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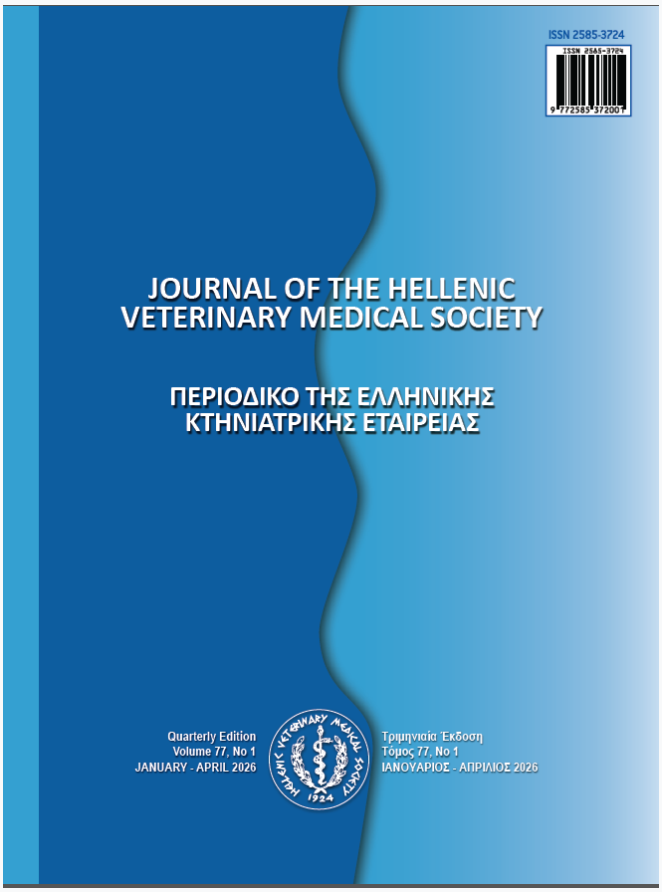

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Research article
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Effects of elderberry (*Sambucus ebulus*) feeding on broiler performance

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ABSTRACT: This study aimed to evaluate the effects of elderberry (*Sambucus ebulus*) feeding on performance, carcass characteristics, biochemical and antioxidant blood parameters, immune system, sensory and taste traits and fatty acid profile of meat in broiler chickens. Biological experiments were planned in a completely randomized design including three treatments of 0, 0.5 and 1% elderberry (*Sambucus ebulus*) fruit powder with 5 replications and 10 birds per replication. In total, 150 broiler chickens of the Ross 308 strain were fed corn and soybean meal-based diets during three periods: starter (1-11 d), grower (12-21 d) and finisher (22-42 d). The performance results showed that in the finisher period (22 to 42 d) a significant difference was observed for body weight and feed conversion ratio (FCR), so that for body weight in this period, the best performance was related to the 0.5% elderberry treatment ($P<0.05$). Also, the worst (highest) FCR was observed for the group fed with the 1% elderberry group, which was significantly higher in terms of quantity than the control and 0.5% elderberry groups ($P<0.05$). In addition, performance in the entire period (age 1 to 42 d) also showed a significant difference between FCR in the 1% elderberry group (1.616) versus the control treatment (1.583) and the 0.5% elderberry treatment (1.577) ($P<0.05$), and the linear equation for this trait was also significant ($y = 0.033x + 1.5755$ with a coefficient of determination of 0.62 and a significance level of 0.037). In addition, the results of blood biochemical parameters for the traits glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), atherogenic index, uric acid, albumin, total protein, iron, calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) and total antioxidant capacity (TAC) showed significant differences (improving trend) among the experimental treatments ($P<0.01$). Also, in all traits (except blood triglycerides, phosphorus and uric acid) a significant difference was observed between the control group compared to the effects of contrast with elderberry (zero group compared to two levels of 0.5 and 1% elderberry) and also for all mentioned blood traits except blood triglycerides, phosphorus and uric acid, there were significant linear equations between the effects of the treatments. Overall, the results showed that dietary supplementation with elderberry has a positive and promising role on blood biochemical parameters and immunity and in improving the health of chickens as well as maintaining product quality, but in choosing high levels of intake with the source, bird performance must be managed.

Keyword: Atherogenic index; Broiler chickens; Cholesterol; Elderberry; Health; Total antioxidant capacity

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INTRODUCTION

In the discussion of food security, poultry, as one of the largest livestock groups, has been able to play a significant role in food security and subsequently national security of countries (Nijdam et al., 2012; Abd El-Hack et al., 2020). In recent years, the rate of growth and improvement of production has led to sensitivity, stress, and pressure on the immune system of poultry. On the other hand, scientific research and historical events during the evolution of the planet show that the growth and evolution of diseases and infectious agents is faster than the growth and evolution of immunity-related parameters (Mocanu and Amariei, 2022; Hosseintabar-Ghasemabad et al., 2024abc). Therefore, in the context of the poultry industry, nutritional solutions with priority to balance nutrients and then the use of a series of additives to improve performance and flock health are always of interest (Korver, 2012; Philips et al., 2023; Seidavi et al., 2023ab; Madhulika et al., 2025). New reports on the positive effects of feed additives in humans (Liu et al., 2023; Chuai et al., 2023; Zhen et al., 2024) and animals (Baghban-Kanani et al., 2019, Chand et al., 2021; Das et al., 2023). According to botanical studies, the *Caprifoliaceae* family includes 20 genera and more than 300 plant species. The important genera of this family are *Sambucus* (80 species), *Viburunum* (150 species), *Lonicera* (150 species) and *Linnacea* (12 species) (Zargari, 1997). A review of the scientific literature shows that *Sumbucus ebulus*, which is also abbreviated as SE in scientific reports, is one of the most important and valuable species of this family in this group of plants. SE is a herbaceous plant with a height of 1 to 2 meters that grows in many temperate and subtropical countries. The leaves of the plant have 5 to 9 long, elliptical leaflets, sharp and toothed. Its flowers are white, which turn into small spherical fruits (0.4 to 0.6 cm). cm) and with a purple-black color that is completely recognizable. The entire plant body naturally has an unpleasant odor that is easily perceived. This plant has few requirements in terms of ecological conditions and growth requirements and has good growth in eutrophic, loamy and clay-sandy soils (Zargari, 1997). Due to the high content of phenolic compounds and medicinal and health-promoting properties of this plant, it has been considered in the pharmaceutical and food industries (Ebadi and Hisoriev, 2011). Given the increasing demand for fruits containing high antioxidants in human and animal diets, for supplementation and production of functional foods as preventive and therapeutic, the use of plant sources such as SE could be an attractive option for consumers in the future (Pascariu and Israel-Roming, 2022). Phytochemical compounds in the *S. ebulus* plant include flavonoids, steroids, tannins, Glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, ebulin 1and volatiles substances (Saeedi, 2010). 0.8% of valuable flavonoid compounds have been reported in this plant. Caffeic acid and its derivatives, including chlorogenic acid, p-coumaric acid and quinic acid, are well-known and recognized as antioxidant sources in many plants including this plant (Bonita et al., 2007; Saeedi et al., 2010). Additional research showed that SE has a unique active compound including ebulosid (7-oxo-8-deoxyvaleroside) with the molecular formula $C_21H_{32}O_{10}$, which can be extracted from its leaves and fruits (Saravi and Shokrzadeh, 2009). Tasinov et al. (2021) reported that among the phytochemical compounds identified in the aqueous extract SE fruit contains 15 amino acids, 10 organic acids, 36 sugar acids and alcohols, 25 mono-, di- and trisaccharide, 13 fatty acids (saturated and unsaturated) and their esters, and 38 phenolic compounds. The amount of hydroxycinnamic acid in SE extract contains 51.45% of its total phenolic compounds. In general, hydroxycinnamic acids are the most abundant phenolic acids in fruits, vegetables, and coffee beans, which is also significant in SE fruit (more than 50%) (Herrmann and Nagel, 1989). Among the beneficial effects of hydroxycinnamic acids, they can be mentioned as potential chemical preventives with high antioxidant activity (Weng and Yen, 2012). Research investigating the active compounds indicates that SE fruit is rich in beneficial natural compounds including hydroxycinnamic acids, Anthocyanins, proanthocyanidins, and resveratrol, with strong antioxidant, anti-inflammatory, and stress-reducing potential, as well as the presence of a variety of essential amino acids, organic acids, alcohols, and saturated and unsaturated fatty acids and esters, some of which were reported for the first time in SE fruits, indicate the nutritional value of this source in dietary programs (Tasinov et al., 2021). Research has shown that flavonoids in SE fruit can prevent virus entry into host cells and also prevent influenza pathogenesis (Mocanu et al., 2022). In addition, agglutination of SE flavonoids can stop influenza infection by competitively inhibiting the virus and through endocytosis (Akram et al., 2018). Also, the effectiveness of the fruit against a number of infections may be due to its immune-stimulating properties (immunostimu-

latory) (Kinoshita et al., 2012). The fruit extract of this plant can affect the immune system by increasing the production of cytokines by monocytes, and the immunomodulatory properties of the system are revealed by increasing the expression of IL-6, IL-8 and TNF35 (Mocanu et al., 2022). Karimi et al. (2014) reported that the extract of the elderberry family has potent direct antiviral effects against the H9N2 avian influenza virus in vitro and that administration of this extract via drinking water to poultry could potentially have beneficial effects in the prevention and treatment of avian influenza and possibly other viral infections. However, avian influenza caused by H9N2 is spreading in poultry worldwide, especially in Asia, and can even infect humans and in some cases even combine with highly pathogenic H5 and H7 influenza viruses, indicating a potential and serious threat to human and livestock health. The high rate of antigenic drift in influenza viruses and the short rearing time of commercial broilers are potential concerns for the poultry industry. On the other hand, according to international livestock regulations, due to the possibility of resistant virus mutations, existing synthetic anti-influenza drugs are only fully approved for human use, and there are still restrictions and concerns about the use of antiviral drugs, especially influenza, in the poultry industry (Karimi et al., 2014). Therefore, the use of natural resources of plant origin, whose antiviral effects are confirmed and without any side effects, can be effective in improving performance, health and effectiveness of drugs and preventive measures, can be considered a low-cost and risk-free solution in the poultry industry. The edible plant, elderberry (*Sambucus ebulus*), is known as a valuable source of antioxidants, immune stimulants, anti-influenza and antibiotic substitutes, and a strong antiviral, and plays a significant role in reducing various metabolic and non-metabolic diseases. On the other hand, the high growth power, the significant abundance of the aforementioned resource, and the distribution and accessibility of this plant in many parts of the world are some of the notable and valuable points of this food source, which can show its valuable position in the future of this industry by entering poultry nutrition as an additive with high potential (Shokrzadeh and Saravi, 2010; Karimi et al., 2014, Durakova et al., 2025). The high diversity of bioactive and unique compounds in the elderberry plant, most of which act as a preventive against oxidative stress, a preventive against various known and unknown viral diseases, enhance anti-inflammatory and anti-infective properties, and also have significant power in eliminating free radicals, have been proven in many human and laboratory animal studies, and its application in the food-pharmaceutical field is always of interest (Tasinov et al., 2021, Merecz-Sadowska et al., 2024). However, research on the use of this source in the field of poultry nutrition is very limited, and considering the functional potential of this food source and also the concerns mentioned in the future of humanity regarding food security, it seems that the time has come to pay more attention to the use of elderberry as a new source of feed additive, and it is hoped that with further research and awareness of its optimal use, the nutrition of this edible plant source can be placed on the agenda of activists in this industry.

MATERIALS AND METHODS

This study was conducted in compliance with the guidelines for the welfare of laboratory animals by the Ethics Committee of Islamic Azad University, Rasht-Gilan Branch, with the ethics code IR.IAU.RASHT.REC.1402.021, in 2024. Biological experiments were planned in a completely randomized design using 3 treatments with 5 replicates and 10 Ross 308 strain broiler chickens in each replicate, and a total of 150 birds with similar average weight (41 ± 1 g). The dimensions of the chicken pens were (1.2m✕1.5m✕2m) and access to feed and water was *ad libitum*. Light management, room temperature, and vaccination schedule were planned based on the recommendations of the breeder reference and in accordance with the guide catalog. Experimental diets were formulated based on corn and soybean meal using Amino Feed 5.0 software from Evonik according to Table 1. The test ingredient of the present study was the dried and powdered fruit part of the plant species Sambucus ebulus, which was obtained from Darvash Giah Khazar medicinal herbs complex company (Ltf) (Iran-Guilan-Rasht). The experimental treatments included three levels of fruit powder SE 0, 0.5 and 1%. The growth period was planned in three periods: starter (1-11 d), grower (12-21 d) and finisher (22-42 d), and the total period was 42 days. The research location was Maaf Research and Development (R&D) farm belonging to Sepid Makian Company (Soumeh-Sara-Guilan-Iran). The apparent metabolizable energy corrected to zero nitrogen balance (AME_n) values of the test ingredient were calculated based on the equation proposed by the World Poultry Science Association (WPSA) according to formula (1).

Formula (1): AME_n (kcal/kg DM) = 15.51 (Crude Protein) + 34.31 (Ether Extract) + 16.51 (Strach) + 13.01 (Sugar)

Analysis of the chemical composition of elderberry was also carried out at Viromed Laboratory (Iran-Guilan-Rasht). Based on the Iranian National Standard Method (Laboratory Method Reference Code) for the items dry matter (Method: 8438), fat (Method: 10700), protein (Method: 10703-1), total sugar (Method: 8986-2), starch (Method: In House), crude fiber (Method: 3105), neutral-detergent fiber (NDF) (Method: 8917), acid detergent fiber (ADF) (Method: 8917), amino acid profile (Method: ISO 13903), total phenolic compounds (Method: 8986-1), phosphorus (Method: 513), calcium (Method: 10701-1), iron (Method: In House), magnesium (Method: In House), phosphorus (Method: In House), vitamin A (Method: ISIRI 7432), vitamin D3 (Method: ISIRI 13579) and vitamin K3 or menadione (Method: In House), the values was measured and reported in Table 2. In addition, the fatty acid profile was analyzed at Techno Azma Laboratory (Tehran, Iran) based on the method (Method: 13126-1-2).

Sampling

Feed intake (FI) and body weight (BW) were measured in three rearing periods: starter, grower and finisher, as well as the entire period, and FCR was calculated. On day 42 of rearing, after four hours of starvation, two birds from each replicate with the average weight of their experimental unit were randomly selected and slaughtered. By separating the carcasses, the following were weighed: live weight, defeather body, eviscerated carcass, breast, thigh, gizzard, crop, liver, heart, pancreas, spleen, bursa of Fabricius and abdominal fat, respectively.

For sampling and testing of blood biochemical parameters on day 42 of rearing, three birds were randomly selected from each replicate and sampling was performed from the wing vein with 5 cc sterile syringes. The samples were packaged and prepared for transport to the laboratory in accordance with the recommendations of the reference laboratory, under cool conditions and centrifuged (3000 rpm) in commercial kits also used by Pars Azmoon Company (made in Iran).

Measurements were made using the colorimetric method for glucose (Kianfar et al., 2023), triglycerides (Baghban-Kanani et al., 2023), total cholesterol (Janmohammadi et al., 2023), HDL (Tufarelli et al., 2021), LDL (Baghban-Kanani et al., 2018), total protein (Hosseintabar et al., 2015), albumin (Hosseintabar, 2015), uric acid (Feshanghchi et al., 2022), calcium (Selim et al., 2022), iron (Hosseintabar et al., 2015), total antioxidant capacity (Tufarelli et al., 2022), and malondialdehyde (MDA) (Baghban-Kanani et al., 2019a), respectively. The atherogenic index (LDL to HDL ratio) was calculated and reported as a health index (Baghban-Kanani et al., 2019a). Liver enzymes including aspartate aminotransferase (AST) (Tufarelli et al., 2021) and alanine aminotransferase (ALT) (Hosseintabar-Ghasemabad et al., 2023) were measured using the enzymatic method, and phosphorus (P) was measured using the photometric method (Mohamed et al., 2024).

On days 28 and 36 of rearing, sheep red blood cells (SRBC) were injected at a 5% dilution into the pectoral muscle area (Seidavi et al., 2014). On days 35 and 42 (seven days after SRBC injection), samples were taken from the wing vein of the same injected birds using 3-cc sterile syringes. The aim of this sampling was to evaluate the antibody levels of the samples against SRBC by hemagglutination method in order to evaluate Newcastle disease (NDV) and influenza (AIV) titers for immunological parameters (Shabani et al., 2015; Omidi et al., 2021; Amirdahri et al., 2023).

Sampling of the whole bird breast was performed to evaluate the fatty acid profile of the meat according to the method reported by Zaker-Esteghamati et al. (2021) and Belali et al., (2021). Calculation of meat health indices including omega-6 to omega-3 ratio, atherogenic index (AI), thrombogenic index (TI), hypocholesterolemic index (HI) and hypocholesterolemic to hypercholesterolemic ratio was performed based on formulas (2, 3, 4 and 5) (Attia et al., 2022). Due to technical limitations of laboratory activities, only one sample was taken from each treatment.

Formula (2): $AI = (4 \times C14:0) + C16:0/(\Sigma MUFA + \Sigma PUFA - \omega\text{-}6 + \Sigma PUFA - \omega\text{-}3)$

Formula (3): $TI = (C14:0 + C16:0 + C18:0)/0.5 \times \Sigma MUFA + 0.5 \times \Sigma (\omega\text{-}6) + 3 \times \Sigma(\omega\text{-}3) + \Sigma(\omega\text{-}3)/\Sigma(\omega\text{-}6)$

Formula (4): $HI = (C18:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:6)/(C14:0 + C16)$

Formula (5): Hypocholesterolemic/Hypercholesterolemic index = $[(C18:1\ \omega\text{-}9 + C18:1\ \omega\text{-}7 + C18:2\ \omega\text{-}6 + C18:3\ \omega\text{-}6 + C18:3\ \omega\text{-}3 + C20:3\ \omega\text{-}6 + C20:4\ \omega\text{-}6 + C20:5\ \omega\text{-}3 + C22:4\ \omega\text{-}6 + C22:5\ \omega\text{-}3 + C22:6\ \omega\text{-}3)/(C14:0 + C16:0)]$

Table 1. Ingredients and calculation of nutrient composition of the assay diets in the experimental treatments (T_1, T_2 and T_3)[1]

Items	Starter (1-11 d)			Grower (12-21 d)			Finisher (22-42 d)		
Ingredients (%)	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Corn	53.54	53.01	52.48	61.78	61.31	60.78	67.59	67.06	66.53
Soybean meal 44%	40.95	40.90	40.85	33.23	33.17	33.13	27.35	27.30	27.25
Elderberry (SE[2])	0.00	0.50	1.00	0.00	0.50	1.00	0.00	0.50	1.00
Vegetable oil	1.53	1.61	1.70	1.39	1.46	1.55	1.91	1.99	2.08
Methionine	0.35	0.35	0.35	0.28	0.28	0.28	0.22	0.23	0.23
Lysine hydrochloride	0.21	0.21	0.20	0.21	0.20	0.20	0.20	0.20	0.20
Threonine	0.11	0.11	0.11	0.09	0.09	0.09	0.07	0.08	0.08
Valine	0.04	0.04	0.04	0.02	0.02	0.02	0.01	0.01	0.01
Choline chloride	0.01	0.01	0.01	0.07	0.04	0.04	0.07	0.07	0.07
Monocalcium phosphate	1.11	1.11	1.11	0.90	0.90	0.90	0.69	0.69	0.69
Calcium carbonate	1.19	1.19	1.18	1.06	1.05	1.05	0.92	0.91	0.91
Sodium bicarbonate	0.24	0.24	0.24	0.27	0.26	0.26	0.27	0.27	0.27
Sodium chloride	0.21	0.21	0.21	0.19	0.20	0.20	0.19	0.19	0.19
Vitamin and Mineral Premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated nutrient composition (%)									
ME (kcal/kg)[4]	2853	2853	2853	2937	2937	2937	3040	3040	3040
Crude protein	22.91	22.89	22.87	19.98	19.96	19.94	17.70	17.68	17.67
Lysine	1.26	1.26	1.26	1.09	1.09	1.09	0.96	0.96	0.96
Methionine + Cysteine	0.94	0.94	0.94	0.81	0.81	0.81	0.71	0.71	0.71
Threonine	0.83	0.83	0.83	0.72	0.72	0.72	0.63	0.63	0.63
Tryptophan	0.25	0.25	0.25	0.21	0.21	0.21	0.18	0.18	0.18
Arginine	1.40	1.40	1.40	1.20	1.19	1.19	1.04	1.03	1.03
Isoleucine	0.86	0.86	0.86	0.74	0.74	0.74	0.65	0.65	0.65
Valine	0.97	0.97	0.97	0.84	0.84	0.84	0.74	0.74	0.74
Calcium	0.95	0.95	0.95	0.84	0.84	0.84	0.73	0.73	0.73
Available Phosphorus	0.48	0.48	0.48	0.42	0.42	0.42	0.37	0.37	0.37
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Potassium	1.01	1.02	1.02	0.88	0.88	0.89	0.78	0.78	0.79
Chlorine	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
DCAB (mEq/kg)	269	270	271	235	236	238	209	210	211
Choline (g/kg)	1.47	1.47	1.47	1.59	1.47	1.47	1.48	1.47	1.47
Ether extract	4.19	4.33	4.47	4.24	4.36	4.50	4.87	5.00	5.14
Linoleic acid	1.99	2.23	2.48	2.02	2.25	2.50	2.34	2.58	2.83
Neutral Detergent Fiber	9.65	9.77	9.89	9.70	9.83	9.96	9.70	9.82	9.95
Acid Detergent Fiber	4.19	4.30	4.41	3.97	4.08	4.19	3.79	3.89	4.00
Crude fiber	3.10	3.16	3.23	2.94	3.00	3.07	2.81	2.87	2.93
Ash	6.17	6.19	6.21	5.44	5.45	5.47	4.77	4.79	4.77
Starch	34.20	33.92	33.64	39.38	39.14	38.86	43.03	42.76	42.48

[1]T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
[2] SE: *Sambucus ebulus* (Dried fruit powder)
[3]The values of vitamins and minerals per kg of the assay diet: Vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 18 IU; vitamin K_3, 3 mg; vitamin B_1, 1.8 mg; vitamin B_2, 6 mg; vitamin B_6, 3 mg; vitamin B_{12}, 0.012 mg; vitamin B_3, 30 mg; vitamin B_9, 1 mg; vitamin H_3, 0.24mg; vitamin B_5, 10 mg; 500 mg; Choline,100 mg; Mn, 100 mg; Zinc, 80 mg; Iron, 10 mg; Cu, 1 mg.
[4]ME: Metabolizable Energy
[5]DCAB: Dietary Cation- Anion Balance

Table 2. Analysis of chemical composition, minerals, vitamins, total phenolic compounds, amino acid profile and fatty acid profile in elderberry dried fruit powder (*Sambucus ebulus*)

Item	Value	Item	Value (g/100g)	Item	Value (%)
Dry matter (%)	91.20	Proline	0.10	C12:0	0.18
Ether extract (%)	14.17	Leucine	0.61	C14:0	0.27
Crude Protein (%)	8.66	Isoleucine	0.49	C16:0	7.78
Sugar (%)	18.73	Valine	0.46	C16:1	0.28
Starch (%)	11.7	Lysine	1.15	C17:0	0.07
Crude fibire (%)	15.60	Methionine	0.46	C17:1	0.08
NDF (%)	34.40	Arginine	0.07	C18:0	2.43
ADF (%)	24.80	Threonine	0.06	C18:1c	20.93
Total phenolic compounds (%)	3.75	Histidine	0.22	C18:2c	42.50
Phosphorus (%)	0.29	Tryptophan	0.1>	C18:3t	0.10
Calcium (%)	0.56	Phenylalanine	0.73	C18:3c	25.51
Iron (mg/kg)	40	Tyrosine	0.10	C20:0	0.14
Magnesium (%)	0.14	Taurine	0.10	C20:1	0.20
Vitamin A (mg/kg)	0.03	Glycine	0.13	C22:0	0.14
Vitamin D_3 (mg/kg)	1.60	Alanine	0.04	C22:2	0.08
Vitamin K_3 (mg/kg)	0.1>	Glutamic acid	0.07	C24:0	0.21
		Aspartic acid	0.04	C24:1	0.10
		Serine	0.03		

Subsequently, by sampling breast meat from each replicate and cooking them without spices, the sensory and taste attributes of breast meat were evaluated to evaluate aroma, flavor, odor, crunchiness, color, and overall desirability by six panels (food testers) of evaluators through a questionnaire with a score from 1 to 10 (Azizi et al., 2021). All analyses were performed on blood and meat samples at the Viromed Laboratory (Rasht-Gilan-Iran).

Data from the samples studied in this study were collected in Excel software and the results were analyzed with statistical software (SAS 9.3). Comparisons of treatment means were reported with Duncan's multiple range test. Linear and nonlinear equations were also reported using quadratic, linear, and orthogonal equations, and the "Solver" add-on of Excel software was used to find the turning point of quadratic equations. Due to the sampling situation (one sample from each treatment), statistical analysis was not performed for traits related to fatty acids in breast meat, and the results of this section only included laboratory report figures and calculations of a number of indicators.

RESULTS

In Table 3, the performance results of broiler chickens at the age of 1 to 11 days showed that there was no significant difference between the performance of different treatments in terms of feed intake, body weight and FCR. Also, the comparison of the effects of the contrast of control with the groups fed with elderberry was not significant in the linear and quadratic models.

In Table 4, the performance results of broiler chickens at the age of 12 to 21 days for feed intake, body weight and FCR showed that there was no significant difference between the performance of different treatments in terms of feed intake, body weight and FCR. Also, comparing the contrast effects of the control group with the groups fed with elderberry for the linear and quadratic models, no significant difference was observed.

In Table 5, the performance results of broiler chickens at the age of 22 to 42 days showed that there was a significant difference between body weight performance and FCR in the tested groups ($P<0.05$). So that for body weight in this period, the

Table 3. Performance results of experimental treatments for broiler chickens in the starter period (1-11 d)

Treatments	Feed Intake (g)	Body Weight (g)	Feed Conversion Ratio
T_1	472.00	437.80	1.078
T_2	462.60	431.00	1.073
T_3	488.30	445.70	1.095
SEM	11.853	9.163	0.012
P value	0.334	0.543	0.442
Control Vs. Elderberry	0.816	0.962	0.743
Linear	0.350	0.554	0.371
Quadratic	0.250	0.357	0.366

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 4. Performance results of experimental treatments for broiler chickens in the grower period (12-21 d)

Treatments	Feed Intake (g)	Body Weight (g)	Feed Conversion Ratio
T_1	570.90	402.20	1.420
T_2	569.80	398.90	1.429
T_3	576.40	408.50	1.413
SEM	13.762	11.618	0.015
P value	0.936	0.840	0.755
Control Vs. Elderberry	0.897	0.920	0.999
Linear	0.781	0.709	0.711
Quadratic	0.824	0.657	0.524

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 5. Performance results of experimental treatments for broiler chickens in the finisher period (22-42 d)

Treatments	Feed Intake (g)	Body Weight (g)	Feed Conversion Ratio
T_1	2882.8	1640.6^{ab}	1.759^{b}
T_2	2972.8	1710.2^{a}	1.74^{b}
T_3	2792.4	1532.9^{b}	1.82^{a}
SEM	63.784	39.118	0.016
P value	0.178	0.023	0.007
Control Vs. Elderberry	0.998	0.697	0.306
Linear	0.336	0.075	0.015
Quadratic	0.109	0.024	0.018

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

best performance was related to the 0.5% elderberry treatment and there was a significant difference with the 1% elderberry group. Also, the worst (highest) FCR was also for the group fed with the 1% elderberry group, which was significantly higher in terms of quantity than the control and 0.5 elderberry groups ($P<0.05$). Also, the linear equation for FCR ($y = 0.062x + 1.7417$ with a coefficient of determination of 0.52) and the quadratic equation ($y = -493.8x2 + 386.1x + 1640.6$ with a turning point of 0.39) for body weight were significant ($P<0.05$).

In Table 6, the performance results of broiler chickens for the entire period (age 1 to 42 d) showed that there was a significant difference between FCR in the 1% elderberry group (1.616) compared to the control treatment (1.583) and the 0.5% elderberry treatment (1.577) ($P<0.05$), and the linear equation for this trait was also significant ($y= 0.033x + 1.5755$ with a coefficient of determination of 0.62 and a significance level of 0.037).

Tables 7 and 8 for carcass traits and relative weight in the experimental broiler chickens showed that there was no significant difference between the treatments.

In Table 9, the results of the analysis of blood biochemical parameters in broilers showed that in all traits (except triglycerides and blood phosphorus) there was a significant difference between the tested treatments ($P<0.01$). Also, in all traits (except triglycerides, phosphorus and uric acid), a significant difference was observed between the control group compared to the contrast effects of the group fed with elderberry (zero group compared to two levels of 0.5 and 1% elderberry). For all traits except

Table 6. Performance results of experimental treatments for broiler chickens in the entire period (1-42 d)

Treatments	Feed Intake (g)	Body Weight (g)	Feed Conversion Ratio
T_1	3925.70	2480.70	1.583[b]
T_2	4005.30	2540.10	1.577[b]
T_3	3857.20	2387.10	1.616[a]
SEM	80.859	52.087	0.010
P value	0.455	0.154	0.037
Control Vs. Elderberry	0.957	0.794	0.287
Linear	0.560	0.228	0.037
Quadratic	0.273	0.122	0.092

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 7. Results of carcass weight traits of experimental treatments in broiler chickens (g)

Treatments	Live body	Defeather body	Eviscerated carcass	Breast	Thigh	Abdominal fat	Gizzard	Heart	Crop	Liver
T_1	2554.0	2180.0	1904.0	688.0	506.0	23.32	37.64	12.16	9.88	52.04
T_2	2596.0	2204.0	1926.0	726.0	524.0	19.18	39.08	11.62	5.86	47.74
T_3	2474.0	2120.0	1876.0	710.0	502.0	27.08	38.68	11.32	6.54	48.42
SEM	48.436	37.220	45.927	33.813	17.851	3.631	3.612	0.677	1.745	2.373
P value	0.235	0.296	0.748	0.733	0.660	0.339	0.959	0.682	0.258	0.415
Control Vs. Elderberry	0.754	0.700	0.958	0.483	0.754	0.967	0.784	0.422	0.111	0.198
Linear	0.266	0.277	0.674	0.654	0.877	0.478	0.842	0.398	0.201	0.302
Quadratic	0.192	0.259	0.534	0.527	0.378	0.201	0.839	0.887	0.293	0.408

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 8. Results of relative weight percentage of carcass traits of experimental treatments in broiler chickens (%)

Treatments	Defeather body	Eviscerated carcass	Breast	Thigh	Abdominal fat	Gizzard	Heart	Crop	Liver
T_1	85.37	74.49	26.88	19.78	0.905	1.468	0.478	0.389	2.045
T_2	84.92	74.20	27.94	20.19	0.741	1.509	0.447	0.225	1.835
T_3	85.71	75.86	28.72	20.29	1.092	1.567	0.458	0.264	1.955
SEM	0.647	1.052	1.107	0.492	0.138	0.144	0.029	0.069	0.088
P value	0.696	0.513	0.516	0.740	0.241	0.889	0.762	0.262	0.279
Control Vs. Elderberry	0.945	0.681	0.305	0.455	0.959	0.706	0.494	0.116	0.193
Linear	0.718	0.376	0.261	0.471	0.365	0.638	0.634	0.224	0.491
Quadratic	0.450	0.467	0.920	0.804	0.153	0.951	0.583	0.267	0.152

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 9. Results of blood biochemical parameters of experimental treatments in broiler chickens

Items	Treatments			SEM	P value	Control Vs. Elderberry	Linear			Quadratic
	T_1	T_2	T_3				P value	Equal	R^2	
Glucose (mg/dl)	186.20^a	184.20^a	176.20^b	1.306	0.0004	0.0028	0.0002	y = -10x + 187.20	0.89	0.0853
Cholesterol (mg/dl)	183.40^a	165.00^b	163.20^b	1.238	0.0001	0.0001	0.0001	y = -20.2x + 180.63	0.83	0.0001
HDL (mg/dl)	37.16^c	43.24^b	46.60^a	0.795	0.0001	0.0001	0.0001	y = 9.44x + 37.61	0.97	0.1880
LDL (mg/dl)	102.34^a	98.74^b	94.68^c	0.916	0.0003	0.0003	0.0001	y = -7.66x + 102.42	0.99	0.8410
Atherogenic Index	2.76^a	2.29^b	2.03^c	0.062	0.0001	0.0001	0.0001	y = -0.732x + 2.72	0.96	0.1588
Triglycerides (mg/dl)	77.40	76.58	75.50	0.722	0.2169	0.1502	0.0876	-	-	0.8856
Uric Acid (mg/dl)	5.74^b	6.43^a	5.76^b	0.145	0.0086	0.0689	0.9166	y = 0.022x + 5.96	0.01	0.0025
Albumin (g/dl)	2.12^c	2.24^a	2.19^b	0.010	0.0001	0.0001	0.0002	y = 0.070x + 2.14	0.34	0.0001
Total Protein (g/dl)	4.38^b	4.46^a	4.44^a	0.013	0.0022	0.0009	0.0094	y = 0.056x + 4.40	0.44	0.0049
Phosphorus (mg/dl)	3.82	3.91	3.87	0.037	0.2702	0.1402	0.3186	-	-	0.1997
Fe (µg/dl)	131.80^b	138.00^a	141.40^a	2.000	0.0162	0.0073	0.0053	y = 9.60x + 132.27	0.97	0.5782
Calcium (mg/dl)	10.63^c	10.90^b	11.14^a	0.030	0.0001	0.0001	0.0001	y = 0.51x + 10.63	0.99	0.6539
Alanine transaminase (u/l)	14.20^a	12.30^b	10.90^b	0.467	0.0011	0.0007	0.0003	y = -3.30x + 14.12	0.99	0.6697
Aspartate Aminotransferase (u/l)	360.40^a	335.80^b	325.60^c	2.778	0.0001	0.0001	0.0001	y = -34.8x + 35	0.94	0.0559
Malondialdehyde (nmol/ml)	20.04^b	22.00^a	20.34^b	0.360	0.0048	0.0248	0.5663	-	0.02	0.0015
Total antioxidant capacity (nmol/ml)	707.20^c	784.20^b	820.80^a	5.956	0.0001	0.0001	0.0001	y = 113.6x + 713.93	0.95	0.0170

Means without letter or with the same letter for each row are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

triglycerides, phosphorus and uric acid, there were significant linear equations between the effects of the treatments. Also, for the MDA trait, a significant quadratic equation was observed (MDA= -7.24x2 + 7.54x + 20.04 and Max=0.521).

It should be noted that for the parameters of blood cholesterol and ALT, a significant difference was observed between the control treatment compared to each of the treatments containing elderberry (0.5 and 1 percent), and the values in the control treatment were the highest (P<0.01). For the parameters of LDL, Atherogenic Index and AST, a significant difference was observed between the control treatment compared to each of the elderberry treatments of 0.5 and 1 percent, and with the decrease in the percentage of elderberry in the diet, the numerical value of these parameters had a decreasing trend and was affected by the type of treatment (P<0.01).

For the parameters of HDL, Ca and TAC, with the increase in the elderberry level, a significant upward trend was observed, so that the control treatment compared to the two elderberry treatments of 0.5 and 1 percent and also the 0.5 percent treatment compared to 1 percent showed a lower and significant performance (P<0.01). For blood glucose, the lowest value was related to the 1% elderberry treatment, and a significant difference was observed with the control treatment and the 0.5% treatment (P<0.01).

The use of elderberry (0.5 and 1%) had a positive and significant effect on iron and total blood protein (P<0.01), so that the amount of these two blood parameters for each of the groups fed with elderberry was significantly different from the control treatment (P<0.01). Also, for blood albumin, a significant difference was observed between the 0.5% elderberry treatment compared to the control treatment and the 1% elderberry treatment (P<0.01), so that the lowest blood albumin value was observed in the control group. Finally, for the uric acid and MDA parameters, a significant difference was observed between the 0.5% treatment compared to the other two treatments (P<0.01).

In Table 10, the results of the analysis of traits related to the immune system of broiler chickens showed that the type of treatment had a significant effect on antibody titer (35day-SRBC test) (P<0.05), so that the control treatment had a significantly higher antibody titer (35day-SRBC test) than each of the 0.5 and 1 percent elderberry treatments. Also, the contrast effect of the control group was significantly higher than the groups fed with elderberry (P<0.01). For the spleen weight parameter, the lowest value was observed for the 1 percent elderberry treatment and a significant difference was observed with the control and 0.5 percent groups (P<0.05). The linear equation of the significant parameters' antibody titer (35day-SRBC test) and spleen weight was as follows:

Antibody Titer (35 day- SRBC test) = -2x + 2.067 R^2=0.94
Spleen weight (g)= -0.52x + 3.313 R^2=0.49

In Table 11, the results showed that among the

Table 10. Results of immunological parameters of experimental treatments in broiler chickens

Treatments	Antibody Titer (35 day- SRBC test)	Antibody Titer (42day-SRBC test)	Pancreas weight (g)	Relative weight of the pancreas (%)	Spleen weight (g)	Relative weight of the spleen (%)	Fabricius weight (g)	Relative weight of the Fabricius (%)
T_1	2.20a	5.00	6.100	0.24	3.160[a]	0.12	2.00	0.078
T_2	0.80b	4.20	7.320	0.28	3.360[a]	0.13	2.06	0.08
T_3	0.20b	4.80	5.420	0.22	2.640[b]	0.10	1.60	0.06
SEM	0.416	0.653	0.656	0.025	0.159	0.007	0.192	0.007
P value	0.015	0.675	0.159	0.235	0.021	0.072	0.225	0.324
Control Vs. Elderberry	0.006	0.544	0.743	0.735	0.427	0.505	0.484	0.527
Linear	0.005	0.832	0.478	0.567	0.039	0.091	0.167	0.224
Quadratic	0.448	0.399	0.076	0.113	0.036	0.096	0.291	0.378

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

Table 11. Results of sensory taste attributes of breast meat of experimental treatments in broiler chickens

Treatments	Meat perfume	Meat taste	Meat smell	Meat crispy	Meat color	Desirability and acceptance of meat
T_1	7.00	7.60	7.40[b]	7.20	7.00	7.60
T_2	7.60	8.00	8.60[a]	7.60	7.60	8.00
T_3	7.20	7.60	7.80[b]	7.40	7.40	8.00
SEM	0.365	0.271	0.294	0.294	0.271	0.346
P value	0.516	0.503	0.039	0.641	0.315	0.651
Control Vs. Elderberry	0.389	0.558	0.047	0.422	0.158	0.364
Linear	0.705	0.999	0.356	0.640	0.317	0.430
Quadratic	0.285	0.251	0.017	0.422	0.251	0.646

Means without letter or with the same letter for each column are not significantly different.
T_1: 0% Elderberry level or control group, T_2: 0.5% Elderberry level and T_3: 1% Elderberry level.
SEM: Standard Error of Means

taste and sensory attributes of chicken breast meat, only meat smell was affected by the experimental treatments, so that a significant difference was observed between the meat smell of the 0.5% treatment compared to the control treatment and the 1% treatment ($P<0.01$). Also, the results of the contrast of the control group compared to the elderberry treatments, the quadratic equation ($y = -4x2 + 4.4x + 7.4$ with a turning point of 0.55) for meat smell was significant ($P<0.05$).

In Table 12, the fatty acid profile of broiler breast meat, as well as the ratio and indicators related to product health, were presented in the materials and methods section of the present report, according to formulas (2, 3, 4, and 5). Given that one sample from each treatment was selected for evaluation and reported, statistical analysis and comparisons were not possible. In general, by examining the numerical values reported in this table, it can be acknowledged that the meat health indicators in the groups fed with elderberry showed an improving trend, which can be considered by researchers as a main goal in future research, using this food source to improve product quality, and a proposal to evaluate it by observing all statistical principles is proposed and put on the agenda.

DISCUSSION

Regarding the role of this food source in supplementing functional diets and feeding broilers, the authors of the present study did not find any direct specific research or reports. It seems that the use of this valuable food source has been conducted for the first time in this animal model. Available evidence and findings also indicate the clinical effectiveness of this plant on humans, fish and other laboratory animals, which can be generalized to some extent with the results of this study and the mechanisms discussed and analyzed.

In the research of Hosseini Shekarabi et al. (2021) elderberry (*Sambucus ebulus*) extract at levels of 0, 2.5, 5 and 10 g of extract per kg of feed on the growth performance of carp (Cyprinus carpio), the results showed that the group fed with 10 g of SE extract showed improvement in weight and FCR, which was not in line with the results of this study to some extent and the reason for this is the difference in consumption levels and variation in the animal model tested. Perhaps the relative decrease in FCR at high levels of *Sambucus ebulus* consumption in the present study can be attributed to the high content of crude fiber, NDF and ADF reported in Table 2. Binaii et al. (2022) evaluated the dietary administration of elderberry (*Sambucus ebulus*) and stinging nettle (Urtica dioica) on carp (Cyprinus carpio) and concluded that feeding 50 g of elderberry along with 120 g of stinging nettle led to an increase in body weight and a decrease in FCR. Immune system-related parameters increased in the fourth and eighth weeks after feeding these two sources. In addition, blood triglycerides decreased in the eighth week. Overall, the improvement in growth and immunity after feeding these sources to fish was quite noticeable. Despite the differences in animal model and diet in details, the overall outcome of this study was consistent and the animal health after consuming

Table 12. Results of fatty acid profile of breast meat of experimental treatments in broiler chickens

Items	Treatments		
	T_1	T_2	T_3
Caprylic acid (C8:0)	0.01	0.22	0.04
Capric acid (C10:0)	0.01	0.19	0.03
Lauric acid (C12:0)	0.06	1.42	0.24
Myristic acid (C14:0)	0.56	1.23	0.65
Pentadecanoic acid (C15:0)	0.07	0.21	0.07
Palmitic acid (C16:0)	23.86	24.88	26.07
Margaric acid (C17:0)	0.12	0.17	0.10
Stearic acid (C18:0)	7.47	7.81	6.53
Arachidic acid (C20:0)	0.26	-	0.22
Behenic acid (C22:0)	0.34	0.34	0.38
Total SFA	32.76	36.47	34.33
Myristoleic acid (C14:1)	0.12	-	0.16
Palmitoleic acid (C16:1)	4.16	3.14	5.14
Elaidic acid (C18:1t)	0.13	0.16	0.15
Oleic acid (C18:1c)	33.87	32.51	34.21
Gondoic acid (C20:1)	0.09	0.37	0.08
Erucic acid (C22:1)	0.02	-	-
Total MUFA	72.35	69.06	74.03
Linoleic acid (C18:2c)	25.22	23.56	22.69
Linolelaidic acid (C18:2c)	22.43	27.33	24.13
Cis-11,14-Eicosadienoic acid (C20:2)	0.25	-	-
Arachidonic acid (C20:4)	0.07	0.58	-
Cervonic acid (22:6)	0.18	-	-
Total PUFA, n-6	50.94	47.70	45.38
Dihomo-γ-linolenic acid (C20:3)	1.13	1.08	1.23
Linolenic acid (18:3)	1.85	1.85	1.67
Total PUFA, n-3	2.98	2.93	2.90
Total PUFA, n-6/Total PUFA, n-3	17.09	16.27	15.64
Other	0.13	0.44	0.34
UFA	123.42	117.20	119.75
UFA/SFA	3.76	3.21	3.48
Atherogenic index (AI)	2.43	5.12	2.81
Thrombogenic index (TI)	4648.95	4717.731	4954.45
Hypocholesterolemia index (HI)	2.55	2.28	2.23
Hypocholesterolemic / Hypercholesterolemic	5.05	4.49	4.48

PUFA: polyunsaturated fatty acids, MUFA: monounsaturated fatty acids, UFA: unsaturated fatty acids, SFA: Saturated fatty acids fatty acids

elderberry was promising and positive. However, the improvement in blood biochemical parameters and immunity after consuming elderberry in the present study indicates the potential of this dietary source as a phytogenic and antioxidant-rich source.

Research has proven that in animal models, methanolic and n-hexane extracts of SE fruits can help enhance anti-inflammatory effects by inhibiting the activity of the pro-inflammatory enzyme cyclooxygenase (COX). The analgesic effects of *S. ebulus* extract are mediated by the release of endogenous glucocorticoids and exogenous steroids, through interaction with adrenergic receptors in the serotonergic system derived from L-arginine, and also by affecting the nitric oxide (NO)-related pathway and interacting with the tachykinin pathway, which may contribute to reducing the effects of pain and strengthening the immune system (Ahmadiani et al., 1998; Shokrzadeh and Saravi, 2010). Given that chickens and humans are monogastric, the effectiveness in improving the immune system in the present study seems to be in line with the aforementioned mechanism and is a plausible reason for the effectiveness of this dietary source on poultry. A series of studies have also shown that the anti-Helicobacter pylori activity of SE plant in order to heal stomach ulcers and reduce the effects of antibiotic use, as a result of consuming the fruit of this plant has had promising results that can be extended to reduce the use of growth-promoting antibiotics and other chemical additives in poultry nutrition (Yesilada et al., 1999). Also, the anthocyanin present in the fruit of this plant is of interest as an anti-inflammatory and health-promoting antioxidant. Cytotoxic studies of SE fruit extract show that anthocyanins are useful and effective in preventing and treating many inflammatory diseases and utilizing rich sources of anthocyanins can be an effective strategy to prevent various diseases and improve the health of the consuming host. Kiselova-Kaneva et al. (2023) also believe that SE fruit has immunomodulatory activity and plays a pivotal role as a preventive and therapeutic treatment in case of infection by anthocyanins, in which cyanidin-3-O-galactoside and cyanidin-3-sambobioside are the most abundant.

However, the development of natural products extracted from medicinal plants such as *S. ebulus*, which has anti-inflammatory, analgesic, anticancer, antioxidative, etc. functions, can always be a safe nutritional solution to help human and animal health (Ayoob et al., 2023; Afiouni et al., 2023). In a research study, Ivanova et al. (2014) showed that consumption of SE fruit infusion for one month in 21 volunteers aged 20 to 59 years led to a decrease in blood triglycerides (14.92%), cholesterol (15.04%), LDL (24.67%), and an improvement in the HDL to LDL ratio of 42.77%. In addition, the improvement in the antioxidant status of the blood of the consumers was quite noticeable for the volunteers. Overall, the improvement of lipid and blood antioxidant profiles indicated the potential of this plant to improve the health of its consumers and it was suggested that the consumption of SE fruit infusion could have a preventive role on some metabolic disorders, including lipid metabolism disorders and reduction of oxidative stress such as CVD, metabolic syndrome and type 2 diabetes. The results of these results were fully consistent with the overall results of this study on the health effects of animals. Jiménez et al. (2015) also stated in a review study that the dietary consumption of the medicinal plant *Sambucus ebulus* is limited mainly due to the complexity of the active compounds and low palatability. SE fruit has been shown to contain structural proteins (lectins) bound to sugars with the activity of single-chain ribosome inactivating proteins (RIPs). RIPs are enzymes that inhibit protein synthesis by stopping the elongation step of the polypeptide chain. The biological role of these proteins is still unknown. Evidence suggests that they may play an important role in plant defense against predators and viruses, or in nitrogen storage, immunogenic, and nutritional properties. However, the toxicity of the active compound ebulin and the immunotoxin properties of this plant need to be considered so that the side effects of its high consumption do not become a problem. Because lectins are proteins that bind sugars together, and RIPs are enzymes that hydrolyze the N-glycosidic bond and attach adenine (4234) to the ribose phosphate backbone of 28S rRNA. This property suggests that the protein can be described as an antiviral protein.

RIPs are classified into types 1 and 2 in SE depending on their structure. Type 1 RIPs are single-chain (A chain) and have only enzymatic activity. Type 2 RIPs are two- and four-chain A and B proteins, in which the A chain is an enzyme and the B chain is a lectin.

In the unique and active composition of alpha ebulitins, type 1 RIP is found, and in ebulin, beta and gamma RIP forms are found, each of which has an important role in enhancing immunity and antiviral properties. Tasinov et al. (2021) also report-

ed in a review report examining the phytochemical compounds and anti-inflammatory and anti-stress properties of the fruit of *Sambucus ebulus* that SE fruits have high potential for stimulating immunity, hematopoiesis and antiviral effects. Recently, the potential to reduce endoplasmic reticulum (ER) stress to modulate immunity and enhance the anti-inflammatory activities of Fruit Aqueous Extract's (FAE) under lipopolysaccharide (LPS) stimulation conditions has been considered. In addition, the dominant phytochemicals in SE fruit extract included hydroxycinnamic acids, proanthocyanidins, and anthocyanins. The extract can enhance immune system stimulation conditions by stimulating transcription of COX2, Ccl2, TNFα, IL-6, and iNOS enzymes without causing stress in the ER system.

It has also been shown that the fruit of the plant, in the form of an extract, can suppress the transcription of inflammatory-related genes including IL-1β, IL-6, TNFα, Ccl2, Icam-1, Fabp4, COX2, iNOS, Noxo1, IL-1ra, Sirt-1 induced by lipoprotein saccharide (LPS) and reduce their protein levels. It has been proven that the active compounds of SE fruit can suppress LPS-stimulated inflammatory markers at the transcriptional and translational levels. In fact, by targeting ER stress, an anti-inflammatory background has been provided, indicating the potential of SE fruit to treat and reduce inflammation, especially in pathological and stress conditions. These properties and mechanisms can be very effective in helping modern broilers, which are always under silent stress. The aqueous extract of SE fruit acts as a modulator of antioxidant gene transcription (Hosseintabar-Ghasemabad et al, 2024a). Research shows that in macrophages fed with ethanol and lipopolysaccharides (LPS), it can stimulate transcription and suppress glutamate-cysteine ligase, glutathione peroxidase, and nuclear factor kappa B (NFκB) through ethanol and LPS, respectively (Tasinov et al., 2020ab). Acetone extracts, hydrophilic and anthocyanin-rich fractions of SE fruits, have high antioxidant activity and protect macrophages against cytotoxicity caused by oxidative stress caused by tert-butyl hydroperoxide in vitro (Todorova et al., 2019). Also, ethyl acetate of SE fruits has been shown to have cytoprotective and anti-inflammatory activities, reducing ethanol-induced cell death and increasing pro-inflammatory gene transcription in macrophages (Tasinov et al., 2021). The antiemetic, neuroprotective and antiviral activities of SE fruit extract have been confirmed in some reports (Zahmanov et al., 2015; Fathi et al., 2015). In one study, consumption of SE fruit tea significantly improved serum antioxidant status and blood lipid profile (Ivanova et al., 2014), which was consistent with the present report. The reduction in serum levels of CRP, IL-1β, leptin and adiponectin after consumption of SE fruit extract led to increased immunomodulatory activity and lipid metabolism. It has been shown that macrophages are a source of various proinflammatory cytokines, chemokines and may act in a paracrine and endocrine mode. In low-grade inflammation, such as obesity, where activation and release of chemokines by macrophage recruitment and the establishment of a feeding inflammatory process leads to complications such as diabetes and atherosclerosis (Olefsky and Glass, 2010). It has been shown that other released cytokines and chemokines such as TNFα, IL-6, IL-1β and NO as iNOS, can activate signaling pathways that are mediated by Jun N-terminal kinase (JNK) and Inhibitor of β-kinase (IKKβ) and other kinases leading to the activation of NFκB and subsequent induction of proinflammatory genes (Nguyen et al., 2007). On the other hand, research shows that in protein synthesis, the endoplasmic reticulum (ER) plays an important role in nutrient absorption and has a positive effect on activating protein synthesis in stress and disease conditions such as insulin resistance and cardiovascular diseases (Ozcan et al., 2004; Tasinov et al., 2021). Following increased ER stress, inflammation by iNOS can increase (Zhang and Kaufman, 2008; Anthony and Wek, 2012). Therefore, the iNOS enzyme, as a factor in inflammation and ER stress, can be considered a therapeutic target. A series of studies show that the host in a state of ER stress and inflammation as well as in various pathological conditions can be reduced by compounds such as resveratrol (Li et al., 2011; Sun et al., 2020), epigallocatechin gallate (Karthikeyan et al., 2017), and proanthocyanidins found in SE plant extracts. Given that SE fruits are rich in polyphenols, anthocyanins, and stilbenes, they can be very effective in combating ER stress and inflammation. Therefore, the role of SE in preventing many infectious and viral diseases, as well as helping to regulate bird health and improve blood biochemical status based on the aforementioned mechanisms, can be generalized, and the consumption of this valuable resource can be included in poultry feeding programs, including broilers, at optimum levels, considering and controlling production performance without any concerns, and it is assured that the functional effects of elderberry in the future perspective of poultry

nutrition and diet formulation can be considered and consumed under acceptable conditions.

CONCLUSION

In summary, the overall conclusion of this study showed that dried elderberry fruit powder (SE) is a valuable dietary supplement according to the analysis of the compounds presented in Table 2 for a laboratory sample, while having fiber (15.6%), containing 14% fat with a rich content of oleic acid (20.93%), linoleic acid (42.50%), and linolenic acid (25.51%), and also the ratio of omega-6 to omega-3 was reported to be about 1.66. In addition, in terms of other nutritional components, the amino acid profile was in an acceptable state. The content of lysine (1.15), methionine (0.46), as well as branched-chain amino acids including leucine (0.61), isoleucine (0.49), and valine (0.46) in terms of g/kg were among the richest amino acids in this dietary source.

The functional and clinical effects of nutritional use of this food source in an animal model of broiler chickens showed that consumption of this source leads to improvement of biochemical and immunological parameters related to health and, in parallel, at low consumption levels, it will not have a negative impact on performance and production.

Considering the growth potential and accessibility of the fruit of this plant in many territories, the use of this valuable food source in nutritional programs can be a unique opportunity in the future perspective of natural herbal supplements for the poultry industry.

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