leaf extract at 1%, while future studies conducting on-farm conditions may pronounce its impact on growth performance. Olive leaf can be used in diets of broilers and further studies on-farm conditions may justify its impact on growth performance.

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Introduction

Today, the possible side effects of chemical medicines in the treatment of various diseases, on the one hand and the positive effects of herbal medicines and reducing the negative effects of their use on the other hand, have led researchers to identify, analyze, purify and use beneficial compounds in such plants. The olive tree (*Olea europaea* L.) is an evergreen plant that has been cultivated for more than 7,000 years and is found throughout the world. The olive tree is of the most important crops in Mediterranean countries. Olive leaves and wastes from the olive lubrication process are both compounds of lignocellulose, if not used, they cause environmental pollution but have the potential to be used in animal and poultry feed, so that it is economically and socially important [1-6]. In traditional medicine, this plant has been mentioned as an antihypertensive, anti-atherosclerosis drug, laxative, antipyretic, invigorating, effective in treating urinary tract infections, relieving headaches and with antioxidant activity [7].

Olive leaves contain starch, crude protein, crude fiber, crude fat, crude ash, as well as chemical elements such as calcium, phosphorus, potassium, sodium, chlorine, magnesium, iron, zinc and copper [6, 8]. Olive leaves also contain sugar compounds, resin compounds, wax, chlorophyll and tannins, saponins, gallic acid, mannite and three types of alcohols called oleasterol, oleastranol and homo oleranotranol [9]. Olive leaves contain significant levels of polyphenols, especially oleuropein, riotin, veriacoside, apigitin-7-glucoside and lyoteolin-7-glucoside [10-13]. Oleuropein is the most abundant and most influential phenolic compound in olive leaves, belonging to squirreidoids, and found in the olive genus and many other plants [10, 14].

The extract of fresh olive leaves contain polyphenolic, flavonoid compounds, which show highest antioxidant activity and free radical scavenging power reducing the types of low and very low density lipoproteins (LDL.VLDL) and prevents atherosclerosis by preventing lipid peroxidation, through helps to stabilize the cell membrane [15-17]. The bark and leaves of the olive tree have a bitter taste and a diuretic, tonic, and blood pressure lowering effect [16, 18], are having through blood pressure by lowering the superficial arteries, without having a debilitating effect on the heart [16, 19]. This antihypertensive property may be due to the anti-angiotensin activity of oleasin and oleuropein compounds [16, 20].

Alcoholic extract of olive leaf has showed a hypoglycemic effect compared to gliben glamide and decreased blood sugar and increased serum insulin in diabetic rats, but no effect on healthy animals due to the oleuroposide [11, 16, 21, 22]. In a study, diethyl ether extract of olive leaves was administered to animals on a high-cholesterol diet which reduced serum cholesterol levels, increased serum heavy lipoprotein (HDL) and decreased serum light lipoprotein (LDL) [16]. Aqueous extract of olive leaf also caused a significant reduction in serum cholesterol and triglyceride levels of diabetic animals [18, 23, 24]. Oleuropein, oleasin and phenolic compounds in olive leaves, by preventing the oxidation of light lipoproteins (LDL) and reducing total and free-cholesterol, prevents the deposition of lipids and cholesterol on the inner wall of blood vessels [16]. There are no scientific reports on the side effects of olive leaves [25-27].

In 2021, 135 million tons of chicken meat were produced worldwide, which reached approximately 40.4% of the total production of livestock and poultry meat [28]. olive byproducts such as olive cake and other phytogenic leaves or extracts in the industrialised breeding process is yielded and production performance, health and meat quality in chicken birds are severely affected. Olive leaf (*O. europaea* L.), is one kind of potential animal nutrient used for high-quality meat. The safety of Olive leaf extractas (OLE) a sensory additive in feed for all animal species was confirmed by the European Food Safety Authority [29]. The purpose of this study was to investigate the effect of olive leaves in dietary

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treatments on blood traits in different periods of broiler breeding.

Materials and Methods

This research was conducted at Agricultural Research and Natural Resources Station, Sirvan country, Ilam province of Iran; the area with warm and temperate climate, altitude of 1030 m, mean annual rainfall: 500 mm. The required value of olive tree leaves was collected from the farms of Taghtavi village, Chavar country, Ilam province and dried in the shade and at room temperature condition. After grinding, the chemical compounds of crude protein, crude fat, crude fiber, crude ash, calcium and phosphorus (Table1) were measured in the laboratory of the Agricultural Research and Education Center and Natural Resources of Kermanshah province according to the 1990 AOAC method [8].

Table1. *Chemical composition of olive leaves (in percentage)*

Dry matter	Crude protein	Crude fat	Crude fiber	Crude ash	Calcium	Phosphorus
94.78	8.98	16.33	8.67	9.77	2.67	0.19

To perform this experiment, 392 one-day-old male broilers were transferred to the test site. The mean weight of chickens was 39 g and the age of the breeding herd was 43 weeks. To prevent Bronchitis, Newcastle and Gamburo diseases, the vaccination schedule was done according to the veterinarian's instructions (Table 2).

Table 2. *The vaccination schedule applied during the breeding period of broiler chickens*

	Breeding day	Type of vaccine	Description
1	1	Bronchitis +B1	spray
2	7	Dual (Newcastle + Influenza)	injection
3	11	Bronchitis H120 +B1	eye drop
4	16	Gumborough first vaccination	beverage
5	18	Lasotta (Newcastle)	eye drop
6	21	Gumborough second vaccination	beverage
7	23	Bronchitis IB88	eye drop
8	26	Lasotta	beverage

According to the recommendations of Arian broiler strain, diets were prepared in three periods: initial (1 to 14 days), growth (15 to 28 days) and final (29 to 42 days) [26]. In each of these periods, 1 control diet and 1 diet containing 1% of olive leaves were prepared.

Table 3 shows the nutrients and minerals supplied to the diets in different periods. At each period, to prepare a diet with 1% olive leaves, the required olive leaves were ground and added to other ground foods and then mixed. In this experiment, diets were in the form of flour.

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Table 3. *Compositions and nutrients of control and treatment diet contains 1 percent of olive leaf*

Food (percentage)	Initial (1 to 14 days)		growth (15 to 28 days)		Final (29 to 42 days)	
	Control	1% Olive leaf	Control	1% Olive leaf	Control	1% Olive leaf
Corn	51.94	50	51.83	50.35	51.15	49.56
Soybean meal (48%)	37.44	37.92	33.5	33.5	28.3	28.4
Wheat seed	4	4	8	8	14	14
Olive leaf	0	1	0	1	0	1
Vegetable oil	1.99	2.47	2.52	3	2.66	3.14
Shell	1.23	1.22	1.14	1.14	1.14	1.14
Di-calcium Phosphate	1.91	1.92	1.65	1.65	1.66	1.66
Salt	0.35	0.35	0.3	0.3	0.3	0.3
T-Vitamin Supplement	0.35	0.35	0.35	0.35	0.25	0.25
Mineral upplement	0.35	0.35	0.35	0.35	0.25	0.25
Methionine	0.27	0.27	0.23	0.23	0.18	0.19
Lysine	0.16	0.15	0.13	0.13	0.11	0.11
Calculated compounds (percentage)						
Combustible Energy (Kcal / kg)	2850	2850	2920	2920	2970	2970
Crude Protein	21.8	21.8	20.5	20.5	18.8	18.8
Ca	1	1	0.91	0.91	0.9	0.9
Usable phosphorus	0.5	0.5	0.45	0.45	0.45	0.45
Na	0.19	0.19	0.16	0.16	015	0.15
Methionine	0.58	0.58	0.52	0.52	0.46	0.46
Lysine	1.27	1.27	1.16	1.16	1.02	1.02
Methionine + cystine	0.93	0.93	0.86	0.86	0.77	0.77

Per kg of diet: Vitamin A: 1000 IU, Vitamin D3: 1500 IU, Vitamin E: 15 IU, Vitamin B12: 0.008 mg, Thiamine 0.5: mg, Riboflavin: 4 mg, Pantothenic acid: 8 mg, Niacin: 25 mg, Pyridoxine: 1 mg, Folic acid: 0.2 mg, Biotin: 0.1 mg, Manganese: 110 mg, zinc: 100 mg, copper: 9 mg, iodine: 1.3 mg, cobalt: 0.9 mg and selenium: 0.15 mg.

The dietary treatments contained 1% of olive leaves, while experimental treatments were: a) Control treatment, b) Control treatment with propranolol tablet (Control diet with 95. 104. 120 propranolol tablets in the initial, growth and termination periods, respectively), c) 1% olive leaf treatment from 1 to 14 days, d) - 1% olive leaf treatment from 1 to 21 days, e) 1% olive leaf treatment from 1 to 28 days, f)- 1% olive leaf treatment from1 to 35 days and, g) 1% olive leaf treatment from 1 to 42 days.

On the first day, the chickens based on weight randomly were assigned between treatments and replications of each experimental units. So that, in each experimental unit, there were 14 chickens with an average weight of 39 g. Each treatment had 4 replications and in each replication, 14 one-day-old male Arian strains were used (Table 3).

The weight gaining of chicks of each experimental unit were measured after two hours of starvation at the end of each week and average weight and daily weight gaining (chick/ day) were calculated trough group weighing.

At 42 days of age, 2 chickens were randomly selected from each experimental unit (replicate) and blood was taken from the under wings vein. Parameters such as triglyceride, cholesterol, heavy density lipoproteins (HDL), light density lipoproteins (LDL), total protein and blood glucose were measured.

Cholesterol in serum samples was measured by enzymatic method and also with a commercial kit using the colorimetric methods. For this purpose, using a sampler, 10 µL of each serum was transferred to the test tubes. An identical amount of commercial cholesterol was added to each sample (1000 µL) and

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the serum and test reagent solution were mixed together for 20 minutes. In a separate tube, which was considered as a standard, the standard sample was added as much as the serums in other tubes (10 µL), simultaneously, and finally the reagent was added to and placed in the photometer at 546 nm. In another test tube, a blank solution was prepared to calibrate and control the device. In the way that distilled water was used instead of serum samples. After a certain time, first, the blank was placed inside the photometer and was read several times by the device. When the result showed the same figures, the samples were then placed inside the device, in order of number, and their cholesterol levels were evaluated.

The method of measuring plasma triglycerides was similar to the cholesterol method. The value of serum, reagents, standards and light wavelengths were the same as cholesterol, except that triglyceride kits were used. Although the stability of triglyceride in the samples was up to 7 days at room temperature, but after mixing the samples with the reagent, their optical absorption to be measured within one hour.

HDL and LDL levels were measured using a sediment HDL kit. First, 500 µm of the precipitating solution was poured into pre-numbered microtubes for each treatment and then 200 µL of the sample was added and after mixing, were placed at 20 to 25 °C for 10 minutes and then centrifuged at 10,000 rpm for 2 minutes. After centrifugation, 100 µl was added to the samples and measured.

Total serum protein levels were assessed according to the Lowry et al procedure[30]. After separating the serum from the blood samples, 2000 µL of reagent was poured into the test tubes and 50 µL of blood serum of samples, standard and distilled water were added to the test tubes. After mixing, the samples were incubated at 37 ° C for 15 minutes and then their optical absorption at 540 nm was evaluated by spectrophotometer. Due to the fact that the color stability created by the reagent is 60 minutes, all samples were evaluated within this time period.

In order to measure the blood glucose levels in the samples, 10 µL of each serum and standard sample was placed in a test tube and then 1000 µL of reagent was added to each. In a blank tube, 10 µL of distilled water and 1000 µL of reagent were mixed. After 10 minutes of mixing the samples and reagent at 37 ° C, the device was first calibrated with a blank sample and then the samples were read by spectrophotometer at 546 nm and finally the standard was introduced to the device. The resulting data were analyzed using GLM procedure of SAS statistical software for statistical model 1[31] as follow:

$$\text{Model 1) } Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where Yij is the value of each observation from the jth repetition and the ith treatment, the average effect of the population; Ti is the effect of the ith treatment and εij is the effect of the experimental error related to the ith treatment.

The normality of the data was checked using the Kolmogorov Smirnov test. Data that were in the form of percentages were normalized by converting to Arc Sin. Means were compared using Duncan's multiple range test [32].

Results

The effect of using olive leaves in different periods of breeding on daily weight gain has come in Table 4. The effect of experimental treatments on feed intake and weight gaining in periods of one to 14, 14 to 28 and 1 to 42 days was not statistically significant at 5 % level. In the period of 29 to 42 days, birds fed with a diet containing olive leaves in the period of one to 14 days of age compared with the control birds and those in the period of one to 21 days of the hint leaf were gained more weight gain (P>0.05) (Table 4).

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Table 4. *The effect of olive leaf on the growth of broiler*

Experiment*								
Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**SEM**	**P-vale**
Average live weight at the end of the course(gr)	2238.8	2292.5	2086.00	2192.80	2185.90	2056.3	37.285	0.468
Average weight gain								
1 to 14 days	15.05	16.82	13.62	15.24	12.91	14.00	0.498	0.262
15 to 28 days	55.46	54.95	52.70	52.34	49.62	50.83	0.978	0.518
29 to 42 days	70.09^{b}	84.48^{a}	72.94^{b}	76.18^{ab}	79.95^{ab}	75.99^{ab}	1.468	0.049
1 to 42 days	46.87	52.09	46.42	47.92	47.49	46.94	0.630	0.079

Means within a column with different superscript letters are significantly different at P<0.05 SEM: Mean Standard Error

**Experiments: 1: control (without olive leaf), 2: 1 to 14 days, 3: 1 to 21 days, 4: 1 to 28 days, 1 to 35 days and 1 to 42 days.*

Table 5 shows the mean difference of blood biochemical indices such as triglyceride and HDL between different treatments which was not significant statistically (P>0.05), but such difference was significant for LDL and cholesterol levels (P <0.05). No differences were observed in all treatments containing olive leaves compared to the control group, except OLD35 and OLD42 treatments. Compared to the control group, a decrease in LDL in OLD35 treatment and a decrease in cholesterol in OLD42 treatment was observed (P <0.05).

The table 5 shows that no significant difference was observed between the treatments for the mean blood glucose level (P>0.05).

Table 5. *The effect of olive leaf on blood metabolites at the end of the experimental period (ml /dl)*

Characteristics	PRO	OLD0	OLD14	OLD21	OLD28	OLD35	OLD42	SEM	P-vale
Cholesterol	132.50^{abc}	130.50^{abc}	134.20^{a}	125.00^{bc}	122.20^{bc}	123.00^{bc}	116.25^{c}	3.774	0.049
Tri glyceride	67.50	63.75	66/25	65.50	66.00	62.75	61.00	4.024	0.919
HDL	46.25	45.00	51.75	48.00	49.25	50.75	49.00	3.986	0.989
LDL	72.75^{a}	71.75^{ab}	67.25^{ab}	62.90^{ab}	58.80^{bc}	49.95^{c}	53.55^{bc}	3.049	0.007
Glucose	207.70	198.50	206.00	205.60	216.20	206.80	214.10	2.767	0.741
Protein (g/Dl)	4.352	4.237	4.317	4.420	4.072	3.892	3.915	0.083	0.505

PRO, OLD0, OLD14, OLD28, OLD35, OLD 42: propranolol tablets treatment, control (without olive leaf) and use of olive leaves from 1 to 14, 1 to 21, 1 to 28, and 1 to 42 days, respectively.

SEM: Mean standard error

Means within a column with different superscript letters are significantly different at P<0.05

Discussion

The effect of using olive leaves in different periods of breeding showed that the addition of olive leaf did not pose any effect on their weight gain than control groups. The use of plant extracts in the diet of broilers showed positive effects on weight gain compared to the control group, which was not consistent with the results of the present study [33, 34], In some studies, a significant increase in the weight of broiler has been reported in certain periods compared to the control group [35].

According to various sources, the exact mechanism of the reduction effect of olive leaf on LDL was not found, but unsaturated fats seem to decrease LDL cholesterol and increase HDL cholesterol and reduce the incidence of

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cardiovascular disease through a double bond, including oleic acid in olive leaf [36]. The presence of oleuropein in olive leaf prevents LDL oxidation [37]. In animals with high cholesterol diet, HDL levels increased and serum LDL levels decreased significantly, as a result of consuming olive leaf extract [37].

The results of this study on LDL levels were in consistent with the results of recent research but not in case of HDL. Ismaili et al. reported that administration of olive leaf diethyl ether extract was able to reduce serum cholesterol levels in animals on a high-cholesterol diet [37, 38]. Consumption of aqueous extract of olive leaf caused a significant reduction in serum cholesterol and triglyceride levels of diabetic animals [23, 24]. It seems that consuming olive leaf extract is able to reduce the total cholesterol and triglycerides levels in the blood, and increase the high-density lipoprotein levels, regardless of the cause (diabetes or high cholesterol diet) [18, 39]. The results of such reports do not support to the results of our study.

The results of studies show that alcoholic olive leaf extract has a hypoglycemic effect in comparison with gliben glamide drug and reduces blood sugar and increases serum insulin in diabetic rats, but showed no significant effect on healthy animals [22, 24, 40]. Olive leaf extract is having anti-diabetic and hypoglycemic effects [4, 22]. The present results regarding blood glucose levels do not match the results of experiments performed in this field.

The effect of different levels of olive leaves on blood metabolites, the results showed that dietary treatments had no effect on total protein content [24, 41]. The results of the experiment are consistent with the results of this study.

The result of experiments showed that dietary treatments had a significant effect on cholesterol, triglyceride, LDL but did not affect HDL glucose and total protein The results of this study on LDL, HDL, cholesterol, glucose and total protein were consistent with the results of experiments, but did not match on about the triglyceride levels [4, 24, 41].

The mechanism is known to be that oleuropein and hydroxytyrosol derived from olive leaves inhibit the oxidation of LDL and reduce the secretion of the enzyme responsible for cholesterol synthesis (3-hydroxy-3-methylglutaryl-coenzyme A) [5]. Decreased α-amylase activity in the diabetic group may be due to inhibition of pancreatic amylase, which delays carbohydrate digestion and prolongs digestion time, resulting in decreased glucose absorption rate. The increase in plasma glucose is attenuated [16].

As a conclusion, the results of this research showed that the use of 1% olive leaves in broiler chickens' diet does not have a negative effect on functional traits and live weight. Therefore, present results emphasize that broiler chickens can be given olive leaf extract at 1%, while future studies conducting on-farm conditions may pronounce its impact on growth performance.

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Conflict of interest

The authors declare no conflicts of interest.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

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