jotaf Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Eylül/September 2023, 20(3)
Başvuru/Received: 11/02/23
Kabul/Accepted: 10/04/23
DOI: 10.33462/jotaf.1250068

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of Olive Leaves Hydroalcoholic Extract (*Olea Europaea* L.) and LactoFeed® probiotics on Induced Ascites in Male Broilers

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Abstract

The incidence of disease and damage will increase, if environmental control and acceptable management practices are not provided during the rearing period. Ascites affect young broilers with rapid growth, and the most critical factor in causing ascites syndrome is the lack of oxygen in body tissues (hypoxia). This research aimed to investigate the effect of olive leaves hydroalcoholic extract and probiotics (LactoFeed) on experimental ascites caused by levothyroxine in male broiler chickens. The present study was an interventional type, and for its implementation, a single-factor design was used in eight groups with 3 replicates. Data were analyzed based on a one-way analysis of variance. Blood parameters of male chickens were measured after 42 days. Biochemical factors of the blood serum of broilers included AST, ALT, ALT, TSHT, T_4, T_3, Glucose, Cholesterol, Triglyceride, Urea, Uric acid, TP, Albumin, and Globulin. AST, ALT, and ALP levels in the induced ascites group increased by 1.16, 1.35, and 1.16 times, respectively. When the chickens had induced ascites, the levels of all three hormones in the blood serum of broiler chickens increased significantly (P<0.01). AST showed a positive and significant correlation with ALT, T_4, and T_3 (0.76, 0.71, and 0.75, respectively). But there was a very significant negative correlation between TP (-0.86) and albumin (-0.84). T4 had a positive and significant correlation with T_3, glucose, cholesterol, TG, and urea. The olive extract had a positive effect on induced ascites and improved poultry performance. Probiotics, also, had a positive effect on the treatment of birds induced ascites.

Keywords: Olive leaves extract, Probiotic, LactoFeed, Ascites, Levothyroxine.

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Atıf/Citation:. Talebi, E., Haghighat-Jahromi, M. Effect of olive leaves hydroalcoholic extract (*Olea Europaea* L.) and probiotic (LactoFeed) on induced ascites in male broilers. *Journal of Tekirdag Agricultural Faculty,* 20(3): 688-697.

1. Introduction

Extensive genetic selection for growth, meat production, and more acceptable feed conversion ratio has led to many physiological modifications (Willems et al., 2013; Zerjal et al., 2021; Esen et al., 2022). One of these changes is the increased incidence of metabolic diseases such as ascites. Ascites occur in unfavorable environmental conditions and unbalanced nutrition (Hossain and Akter, 2022). This complication emerges when there is an imbalance between the amount of available oxygen and the need for oxygen (hypoxia) (Nasrollahi et al., 2016; Li et al., 2022) and through the accumulation of fluid in the ventricular region of the abdomen driven by events related to the need to supply high amounts of oxygen to the tissue (Gamero et al., 2021). Ascites are more typical in fast-growing male broilers reared at high altitudes and are slightly affected by cold stress (Özkan et al., 2010). However, infectious agents such as *Aspergillosis*, *E. coli*, and bronchitis virus also induce ascites syndrome (Julian, 2000), which is known as a complication of right ventricular dysfunction (Martínez et al., 2021).

Before the bird shows clear symptoms of ascites, which include increased pulmonary blood pressure, pericardial fluid retention, hypertrophy, and dilation of the heart, especially the right ventricle, specific blood, and anatomical changes occur in the body (Hossain and Akter, 2022; Li et al., 2022). Hematocrit, hemoglobin, and red blood cell count are significantly increased in birds with ascites (Luger et al., 2003; Li et al., 2022). The lack of oxygen in birds stimulates the kidneys to produce erythropoietin, which stimulates the production of red blood cells in the bone marrow. This leads to an increase in hematocrit, which leads to an increase in blood viscosity (Hossain and Akter, 2022). In some special conditions, such as when there is a lack of oxygen, increasing the activity of thyroid and metabolic hormones, as well as reducing the amount of cellular antioxidants, the portion of radical's production increases greatly (Ma et al., 2004). Finally, free radicals and other active oxygen particles, due to the peroxidation of fat molecules in cell membranes and important organelles inside the cells of vital body tissues cause the destruction of their membranes and the leakage of fluids outside the cells (Khosravinezhad et al., 2017). Therefore, the change in the antioxidant status of broiler chickens during the development of ascites, characterized by an increase in free radical markers in the damaged tissue, indicates oxidative stress during ascites (Georgieva et al., 2006).

Probiotics are one of the products used in poultry feeding. They are living microorganisms that have many beneficial effects on the microbial population of the gastrointestinal tract and the health of the host. Any change in the microbial flora pattern of the digestive system leads to an increase in the costs related to energy and protein consumption and ultimately increases the bird's oxygen demand (Zheng et al., 2020). Therefore, paying attention to creating a suitable pattern of flora in the digestive system can be effective in reducing the complications related to ascites by improving the immune system and reducing the costs related to oxygen supply (Shimizu et al., 2013). Today, it is known that probiotics, in addition to positive effects on the functioning of the digestive system, such as improving the digestion and absorption of food, and essential minerals, as well as preventing disorders and diseases of the digestive system, reduce symptoms and improve the treatment process of metabolic diseases (Ohashi and Ushida, 2009).

Medicinal plant extracts and essential oils are also used to prevent and help recover birds suffering from ascites. Different parts of medicinal plants are used to stimulate growth, improve immunity, increase digestibility, and prevent and treat diseases in poultry feeding. Among the plants used, we can mention olive tree leaves. Olive leaves contain phenolic compounds, terpenes, fat-soluble compounds, carbohydrates, proteins, minerals, *etc*. (Pereira et al., 2020). They have the highest antioxidant activity and free radical scavenging power among different parts of the olive tree (El and Karakaya, 2009). It has been found that its antioxidant capacity is approximately twice that of green tea and 2.5 times that of vitamin C (Roshanak et al., 2016) and Oleuropein is the most important phenolic compound in olive leaves, which has the capacity of radical adsorption of oxygen ten times as much as green tea with antioxidant and antimicrobial properties (Hayes et al., 2011). This compound has several pharmacological properties, including antioxidant, anti-inflammatory, anti-atherogenic, antimicrobial, and antiviral properties (Omar, 2010).

Due to the limited scientific resources related to the comparison of plant essential oils and the effect of probiotics on ascites, this experiment was performed to investigate the effect of the hydroalcoholic olive extract with probiotics (LactoFeed) on experimental levothyroxine-induced ascites in male broilers.

2. Materials and Methods

Olive leaves were harvested in May 2022. The leaves were identified after being collected at the Medicinal Plants Research Center of Darab Branch, Islamic Azad University. Then they were weighed, washed with distilled water to remove contamination, and spread in the shade. The dried leaves were ground and mixed with 70% ethanol at 40°C. The obtained extract was concentrated with Whatman No. 1 paper and a rotary evaporator (IKA Model RV Basic 05) at 40°C and dried with a freeze dryer (Operon Model FDB 5503). The obtained hydroalcoholic extract was stored away from light at a temperature of 4 °C.

The phenolic amount in the extract was measured by the Folin-Siocalto method and was expressed as mg equivalent of tannic acid per gram of olive leaf extract (Shishehbor et al., 2021; Şahin et al., 2022). Then, different concentrations (50-1000 ppm) were prepared using dried powder, and their antioxidant activity was determined by DPPH and compared with synthetic antioxidants BHA and BHT (Afacan et al., 2014). The net oxidative activity diagram of the extract against the amount of phenolic compounds was determined through Excel and linear equations.

The current experiment was performed at the Darab Branch, Islamic Azad University, Darab, Fars, Iran. In this study, 240 one-day-old male broilers of Ross 308 with an average weight of 47.30±5.00 g were randomly divided into eight experimental groups with three replicates and ten chicks per replication (cage) in a completely randomized design (CRD). Each group had a 4-liter bowl and a separate dining tray.

The experimental treatments included 1) Basal diet (BD), 2) BD+OLE (Olive leaves extract), 3) BD+probiotics, 4) BD+OLE+probiotics 5) Induced ascites+BD, 6) Induced ascites +BD+OLE, 7) Induced ascites+BD+probiotics, 8) Induced ascites+BD+OLE+probiotics. Levothyroxine (a thyroid hormone) was used to induce ascites at a dose of 45 ppm. The concentration of olive leaf extract (OLE) was 150 ppm, and probiotics for ages up to 21 days old and from the age of 21 days to the end (LactoFeed) was 200 and 100 mg/kg, respectively. LactoFeed contained various lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*, which are natural microorganisms found in the digestive system of poultry. A based corn-soybean meal diet was formulated according to Ross 308 requirements for starter (1 to 10 days), grower (11 to 24 days), and finisher periods (25 to 42 days) by UFFDA software (*Table 1*). In all experimental diets, energy, protein, and amino acids were provided at the same level. During the experiment, the light regime was 23 hours and one hour of darkness. The feed was monitored several times during the day and provided to the chickens properly with appropriate drinking water (*ad libitum*).

Table 1. Nutrient contents of basal diet

Feed ingredients	1 to 10 days	11 to 24 days	25 to 42 days
Corn grain	52.55	53.05	54.12
Soybean meal	34.00	34.67	34.86
Corn gluten	5.63	3.00	1.50
Limestone	1.32	1.08	1.04
Dicalcium phosphate	1.76	1.55	1.40
Salt	0.36	0.47	0.42
Threonine	0.10	0.06	0.00
L- Lysine	0.42	0.20	0.00
DL- Methionine	0.32	0.25	0.19
Corn oil lacks antioxidants	3.04	5.17	5.97
Vitamins Mineral premixes[1]	0.50	0.50	0.50
Nutrient composition			
Metabolizable energy (kcal/kg)	3025	3150	3200
Crude protein (%)	23.52	22.00	21.00
Ca (%)	1.05	0.90	0.85
P av. (%)	0.50	0.45	0.42
Methionine + Cystine (%)	1.07	0.95	0.86
Methionine (%)	0.71	0.60	0.53
Lysine (%)	1.44	1.25	1.09
Na (%)	0.16	0.20	0.18

[1]Each kilogram of diet containing vitamin A, 11,000 IU; Vitamin D_3 (Cholecalciferol), 2300 IU; Vitamin E, 121 IU; Vitamin K_3, 2 mg; Vitamin B_{12}, 0.02 mg; Thiamine, 4 mg; Riboflavin; 4 mg; Folic acid, 1 mg; Biotin, 0.03, mg; Pyridoxine 4 mg; Choline chloride, 840 mg; Ethoxyquin, 125 mg; Manganese sulfate, 100 mg; Selenium (sodium selenate), 0.2 mg; Iodine, 1 mg; Copper sulfate, 100 mg; Iron is 50 mg.

At the end of the experiment, two chickens with a weight close to the average of each pen were selected, and approximately 8 ml of blood was taken from each chicken and poured into two test tubes, one containing anticoagulant and the other a simple test tube. The test tubes were stored at a temperature of about 2-4 ° C and transferred to the laboratory in less than 30 minutes, where they were placed in a centrifuge at 3000 rpm for 10 minutes to separate the serum. Samples containing anticoagulants for measuring hemoglobin and hematocrit. The isolated serum samples were kept until the measurement of blood parameters in the freezer at -20 ° C. The dependent variables of blood parameters were included AST, ALT, ALP, TSH, T_4, T_3, glucose, cholesterol, triglyceride, urea, uric acid, total protein, albumin, globulin, RBC, HTC, and body weight, which measured through standard Kits (Pars Azmun, Iran) and a weight scale. The mentioned parameters of blood serum were measured by the standard kits using a spectrophotometer.

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

$$Y_{ij} = \mu + T_i + e_{ij} \quad \text{(Eq. 1)}$$

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

Blood serum enzymes, thyroid hormones, biochemical parameters, RBC, HTC, and body weight were measured based on IU/L, ng/ml, mg/dl, 10^6/µl, percent, and kilogram, respectively. First, the normality of the data was tested through Kolmogorov-Smirnov. The data expressed as a percentage and ratio were first corrected and then subjected to statistical analysis. All statistical analysis were performed using SAS statistical software (version 9.1) with GLM proc and post-hoc Tukey’s range test was used to compare the means at the significance level of 0.05.

3. Results and Discussion

The phenolic compounds and DPPH radical inhibition in olive leaf extract was recorded 164.34±0.35 (mg/gram) and 144.35±0.55 (µg/ml), respectively.

The results of statistical analysis comparing the average of AST, ALT, and ALP enzymes are presented in Table 2. The highest concentration of all three enzymes was recorded in ascites chickens (P<0.001). The levels of AST, ALT, and ALP in the induced ascites group increased by 1.16, 1.35, and 1.16 times, respectively, compared to the control group, which showed the highest amount of enzymes compared to other treatments (p<0.001). The induced ascites group that consumed probiotics exhibited the lowest ratio of all three enzymes compared to the group of ascites chickens without treatment. This finding showed the positive effect of probiotic consumption on liver function in chickens with ascites.

Table 2. Comparison between the mean of different enzymes in broiler serum under experimental conditions

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
1	$20.43±0.49^{c}$	$15.7±0.40^{d}$	$88.33±3.06^{e}$
2	$23.68±1.47^{ab}$	$17.72±0.54^{cd}$	$94.00±1.00^{bc}$
3	$21.1±1.10^{b}$	$16.37±0.45^{d}$	$92.33±4.16^{cde}$
4	$22.83±0.76^{ab}$	$20.50±0.44^{bc}$	$89.00±1.00^{de}$
5	$23.74±0.98^{ab}$	$21.20±1.65^{b}$	$102.67±2.52^{a}$
6	$22.00±0.87^{ab}$	$22.23±1.26^{b}$	$101.67±1.53^{abc}$
7	$22.13±0.42^{ab}$	$16.89±0.30^{d}$	$98.00±1.00^{abc}$
8	$25.10±1.05^{a}$	$25.4±1.10^{a}$	$96.67±0.58^{ab}$
P-value	<0.001	<0.001	<0.001

[1]: Control, [2]: OLE: Olive leaf extract, [3]: Probiotic, [4]: OLE+probiotics, [5]: Induced ascites, [6]: Induced ascites+OLE, [7]: Induced ascites+probiotics, [8]: Induced ascites+probiotics+OLE, AST: Aspartate transaminase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, IU/L: International Unit per Liter, [a-d:] The means with different letters in each column have a significant difference

The occurrence of necrosis or damage to the cell membrane causes the release of AST, ALP, and ALT enzymes into the blood circulation. In the present study, in the group of induced ascites with probiotics, the amount of liver function index enzymes had a significant decrease and showed that probiotics have the most positive effect on liver function in induced ascites and then Olive has a significant positive effect on liver function in induced ascites but less than probiotics. Research showed that probiotics have a positive effect on liver enzymes and improve damaged liver tissue (Hossain and Akter, 2022; Li et al., 2022). The amount of total fatty acid in the liver decreases due to the consumption of probiotics and they reduce the serum level of ALT. These effects have been associated with a decrease in the activity of TNF-regulated stress kinases. In this study, the serum levels of these enzymes did not increase, especially during the consumption of probiotics, which indicates the absence of liver damage under heat stress due to the consumption of probiotics and the interaction of probiotics and prebiotics. It has been determined that probiotics perform this action through the production of butyric acid and hydrogen, which probably have a stimulating role in the production of antioxidants and the destruction of free radicals (Pereira et al., 2020).

Oleuropein is the most important phenolic compound of OLE and a substance with strong antioxidant and antimicrobial properties. This compound is rarely found in free form in nature. Antioxidant compounds from plant sources are known as oxygen or free radical absorbers. Some molecules, including flavonoids and phenolic compounds, are responsible for the antioxidant properties of natural plant extracts. Flavonoids have a strong antioxidant capacity to inhibit free radicals and end oxidative reactions. Today, the use of plants as an alternative to antibiotics as growth promoters has increased and in poultry feed, various compounds have been studied as alternatives to antibiotics as growth promoters in the production of broiler chickens (El and Karakaya, 2009).

Induced ascites chickens and induced ascites chickens that consumed probiotics and OLE demonstrated the highest levels of serum TSH, T_4, and T_3 hormones (*Table 3*). When the chickens suffered from induced ascites, the level of all three hormones in the blood serum of broiler chickens increased significantly (P<0.01). The treatments with OLE were able to significantly decrease TSH, T_4, and T_3 levels compared to other groups.

Table 3 Comparison between means of Thyroid hormone under experimental conditions

Treatment	TSH (ng/ml)	T_4 (ng/ml)	T_3 (ng/ml)
1	26.10±1.61^{c}	7.17±1.05^{c}	1.47±0.21^{c}
2	28.77±1. 27^{b}	7.57±0.26^{bc}	1.63±0.31^{c}
3	30.07±1.12^{a}	8.08±0.36^{bc}	1.90±0.21^{abc}
4	27.03±1.12^{b}	9.47±0.57^{bc}	2.13±0.25^{ab}
5	31.10±2.54^{a}	16.88±2.35^{a}	2.50±0.26^{ab}
6	29.80±2.01^{b}	9.43±0.25^{bc}	1.80±0.10^{bc}
7	30.57±0.76^{a}	11.07±0.47^{b}	1.99±0.10^{abc}
8	30.83±0.35^{a}	16.37±1.45^{a}	2.67±0.32^{a}
P-value	0.016	<0.001	<0.001

[1]: Control, [2]: OLE: Olive leaf extract, [3]: Probiotic, [4]: OLE+probiotics, [5]: Induced ascites, [6]: Induced ascites+OLE, [7]: Induced ascites+probiotics, [8]: Induced ascites+probiotics+OLE, TSH: Thyroid stimulating hormone, T_4: Thyroxin, T_3: Triiodothyronine, ng/ml: Nanograms per deciliter, [a-d:] The means with different letters in each column have a significant difference

Thyroid hormones increased in the group receiving OLE and the probiotic group, both individually and in combination. So that the amount of T_4 and T_3 in olives was significantly affected in induced ascites + OLE compared to induced ascites. Research indicated that the plasma concentration of thyroid hormones plays an important role in increasing the metabolism of chickens affected by ascites disease and the prevalence of ascites. The level of thyroid hormone T_3 decreased in chickens with induced ascites that received olive leaf extract (El and Karakaya, 2009). In rats, the administration of triterpenoids isolated from olive leaves for 6 weeks at a dose of 60 mg/kg prevented the development of hypertension and severe atherosclerosis and improved insulin resistance (Manafi et al., 2017).

This disease, also, affected the levels of glucose, cholesterol, triglyceride, urea, uric acid, TP, albumin, and globulin. So that the levels of these biochemical factors in the blood serum of broiler chickens showed a significant increase in chickens with induced ascites compared to other groups (p<0.001) (*Table 4*). The use of probiotics and probiotics along with olive leaf extract in both healthy and induced ascites groups caused a significant decrease in the levels of biochemical factors.

The results showed that the amount of albumin and total protein recorded a range of 1.2-1.6, and 3.3-4.2 mg/dl, respectively. The reason for the decrease in albumin and total protein in serum can be attributed to the secretion of high amounts of albumin and total protein in ascites fluid and the decrease in the concentration of ions in the blood serum. Some studies focused on the ineffectiveness of probiotics on serum lipids (Talebi et al., 2021). However, in this study, the effect of probiotics on serum lipids was significant. Serum protein and albumin are affected in chronic liver diseases and indicate liver damage (Manafi et al., 2017).

Table 4 Comparison between means of some biochemical traits under experimental conditions

Tr	Glucose (mg/dl)	Cho (mg/dl)	TG (mg/dl)	Urea (mg/dl)	UA (mg/dl)	TP (mg/dl)	Al (mg/dl)	Gl (mg/dl)
1	242.1±10.6^{d}	128.7±8.1^{b}	104.7±4.5^{b}	5.3±0.2^{b}	11.7±0.6^{a}	4.2±0.1^{a}	1.6±0.1^{a}	1.7.1^{a}
2	243.5±22.1^{d}	147.3±5.7^{ab}	115.3±5.1^{ab}	5.4±0.2^{b}	10.6±0.6^{ab}	3.6±0.3^{ab}	1.5±0.1^{b}	1.7±0.1^{a}
3	261.1±14.7^{cd}	154.4±9.5^{ab}	116.7±2.1^{ab}	5.7±0.1^{b}	10.6±0.3^{ab}	3.7±0.2^{ab}	1.5±0.1^{ab}	1.5±0.1^{ab}
4	272.7±2.3^{bcd}	161.3±14.8^{a}	122.7±4.5^{a}	5.8±0.1^{b}	10.6±0.5^{ab}	3.5±0.1^{b}	1.4±0.1^{ab}	1.5±0.1^{ab}
5	324.3±3.8^{a}	177.5±5.7^{a}	123.7±2.5^{a}	6.5±0.2^{a}	5.7±4.7^{b}	3.5±0.2^{b}	1.2±0.1^{b}	1.4±0.1^{b}
6	277.4±5.3^{bcd}	154.7±6.0^{ab}	113.3±0.6^{ab}	5.6±0.2^{b}	9.9±0.2^{ab}	3.7±0.1^{ab}	1.5±0.1^{ab}	1.6±0.1^{ab}
7	301.1±26.2^{abc}	175.3±7.5^{a}	124.7±6.4^{a}	5.8±0.1^{b}	10.5±0.4^{ab}	3.7±0.2^{ab}	1.4±0.1^{ab}	1.5±0.1^{ab}
8	321.3±2.5^{ab}	176.3±7.2^{a}	126.3±2.3^{a}	6.8±0.3^{a}	8.1±0.1^{ab}	3.3±0.2^{b}	1.2±0.1^{b}	1.3±0.1^{b}
P-value	<0.001	<0.001	<0.001	<0.001	0.014	0.002	<0.001	<0.001

[1]**:** Control, [2]: OLE: Olive leaf extract, [3]: Probiotic, [4]: OLE+probiotics, [5]: Induced ascites, [6]: Induced ascites+OLE, [7]: Induced ascites+probiotics, [8]: Induced ascites+probiotics+OLE, TP: Total protein, (mg/dl): milligrams per decilitre, [a-d:] The means with different letters in each column have a significant difference, Tr: Treatment, Cho: Cholesterol, TG: Triglyceride, UA: Uric acid, Al: Albumin, Gl: Globulin

Blood globulin concentration significantly decreased and blood glucose increased ($p<0.001$). These changes are probably due to the increase in food consumption and digestibility of nutrients, glucose retention, increase in gluconeogenesis (through conversion of amino acids to glucose) due to probiotic consumption, decrease in the activity of metabolic enzymes, increase in glycogenolysis and gluconeogenesis. In some studies, it was shown that the serum glucose of broiler chickens was not affected by probiotics, but in the present study, the level of glucose in the blood serum of chickens with induced ascites that consumed probiotics decreased significantly (Manafi et al., 2017; Talebi et al., 2021). Probiotics may cause glucose absorption through insulinotropic polypeptides and the production of glucagon-like peptides. It was reported in the research that probiotic supplements can increase blood serum glucose and albumin in broiler chickens (Pereira et al., 2020; Stanton et al., 2020).

Table 5 Comparison between RBC, HTC, and body weight under experimental conditions

Treatment	RBC (10^6/µl)	HTC (%)	BW (kg)
1	1.82±0.11^{c}	31.99±0.99^{a}	3.33±0.21^{a}
2	1.87±0.06^{c}	31.04±1.06^{ab}	3.17±0.21^{a}
3	2.17±0.12^{bc}	29.20±0.92^{abc}	3.03±0.34^{a}
4	2.27±0.12^{bc}	29.28±0.91^{abc}	3.04±0.07^{a}
5	2.54±0.21^{ab}	26.40±0.78^{bc}	1.93±0.07^{b}
6	2.27±0.15^{abc}	29.57±0.29^{ab}	2.55±0.65^{ab}
7	2.44±0.04^{ab}	28.97±1.01^{bc}	2.30±0.36^{ab}
8	2.67±0.15^{a}	25.03±0.55^{c}	1.87±0.06^{b}
P-value	<0.001	<0.001	<0.001

[1]**:** Control, [2]: OLE: Olive leaf extract, [3]: Probiotic, [4]: OLE+probiotics, [5]: Induced ascites, [6]: Induced ascites+OLE, [7]: Induced ascites+probiotics, [8]: Induced ascites+probiotics+OLE, RBC: Red blood cell, HTC: Hematocrit, BW: Body weight, (10^6/µl): Million/microliter, [a-d:] The means with different letters in each column have a significant difference.

The intestinal microbial population is involved in the regulation of energy homeostasis and the development of metabolic diseases and insulin resistance in the body's cells. The beneficial effects of probiotics on plasma glucose levels may be due to changes in the microbial composition of the gastrointestinal tract, and the correction of intestinal microflora imbalances by increasing gram-positive bacteria, improving the function of the renal defense barrier and its immunomodulatory effects. Improve intestinal integrity and simultaneously reduce TLR_4 (Toll-like receptor-4) messaging (Nemati et al., 2017; Li et al., 2022).

The number of RBC in chickens with induced ascites showed a significant increase, and like other measured factors, OLE and probiotics caused its adjustment in sick chickens ($p<0.001$). However, no significant change in the number of red blood cells was observed in healthy chickens with the consumption of OLE and probiotics. HTC percentage was also affected by induced ascites. Thus, the percentage of HTC decreased in sick chickens, but it was compensated by the use of OLE and probiotics (*Table 5*).

The results of the use of probiotics in the diet are very diverse, from no effect on growth indicators to increase yield and increase product quality to promotion. The groups receiving OLE and probiotics showed a positive effect on the amount of total protein in the treatments with ascites. The amount of total protein is measured as a functional factor of the liver. The percentage of hematocrit (HCT) in induced ascites treatment + olive showed a significant increase compared to induce ascites treatment and showed the most positive effect. In terms of weight index, all treatments recorded a significant increase compared to induced ascites treatment. In this study, levothyroxine caused weight loss. Levothyroxine is a precursor of thyroid hormones, and increasing thyroid hormones in the body increases metabolism and increases metabolic energy consumption in treatments receiving levothyroxine (Nemati et al., 2017; Manafi et al., 2017; Li et al., 2022).

AST showed a positive and significant correlation with ALT, T_4, and T_3 (0.76, 0.71, and 0.75, respectively) ($p<0.05$). But there was a very significant negative correlation with TP (-0.86), and Albumin (-0.84). T_4 had a positive and significant correlation with T_3, Glucose, Cholesterol, TG, and Urea. However, it showed a negative correlation with other measured traits (*Table 6*).

Considering that the body weight in this experiment was affected by different treatments and chickens with induced ascites recorded the lowest body weight (1.93±0.07 kg), this trait had a positive and significant correlation with HTC percentage.

Table 6 Correlation between blood parameters under experimental conditions

	ALT	ALP	TSH	T_4	T_3	Glu	Cho	TG	Ur	UA	TP	Al	Glob	RBC	HTC	BW
AST	0.76*	0.41	0.49	0.71*	0.75*	0.62	0.66	0.72*	0.77*	-0.66	-0.86**	-0.84**	-0.56	0.60	-0.73*	-0.65
ALT		0.49	0.43	0.71*	0.76*	0.68	0.58	0.54	0.76*	-0.63	-0.74*	-0.71*	-0.64	0.72*	-0.77*	-0.70*
ALP			0.82**	0.62	0.45	0.69	0.63	0.41	0.48	-0.72*	-0.42	-0.59	-0.51	0.61	-0.55	-0.79*
TSH				0.70*	0.63	0.75*	0.79*	0.65	0.67	-0.68	-0.62	-0.70*	-0.73*	0.75*	-0.74*	-0.82**
T_4					0.94**	0.95**	0.83**	0.74*	0.97**	-0.92**	-0.69	-0.97**	-0.91**	0.89**	-0.95**	-0.94**
T_3						0.91**	0.88**	0.86**	0.98**	-0.81**	-0.84**	-0.95**	-0.95**	0.92**	-0.98**	-0.85**
Glu							0.92**	0.81**	0.91**	-0.83**	-0.68	-0.93**	-0.94**	0.97**	-0.93**	-0.98**
Cho								0.96**	0.84**	-0.72*	-0.83**	-0.89**	-0.90**	0.94**	-0.87**	-0.88**
TG									0.80*	-0.59	-0.89**	-0.85**	-0.84**	0.86**	-0.82**	-0.74*
Ur										-0.83**	-0.78*	-0.96**	-0.94**	0.90**	-0.99**	-0.88**
UA											0.64	0.89**	0.75*	-0.73*	0.82**	0.82**
TP												0.81**	0.73*	-0.76*	0.81*	0.65
Al													0.88**	-0.87**	0.94**	0.90**
Glo														-0.97**	0.96**	0.88**
RBC															-0.94**	-0.93**
HTC																0.90**

AST: Aspartate transaminase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TSH: Thyroid stimulating hormone, T_4: Thyroxin, T_3: Triiodothyronine, Glu: Glucose, Cho: Cholesterol, TG: Triglyceride, Ur: Urea , UA: Uric acid , TP: Total protein, Al: Albumin, Glo: Globulin, RBC: Red blood cell, HTC: Hematocrit, BW: Body weight, * Significant at $P<0.05$ ** High significant at $P<0.01$.

The liver function index increased in induced ascites compared to the control group. Plasma glucose, cholesterol, triglyceride, globulin and RBC were affected by induced ascites. Liver function factors such as total protein and albumin and blood hematocrit showed a significant decrease, which indicates severe liver damage followed by kidney dysfunction. Blood urea level also increased and blood uric acid level decreased. The basic rations used in this research had the ideal level of protein for chickens, and vegetable food sources were used to estimate the required protein. It seems that the reason for the decrease in blood uric acid levels is the lack of use of animal proteins.

Cholesterol level in induced ascites + olive compared to induced ascites has shown a significant reduction. In people with pulmonary hypertension, the use of olive extract reduced cholesterol, which is consistent with the results of our study (Pereira et al., 2020). The olive extract in male rabbits reduced plasma total cholesterol levels. Other research has shown that the leaves of the olive plant and many medicinal plants contain compounds that have the property of reducing serum lipids.

Triglyceride levels in induction ascites and OLE showed a significant decrease compared to induced ascites treatment. Researchers mention blood parameters as a suitable indicator in the diagnosis of chickens suffering from the challenge of ascites (El and Karakaya, 2009). A positive genetic correlation between hematocrit and ascites. There was a negative relationship between hematocrit and the percentage of oxygen saturation in the blood and the incidence of ascites which was consistent with the results of our research. On the other hand, it, also, affects thyroid function and increases the amount of hormones T_4, T_3, and TSH, which causes weight loss compared to the control group. Weight loss is due to increased basal metabolism and increased energy expenditure. Levothyroxine caused weight loss because levothyroxine is a precursor to thyroid hormones and an increase in thyroid hormones in the body increased metabolism and increased metabolic energy consumption in levothyroxine-receiving treatments.

4. Conclusions

The positive effects of probiotics on the traits examined in this research are quite clear. However, the olive leaf extract is recommended for the prevention of induced ascites due to its reasonable price, availability, and acceptable effectiveness.

References

Afacan, A., Adiloğlu, S. and Hasanghasemi, A. (2014). Determination of antioxidant activity of sunflower growing in Hayrabolu District of Tekirdağ Province. *Journal of Tekirdag Agricultural Faculty*, 11(2): 21-26.

El, S. N. and Karakaya, S. (2009). Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. *Nutrition reviews*, 67(11): 632-638.

Esen, S., Okuyucu, B., Fisun, K. and Özdüven, M.L. (2022). Determination of nutritional quality and aerobic stability of sorghum, maize, and sorghum-maize mixture silages. *Journal of Tekirdag Agricultural Faculty*, 19(1): 61-69.

Gamero, M., Kim, W. S., Hong, S., Vorobiev, D., Morgan, C. D. and Park, S. I. (2021). Multimodal sensing capabilities for the detection of shunt failure. *Sensors*, 21(5): 1747.

Georgieva, N. V., Koinarski, V. and Gadjeva, V. (2006). Antioxidant status during the course of *Eimeria tenella* infection in broiler chickens. *The Veterinary Journal*, 172(3): 488-492.

Hayes, J. E., Allen, P., Brunton, N., O'grady, M. N. and Kerry, J. P. (2011). Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. *Food Chemistry*, 126(3): 948-955.

Hossain, M. E. and Akter, N. (2022). Further insights into the prevention of pulmonary hypertension syndrome (ascites) in broiler: a 65-year review. *World's Poultry Science Journal*, 78(3): 641-688.

Julian, R. J. (2000). Physiological, management and environmental triggers of the ascites syndrome: A review. *Avian pathology*, 29(6): 519-527.

Khosravinezhad, M., Talebi, E., Shivakumar, Z. N. and Nasrollahi, I. (2017). Essential oil composition and antimicrobial, antioxidant activities of Oliveria decumbens Vent. *International Journal of Herbal Medicine*, 5: 102-106.

Li, L., Jia, Q., Chen, L. and Wang, W. (2022). Changes in the expression of MIF and other key enzymes of energy metabolism in the myocardia of broiler chickens with ascites syndrome. *Animals*, 12(19): 2488.

Luger, D., Shinder, D., Wolfenson, D. and Yahav, S. (2003). Erythropoiesis regulation during the development of ascites syndrome in broiler chickens: A possible role of corticosterone. *Journal of Animal Science*, 81(3): 784-790.

Ma, Y., Freitag, P., Zhou, J., Brune, B., Frede, S. and Fandrey, J. (2004). Thyroid hormone induces erythropoietin gene expression through augmented accumulation of hypoxia-inducible factor-1. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 287(3): R600-R607.

Manafi, M., Khalaji, S., Hedayati, M. and Pirany, N. (2017). Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with Escherichia coli in broilers. *Poultry science*, 96(5): 1174-1183.

Martínez, Y., Almendares, C. I., Hernández, C. J., Avellaneda, M. C., Urquía, A. M. and Valdivié, M. (2021). Effect of acetic acid and sodium bicarbonate supplemented to drinking water on water quality, growth performance, organ weights, cecal traits and hematological parameters of young broilers. *Animals*, 11(7): 1865.

Nasrollahi, I., Talebi, E. and Nemati, Z. (2016). Study on *Silybum marianum* seed through fatty acids comparison, peroxide tests, refractive index and oil percentage. *Pharmacognosy Journal*, 8(6): 595-597.

Nemati, M. H., Shahir, M. H., Harakinezhad, M. T. and Lotfalhian, H. (2017). Cold-induced ascites in broilers: effects of vitamin C and coenzyme Q10. *Brazilian Journal of Poultry Science*, 19: 537-544.

Ohashi, Y. and Ushida, K. (2009). Health-beneficial effects of probiotics: Its mode of action. *Animal Science Journal*, 80(4): 361-371.

Omar, S. H. (2010). Oleuropein in olive and its pharmacological effects. *Scientia pharmaceutica*, 78(2): 133-154.

Özkan, S., Takma, C., Yahav, S., Söğüt, B., Türkmut, L., Erturun, H. and Cahaner, A. (2010). The effects of feed restriction and ambient temperature on growth and ascites mortality of broilers reared at high altitude. *Poultry science*, 89(5): 974-985.

Pereira, A. G., Fraga-Corral, M., García-Oliveira, P., Jimenez-Lopez, C., Lourenço-Lopes, C., Carpena, Otero, P., Gullón, P., Prieto, M., A. and Simal-Gandara, J. (2020). Culinary and nutritional value of edible wild plants from northern Spain rich in phenolic compounds with potential health benefits. *Food & Function*, 11(10): 8493-8515.

Roshanak, S., Rahimmalek, M. and Goli, S. A. H. (2016). Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. *Journal of Food Science and Technology*, 53: 721-729.

Şahin, S., Tonkaz, T. and Yarilgaç, T. (2022). Chemical composition, antioxidant capacity and total phenolic content of hazelnuts grown in different countries. *Journal of Tekirdag Agricultural Faculty*, 19(2): 262-270.

SAS Institute. (2004). *SAS/ETS 9.1 User's Guide*. SAS Institute.

Shimizu, K., Ogura, H., Asahara, T., Nomoto, K., Morotomi, M., Tasaki, Matsushima, A., Kuwagata, Y., Shimazu, Y. and Sugimoto, H. (2013). Probiotic/synbiotic therapy for treating critically ill patients from a gut microbiota perspective. *Digestive Diseases and Sciences*, 58: 23-32.

Shishehbor, F., Joola, P., Malehi, A. S. and Jalalifar, M. A. (2021). The effect of black seed raisin on some cardiovascular risk factors, serum malondialdehyde, and total antioxidant capacity in hyperlipidemic patients: A randomized controlled trials. *Irish Journal of Medical Science,* (1971-): 1-10.

Stanton, A. V., James, K., Brennan, M. M., O'Donovan, F., Buskandar, F., Shortall, K., El-Sayed, T., Kennedy, J., Hayes, H., Fahey, A. G. and Pender, N. (2020). Omega-3 index and blood pressure responses to eating foods naturally enriched with omega-3 polyunsaturated fatty acids: a randomized controlled trial. *Scientific Reports*, 10(1): 15444.

Talebi, E., Rowghani Haghighi Fard, E., Navabi, M. and Eatemadi, M. (2021). Evaluating the effect of two types of thyme essential oils (*Zataria multiflora* & *Ziziphora clinopodioides* lam) on some productive traits and blood parameters in broilers. *Poultry Science Journal*, 9(1): 107-119.

Willems, O. W., Miller, S. P. and Wood, B. J. (2013). Aspects of selection for feed efficiency in meat producing poultry. *World's Poultry Science Journal*, 69(1): 77-88.

Zerjal, T., Härtle, S., Gourichon, D., Guillory, V., Bruneau, N., Laloë, D., Pinard-van Der Laan, M. H., Trapp, S., Bed'hom, B. and Quéré, P. (2021). Assessment of trade-offs between feed efficiency, growth-related traits, and immune activity in experimental lines of layer chickens. *Genetics Selection Evolution*, 53(1): 1-17.

Zheng, L. X., Chen, X. Q. and Cheong, K. L. (2020). Current trends in marine algae polysaccharides: The digestive tract, microbial catabolism, and prebiotic potential. *International Journal of Biological Macromolecules*, 151: 344-354.