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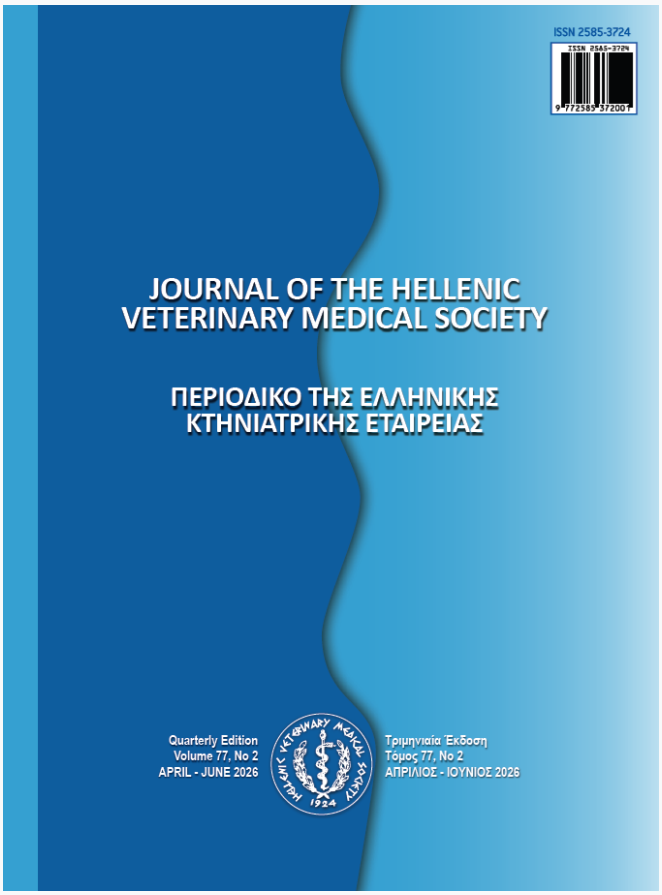

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Research article
Ερευνητικό άρθρο

Evaluation of the dietary effects of *Silybum marianum* on broiler performance

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ABSTRACT: This study was conducted to evaluate the effects of *Silybum marianum* dietary supplements on production performance, carcass characteristics, biochemical parameters and blood antioxidant status, immune system, sensory and taste traits and fatty acid profile of meat in Ross 308 broiler chickens in a completely randomized design including 3 treatments, 5 replications and 10 birds per replication and a total of 150 birds during three growth periods: starter (1-11 d), grower (12-21 d) and finisher (22-42 d). The experimental group treatments included three levels of 0, 4.5 and 6% *Silybum marianum* seed powder in corn and soybean meal-based diets. Performance results from 1 to 11 days of age (starter period) showed that the groups fed with *Silybum marianum* experienced an increase in feed conversion ratio (FCR) ($P<0.05$). Also, from 1 to 42 days of age, the increase in FCR was also noticeable in the 6% group ($P<0.05$) and in parallel, the contrast effect of the control group with the groups fed with *Silybum marianum* for FCR was increased ($P<0.05$). Results of blood parameters except blood uric acid, for glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), atherogenic index, triglyceride, albumin, protein, phosphorus, iron, alanine aminotransferase (ALT), aspartate transaminase (AST), malondialdehyde (MDA) and total antioxidant capacity (TAC) improved health-related status ($P<0.01$). The results of immune system traits also showed that the Antibody Titr trait (42day- SRBC test) decreased with increasing *Silybum marianum* levels in the diet and spleen weight increased ($P<0.05$). The group fed with 4.5 treatment affected meat taste ($P<0.01$). In addition, the contrast results of the control group compared to the groups fed with *Silybum marianum* were significant for the desirability and acceptance of meat trait ($P<0.05$). Overall, the results showed that feeding the studied ingredient as an effective supplement in animal health and meat quality, but further studies are needed to select the optimum levels for performance improvement.

Keyword: Atherogenic index; FCR; Immune system; Meat quality; *Silybum marianum*

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INTRODUCTION

In poultry nutrition, feed quality is significantly dependent on plant nutrients and bioactive compounds. The quality of the plant and the seeds that make up the plant are determined by many factors such as modern agricultural practices, soil quality, climate and grain processing and can affect the content of the nutrient composition and active ingredients of the product (Hosseintabar-Ghasemabad et al., 2020; Janmohammadi et al., 2022; Jabbari and Nazari, 2025). Given global warming and climate change and high stress, the approach of using resistant, low-demand plants that are adaptable to stress conditions is one of the important priorities in selecting plant-based foods in order to create food security and contribute to sustainable and safe programs in the field of food and medicine (Janmohammadi et al., 2023; Seidavi et al., 2023ab; Kadhim et al., 2025). Milk thistle grows in wastelands and around agricultural fields and roads as a dominant plant in many parts of Europe and Asia (Zargari, 1997; Zaker-Esteghamati et al., 2020; Al-Nasrawi et al., 2025). The plant has large, transparent, silvery green leaves, divided, toothed, and prickly. It also has thin and delicate yellow spines at the tips of the leaf teeth and clearly defined white veins. The capitula are large, solitary, and have spherical and purple flowers, and the ripe fruit is black (Zargari, 1997; Feshanghchi et al., 2022;). Given that modern broilers are undergoing significant genetic modifications to produce more meat, studies have shown that these modifications, while creating a series of stresses, may negatively disrupt the expression of genes responsible for the synthesis of ribonucleotide reductase, which plays a role in DNA production or gene repair. This disruption then reduces the regulation of energy production (ATP) in muscle mitochondria and increases cell death (Akbarimehr et al., 2023; Seidavi et al., 2024; Shahsavan, 2025). As a result of the aforementioned process, the normal processes of cell division, collagen production, and vascular network development in the breast muscle are disrupted, which in turn increases fibrosis (tough meat) and breast fat accumulation, losing its natural physical characteristics, nutritional value, and desirable taste for consumers (Shahsavan, 2025). A review of the scientific literature shows that the active ingredient in milk thistle contains silymarin (4-6%) and its extract contains (65-80%) silymarin and also 20-35% fatty acids. It should be noted that silymarin is a combination of phenolic molecules including seven types of flavonolignans including silybin type A, silybin type B, isosilybin type A, isosilybin type B, silychristin, isosilychristin and silydianin and a flavonoid called taxifolin (Scott Luper, 1998; Porwal et al., 2019; Zaker-Esteghamati et al., 2020). In addition, the presence of oleic acid, linoleic acid, palmitic acid, plant sterols such as campesterol and stigmasterol, amines, saponins, and glucose sugars has been confirmed in milk thistle seeds. In today's world, global warming, increased air pollution and oxygen deficiency, the spread of pollution caused by dust and chemicals, the consumption of water and food contaminated with various chemicals and synthetic substances, as well as the presence of various toxins and aflatoxins in foods and medicines, have led to the daily consumption of milk thistle becoming a necessity in the daily routine. In addition to providing nutrients, it can play a significant role in improving the antioxidant activity of the blood, significantly strengthening and improving the health of liver tissue, cleansing the liver from various chemical and metabolic pollution, and improving liver enzymes, reducing fatty liver, reducing various types of cancers with nutritional origin and inhalation of unhealthy air, increasing blood hemoglobin in order to oxygenate tissues, reconstructing damaged tissues, preventing the formation of free radicals in the body, reducing various toxins such as aflatoxin, and stimulating the immune system in the nutrition of humans and domestic animals (Porwal and et al., 2019; Khazaei et al., 2022; Feshanghchi et al., 2022). Exposure of birds to mycotoxins occurs mainly through ingestion of contaminated grains and other raw materials added to animal feed (Chu, 1991; Zaker-Esteghamati et al., 2020; Feshanghchi et al., 2022). The harmful effects of mycotoxins include acute liver damage, mutagenicity, and carcinogenicity, affecting many animal species, including poultry (Chand et al., 2011; Feshanghchi et al., 2022). Several studies have shown that aflatoxins suppress the immune system (Kalorey et al., 2005), decrease blood protein and serum albumin (Oguz et al., 2000), increase uric acid (Kececi et al., 1998), and increase liver enzymes AST, ALT, and alkaline phosphatase (Santurio et al., 2000). A review of the scientific literature indicates that the active ingredient of milk thistle, silymarin, has anti-inflammatory, cytoprotective, hepatoprotective, and anticancer properties and can lead to increased antioxidant, cellular regeneration, and increased protein synthesis (Surai, 2015). In addition, silymarin protects membrane permeability and stability from damage by harmful and toxic compounds (Surai,

2015). It can also prevent the transformation of liver cells into myofibroblasts and prevent collagen fiber deposition and cirrhosis (Fraschini et al., 2002). On the other hand, it has been shown that free radicals, including hydroxyl radicals, superoxide and lipid peroxide, cause fatal liver diseases (Miguez et al., 1994). Silymarin can lead to detoxification and cleansing of the liver by reducing free radicals and increasing antioxidant enzymes (Wisemann, 1996). The cell protective effects of silymarin are mainly considered due to its antioxidant potential. In fact, silymarin acts directly on the cell membrane and prevents abnormal changes in lipid content and maintains the fluidity and normal balance of the cell (Muriel and Mourelle, 1990). Silibinin is another unique and active compound of milk thistle that can reduce the synthesis of leukotriene (B4) and inhibit the 5-lipoxygenase pathway and the leukotriene synthesis pathway, which subsequently reduces inflammatory activities and in fact shifts the synthetic pathways towards increasing anti-inflammatory processes (Saller et al., 2001). Given the ban on the use of antibiotics in poultry nutrition, the use of various natural resources of plant origin has an important place in supplementation (Phillips et al., 2023). Milk thistle (*Silybum marianum*) is becoming popular in animal nutrition and has a significant positive and promising effect on production performance, carcass yield and digestibility in broilers, quails and even growing rabbits (Stastnik et al., 2020; Attia et al., 2025). Therefore, the aim of this study is to use this source as a valuable herbal supplement candidate in functional diets of broiler chickens and to investigate the performance, health benefits, and product quality effects after feeding milk thistle.

MATERIALS AND METHODS

In the present study, biological experiments were conducted on Ross 308 broiler chickens with similar average weight (41±1 g) in a completely randomized design including 3 treatments, 5 replications and 10 birds per replication and a total of 150 birds during three rearing periods: starter (1-11 d), grower (12-21 d) and finisher (22-42 d) in cages with dimensions (1.2 m ✕1.5 m ✕ 2 m) at the Maaf Research and Development Farm of Sepid Makian Company (Somehsara- Guilan- Iran). The experimental treatments included three levels of 0, 4.5 and 6% *Silybum marianum* seed powder, which was obtained from Darvash Giah Khazar medicinal herbs complex company (Ltf) (Iran- Guilan- Rasht). The assay diets were formulated using Amino Feed 5.0 software from Evonik (Table 1). Apparent metabolizable energy corrected to zero nitrogen balance (AMEn) of *Silybum marianum* seeds was calculated based on the equation proposed by the World Poultry Science Association (WPSA) as AME_n (kcal/kg DM) = 15.51 (Crude Protein) + 34.31 (Ether Extract) + 16.51 (Strach) + 13.01 (Sugar) as previously reported. It should be noted that access to feed and drinking water was ad libitum for all birds and temperature management, ventilation and vaccination were planned according to the recommendations of the guide catalogue.

Feed intake (FI), body weight (BW) and FCR were recorded during the starter, grower and finisher periods, respectively. At the end of the period, after four hours of starvation, two birds from each replicate with an average weight close to the average weight of the chicks in each replicate were randomly selected, weighed, and slaughtered. The weights of the defeathered body, eviscerated carcass, breast, thigh, abdominal fat, gizzard, heart, crop, liver, pancreas, spleen, and fabricius were measured with a scale with an accuracy of 0.001. On days 28 and 36, sheep red blood cells (SRBC) were injected in the pectoral muscle area of the birds in an amount of 0.2 cc (5% dilution). After seven days, on days 35 and 42, two birds were selected from each replicate and, by sampling from the wing vein using 3 cc sterile syringes, the antibody levels of the samples against SRBC were evaluated to measure the Newcastle disease (NDV) and influenza (AIV) titers using the hemagglutination method (Amirdahri et al., 2023; Akbari et al., 2025). In order to evaluate the biochemical and antioxidant parameters of the blood, blood parameters were measured by sampling three birds in each replicate using 5 cc sterile syringes and centrifuging the samples at 3000 thousand rpm in commercial kits from Pars Azmoun Company (made in Iran). Glucose, triglycerides, cholesterol, HDL, LDL, total protein, albumin, uric acid, calcium, iron, TAC and MDA were measured by the Colorimetric method (Hosseintabar et al., 2015; Janmohammadi et al., 2023). The atherogenic index (LDL to HDL ratio) was also calculated. AST and ALT were measured by the Enzymatic method. Blood phosphorus (P) was obtained by the Photometric method (Hosseintabar et al., 2015; Hosseintabar-Ghasemabad et al., 2022; Amjadian et al., 2024).

By cooking breast meat without spices from each replicate, the evaluation of aroma, flavor, odor, tenderness, color, and overall desirability was carried

out by six panels (food testers) of evaluators through a questionnaire with a score from 1 to 10, to report the sensory and taste attributes of the meat (Azizi et al., 2021; Samadian et al., 2024). The fatty acid profile content of breast meat was measured in each treatment and the ratio of omega-6 to omega-3, the atherogenic index (AI) {AI= (4 × C14:0) + C16:0/(ΣMUFA + ΣPUFA – ω-6 + ΣPUFA – ω-3)}, the thrombogenic index (TI) {TI = (C14:0 + C16:0 + C18:0)/0.5 × ΣMUFA + 0.5 × Σ (ω-6) + 3 × Σ(ω-3) + Σ(ω-3)/Σ(ω-6)}, the hypocholesterolemic index (HI) {HI = (C18:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:6)/(C14:0 + C16)} and the hypocholesterolemic to hypercholesterolemic ratio { Hypocholesterolemic/Hypercholesterolemic index = [(C18:1 ω-9 + C18:1 ω-7 + C18:2 ω-6 + C18:3 ω-6 + C18:3 ω-3 + C20:3 ω-6 + C20:4 ω-6 + C20:5 ω-3 + C22:4 ω-6 + C22:5 ω-3 + C22:6 ω-3)/(C14:0 + C16:0)]} were reported (Attia et al., 2022). Tests were performed on blood and meat samples at laboratory.

Data were analyzed in excel software with the help of statistical software (SAS 9.3) and mean comparisons between treatments were reported using Duncan's multiple range test. Linear and nonlinear equations were calculated from quadratic, linear and orthogonal equations and the turning point of the quadratic equations was reported in the "Solver" add-on of excel software.

All poultry management activities and laboratory sampling were carried out in compliance with the welfare guidelines appropriate to the Laboratory Animal Ethics Committee of Islamic Azad University, Rasht Branch, Iran, ethics identification code IR.IAU.RASHT.REC.1402.020.

RESULTS

In Table 2, the performance results from 1 to 11 days of age showed that there was a significant difference between the FCR of the control group and each of the *Silybum marianum* fed groups (P<0.05). Also, there was a significant difference between the contrast of the control treatment group compared to the pooled *Silybum marianum* groups (P<0.05). A significant linear equation (y = 0.0074x + 0.9621) with a high coefficient of determination (0.98) was obtained to predict the FCR rate according to the percentage of *Silybum marianum* (P<0.01). Letter y is the target trait, and letter x is the amaranth level.

In Table 3, the performance results from 12 to 21 days of age (for feed intake, body weight and FCR) showed that there was no significant difference between the performance of the control group and the groups fed with different levels of *Silybum marianum*. Also, the contrast, linear and quadratic equation effects between treatments were not significant.

In Table 4, the performance results from 22 to 42 days of age showed that there was no significant difference between the growth performance in the tested groups. Also, the contrast comparison of the control group with respect to the sum of the effects of *Silybum marianum* did not show any significant difference.

In Table 5, the performance results from 1 to 42 days of age for FCR showed that there was a significant difference between the performance of the control group and the 6% *Silybum marianum* group (P<0.05). Also, the contrast effect of the control group with the groups fed with *Silybum marianum* was significant for FCR (P<0.05) and for the linear equation (y = 0.0072x + 1.4843 with a coefficient of determination above 0.99) a significant difference was observed between the different levels of treatments for the FCR trait (P<0.05). Letter y is the target trait, and letter x is the amaranth level.

In Table 6, the results of reported carcass weights showed that there was no significant difference among carcass traits. Also, the quadratic equation was significant for defeather body weight (y = -16.815x2 + 109.89x + 2158 with a turning point of 3.27) and eviscerated carcass (y = -14.667x2 + 90x + 1830 with a turning point of 3.07). In other traits, there was no significant difference between treatments and their linear and quadratic equations. Letter y is the target trait, and letter x is the amaranth level.

In Table 7, the results of the blood parameters analysis showed that in all traits (except blood uric acid) there was a significant difference between the tested treatments in terms of the performance of blood parameters (P<0.01). Also, in all traits (except uric acid and AST), a significant difference was observed between the control group with the contrast of the effects of *Silybum marianum* (zero group compared to two levels of 4.5 and 6 percent *Silybum marianum*). For all traits except (except uric acid and AST), the linear equations were significant. The quadratic polynomial equation for AST was significant (y = 8.228x2 - 55.2x + 420.8) and the turning point of this curve was predicted to be 3.35 percent Letter y is the target trait, and letter x is the amaranth

Table 1. Assay diets and calculated nutrient compositions of treatments

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	54.11	48.74	46.94	61.25	56.21	54.42	67.45	62.09	60.31
Silybum marianum	0.00	4.50	6.00	0.00	4.50	6.00	0.00	4.50	6.00
Soybean meal 44%	40.45	40.76	40.87	33.49	33.80	33.90	27.48	27.79	27.90
Vegetable oil	1.48	2.15	2.37	1.38	2.02	2.24	1.89	2.56	2.78
Lysine hydrochloride	0.21	0.21	0.21	0.21	0.21	0.21	0.20	0.20	0.20
Methionine	0.35	0.34	0.34	0.28	0.28	0.28	0.23	0.22	0.22
Valine	0.04	0.05	0.05	0.02	0.03	0.03	0.01	0.02	0.02
Threonine	0.11	0.11	0.10	0.09	0.09	0.09	0.07	0.07	0.07
Calcium carbonate	1.19	1.12	1.10	1.07	1.00	0.98	0.92	0.86	0.83
Vit and Min Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.01	0.05	0.04	0.07	0.03	0.02	0.07	0.05	0.05
Monocalcium phosphate	1.10	1.09	1.08	0.91	0.90	0.89	0.69	0.68	0.68
Sodium chloride	0.21	0.21	0.21	0.19	0.20	0.21	0.19	0.20	0.20
Sodium bicarbonate	0.24	0.22	0.22	0.27	0.24	0.23	0.27	0.25	0.24
Phytase	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated nutrient compositions (%)									
ME (kcal/kg)	2857	2857	2857	2933	2933	2933	3038	3038	3038
Crude protein	22.72	23.24	23.41	20.08	20.60	20.77	17.76	18.28	18.45
Lysine	1.24	1.40	1.40	1.10	1.10	1.10	0.96	1.05	1.06
Met + Cys	0.93	0.93	0.93	0.81	0.81	0.81	0.71	0.71	0.71
Arginine	1.39	1.39	1.40	1.20	1.21	1.22	1.04	1.05	1.05
Threonine	0.83	0.83	0.83	0.72	0.72	0.72	0.65	0.63	0.63
Isoleucine	0.96	0.85	0.85	0.75	0.75	0.75	0.65	0.65	0.65
Valine	0.94	0.96	0.96	0.85	0.85	0.85	0.74	0.74	0.74
Tryptophan	0.25	0.24	0.24	0.21	0.21	0.21	0.18	0.18	0.18
Calcium	0.95	0.94	0.94	0.85	0.96	0.85	0.74	0.74	0.74
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Potassium	1.00	1.07	1.10	0.88	0.91	0.98	0.78	0.85	0.88
Chlorine	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Av. Phosphorus	0.47	0.47	0.47	0.42	0.42	0.42	0.37	0.37	0.37
Choline (g/kg)	1.47	1.49	1.52	1.60	1.47	1.47	1.48	1.47	1.47
Linoleic acid	1.99	2.70	2.94	2.02	2.72	2.96	2.33	3.05	3.29
Ether extract	4.16	5.59	6.07	4.22	5.63	6.11	4.85	6.28	6.75
Ash	6.13	6.26	6.31	5.47	5.51	5.64	4.79	4.92	4.96
Starch	34.53	31.21	30.09	39.22	35.91	34.80	42.95	39.61	38.50
Crude fiber	3.10	4.46	4.91	2.94	3.40	4.77	2.81	2.90	4.63
NDF	9.65	11.28	11.82	9.70	11.88	10.79	9.70	11.33	11.87
ADF	4.18	5.19	5.52	3.98	5.32	4.65	3.79	4.80	5.14
DCAB[6] (mEq/kg)	267	286	292	237	255	261	210	228	235

T_1: 0% *Silybum marianum* or control group, T_2: 4.5% *Silybum marianum* and T_3: 6% *Silybum marianum*.

Vit and Min Premix: The values of vitamins and minerals per kg in the assay diets: Vitamin A, 9000 IU; vitamin E, 18 IU; vitamin D_3, 3000 IU; vitamin K_3, 3 mg; vitamin B_1 (thiamine), 1.8 mg; vitamin B_2 (riboflavin), 6 mg; vitamin B_3 (niacin), 30 mg; vitamin B_6 (pirodoxine), 3 mg; vitamin B_5 (pantothenic acid), 10 mg; vitamin B_9 (folic acid), 1 mg; vitamin B_{12} (cobalamin), 0.012 mg; vitamin H_3, 0.24mg; 500 mg; Choline,100 mg; Mn, 100 mg; Zinc, 80 mg; Iron, 10 mg; Cu, 1 mg.

ME: Metabolizable Energy, Met + Cys: Methionine + Cysteine, NDF: Neutral-Detergent Fibre, ADF: Acid Detergent Fibre, Av. phosphorus: Available Phosphorus and DCAB: Dietary Cation-Anion Balance

Table 2. Performance results from 1 to 11 d in broiler chickens fed *Silybum marianum*

Treatments	Feed Intake (g)	Body Weight (g)	FCR
0%	282.60	294.40	0.96[b]
4.5%	280.20	281.60	1.00[a]
6%	292.28	290.59	1.01[a]
SEM	9.573	11.507	0.010
P value	0.650	0.728	0.012
Control Vs. *Silybum marianum*	0.761	0.567	0.004
Linear	0.488	0.819	0.005
Quadratic	0.548	0.455	0.335

Means with the same letter for each column are not significantly different

Table 3. Performance results from 12 to 21 d in broiler chickens fed *Silybum marianum*

Treatments	Feed Intake (g)	Body Weight (g)	FCR
0%	687.00	473.40	1.451
4.5%	719.60	498.22	1.445
6%	706.33	489.00	1.448
SEM	14.177	12.527	0.021
P value	0.299	0.396	0.964
Control Vs. *Silybum marianum*	0.161	0.212	0.818
Linear	0.354	0.396	0.894
Quadratic	0.211	0.289	0.818

Table 4. Performance results from 22 to 42 d in broiler chickens fed *Silybum marianum*

Treatments	Feed Intake (g)	Body Weight (g)	FCR
0%	3109.60	2074.00	1.50
4.5%	3173.59	2066.22	1.53
6%	3205.51	2072.65	1.54
SEM	79.614	46.894	0.014
P value	0.694	0.992	0.120
Control Vs. *Silybum marianum*	0.428	0.938	0.056
Linear	0.411	0.984	0.055
Quadratic	0.872	0.904	0.462

Table 5. Performance results from 1 to 42 d in broiler chickens fed *Silybum marianum*

Treatments	Feed Intake (g)	Body Weight (g)	FCR
0%	3796.6	2547.4	1.491[b]
4.5%	3893.2	2564.4	1.518[ab]
6%	3911.8	2561.7	1.526[a]
SEM	91.067	57.475	0.010
P value	0.641	0.975	0.050
Control Vs. *Silybum marianum*	0.361	0.828	0.021
Linear	0.388	0.864	0.018
Quadratic	0.733	0.890	0.572

Means with the same letter for each column are not significantly different

Table 6. Results of carcass weight traits in broiler chickens fed with *Silybum marianum*

Treatments	Live body (g)	Defeather body (g)	Eviscerated carcass (g)	Breast (g)	Thigh (g)	Abdominal fat (g)	Gizzard (g)	Heart (g)	Crop (g)	Wing (g)
0%	2620.0	2158.0	1830.0	670.0	504.0	28.10	42.36	10.34	6.88	140.00
4.5%	2762.0	2312.0	1938.0	712.0	528.0	28.88	44.64	11.10	7.94	144.00
6%	2668.0	2212.0	1842.0	662.0	512.0	27.84	47.94	11.84	10.77	152.00
SEM	54.766	40.382	36.642	25.153	11.460	3.638	4.200	0.665	1.360	5.477
P value	0.217	0.055	0.115	0.352	0.353	0.978	0.650	0.315	0.155	0.323
Control Vs. *Silybum marianum*	0.182	0.057	0.206	0.591	0.277	0.954	0.460	0.190	0.163	0.256
Linear	0.547	0.363	0.821	0.826	0.631	0.961	0.366	0.137	0.066	0.147
Quadratic	0.104	0.025	0.042	0.161	0.180	0.842	0.923	0.990	0.605	0.771

Table 7. Results of biochemical and antioxidant parameters of blood of broiler chickens fed with *Silybum marianum*

Items	Treatments			SEM	P value	Control Vs. *Silybum marianum*	Linear			Quadratic
	0%	4.5%	6%				P value	Equal	R^2	
Glucose (mg/dl)	145.20^{a}	128.20^{b}	132.80^{b}	1.9253	0.0001	0.0001	0.0007	y = -2.46x + 144	0.76	0.0006
Cholesterol (mg/dl)	140.60^{a}	129.80^{b}	120.80^{c}	0.5597	0.0001	0.0001	0.0001	y = -38.99x + 333	0.95	0.2338
HDL (mg/dl)	33.12^{c}	38.34^{b}	40.30^{a}	0.2683	0.0001	0.0001	0.0001	y = 1.18x + 33	0.99	0.0003
LDL (mg/dl)	81.92^{a}	64.16^{b}	53.72^{c}	0.6015	0.0001	0.0001	0.0001	y = -4.52x + 82	0.98	0.0003
Atherogenic Index	2.47^{a}	1.67^{b}	1.33^{c}	0.0187	0.0001	0.0001	0.0001	y = -0.18x + 2.48	0.99	0.0001
Triglycerides (mg/dl)	83.52^{b}	90.04^{a}	90.80^{a}	0.4099	0.0001	0.0001	0.0001	y = 1.26x + 83.6	0.97	0.0001
Uric Acid (mg/dl)	5.23	5.17	5.25	0.1016	0.8400	0.8380	0.9240	-	-	0.5682
Albumin (g/dl)	1.96^{c}	2.14^{b}	2.24^{a}	0.0266	0.0001	0.0001	0.0001	y = 0.046x + 1.95	0.98	0.2220
Total Protein (g/dl)	4.07^{b}	4.44^{a}	4.63^{a}	0.0683	0.0003	0.0001	0.0001	y = 0.09x + 4.06	0.99	0.3034
Phosphorus (mg/dl)	4.87^{c}	5.84^{a}	5.16^{b}	0.0501	0.0001	0.0001	0.0014	y = 0.08x + 4.98	0.3	0.0001
Fe (µg/dl)	121.40^{b}	128.00^{a}	129.00^{a}	0.6481	0.0001	0.0001	0.0001	y = 1.31x + 121	0.98	0.0042
Ca (mg/dl)	10.79^{c}	11.24^{b}	11.92^{a}	0.0690	0.0001	0.0001	0.0001	y = 0.167x + 10.7	0.84	0.1882
ALT (u/l)	18.06^{b}	18.84^{b}	22.00^{a}	0.5472	0.0006	0.0042	0.0003	y = 0.54x + 17.7	0.66	0.1011
AST (u/l)	373.80^{a}	339.00^{b}	385.80^{a}	5.5791	0.0002	0.1211	0.1542	y = -0.24x + 367	0.1	0.0001
MDA (nmol/ml)	21.08^{a}	19.64^{b}	18.68^{c}	0.2831	0.0002	0.0001	0.0001	y = -0.38x + 21	0.97	0.5020
TAC (nmol/ml)	602.40^{c}	778.80^{b}	806.60^{a}	5.2269	0.0001	0.0001	0.0001	y = 35.2x + 606	0.98	0.0001

Means with the same letter for each row are not significantly different

level. For blood glucose parameters, a significant difference was observed between the control treatment compared to each of the *Silybum marianum* treatments (4.5 and 6 percent), and the performance of the control treatment was higher (P<0.01). For the parameter's cholesterol, LDL, atherogenic index and MDA, a significant difference was observed between the control treatment compared to each of the *Silybum marianum* 4.5 and 6% control treatments, and with a decrease in the percentage of *Silybum marianum* in the diet, the amount of cholesterol, LDL, atherogenic index and MDA had a decreasing trend (P<0.01). For the parameters HDL, Albumin, Ca and TAC, a significant upward trend was observed with an increase in the level of *Silybum marianum*, so that the control treatment showed a lower and significant performance compared to the two *Silybum marianum* 4.5 and 6% treatments and also the 4.5% treatment compared to 6% (P<0.01). For the parameter's triglycerides, total protein and Fe, the highest performance was related to the *Silybum marianum* treatments and had a significant difference with the control treatment (P<0.01). For blood phosphorus, a significant difference was observed between the 4.5% *Silybum marianum* treatment compared to the 6% treatment and the control treatment (P<0.01). Also, a significant difference was observed between the 6% *Silybum marianum* treatment compared to the control treatment, such that the blood phosphorus level for the 6% *Silybum marianum* treatment was higher than the control treatment. For the ALT parameter, a significant difference was observed between the 6% treatment compared to the other two treatments (P<0.01). While for the AST parameter, a significant difference was observed between the 6% treatment and the control compared to the 4.5% treatment (P<0.01).

In Table 8, the results of the analysis of immune system traits showed that the type of treatment had a significant effect on the performance of Antibody Titr (42day- SRBC test), spleen weight (g) and relative weight of the spleen (%) (P<0.05). For the Antibody Titr (42day- SRBC test) trait, with an increase in the percentage of *Silybum marianum* in the diet, the value of this parameter had a significant downward trend (P<0.05). While with an increase in the percentage of *Silybum marianum* in the diet, the spleen weight (g) trait showed a significant upward trend (P<0.05). For the relative weight of the spleen (%), a significant difference was observed between the 6% *Silybum marianum* treatment and the control treatment (P<0.05), so that the value of this trait was highest for the 6% *Silybum marianum* group. Also, comparing the effect of the control treatment with the contrast of the effects of *Silybum marianum* and the linear equation in the traits antibody titer (42day-SRBC test), spleen weight (g) and relative weight of the spleen (%) was significant. In addition, a significant linear model was observed for the parameter Fabricius weight (g). Letter y is the target trait, and letter x is the amaranth level. The significant linear equations were as follows:

Table 8. Results of parameters related to the immune system of broiler chickens fed with *Silybum marianum*

Treatments	Antibody Titr (35 day-SRBC test)	Antibody Titr (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 (%)
%0	3.600	7.000^{a}	6.180	0.239	2.260^{b}	0.086^{b}	1.780	0.068
%4.5	3.000	4.332^{b}	5.820	0.211	2.880^{a}	0.104^{ab}	1.700	0.037
%6	1.800	3.200^{c}	5.480	0.205	3.320^{a}	0.125^{a}	1.326	0.040
SEM	0.837	0.353	0.511	0.023	0.174	0.007	0.140	0.012
P value	0.335	0.0001	0.637	0.534	0.004	0.007	0.088	0.160
Control Vs. *Silybum marianum*	0.264	0.0001	0.414	0.277	0.002	0.006	0.145	0.062
Linear	0.154	0.0001	0.352	0.305	0.001	0.002	0.041	0.119
Quadratic	0.775	0.101	0.988	0.681	0.680	0.887	0.408	0.249

Means with the same letter for each column are not significantly different

Antibody Titr (42day- SRBC test): $y = -0.624x + 7.028$ $R^2=0.98$
Spleen weight (g): $y = 0.1677x + 2.2331$ $R^2=0.96$
Relative weight of the spleen (%): $y = 0.0059x + 0.0846$ $R^2=0.9$
Fabricius weight (g): $y = -0.0623x + 1.8201$ $R^2=0.64$

In Table 9, the results showed that among the taste and sensory traits of meat, only meat taste was affected by the experimental treatments, such that a significant difference was observed between the meat taste of the 4.5% treatment compared to the other treatments ($P<0.01$). Also, the results of the contrast of the control group compared to the effects of *Silybum marianum* for the trait desirability and acceptance of meat were significant ($P<0.05$). Letter y is the target trait, and letter x is the amaranth level. The quadratic equation for the traits meat perfume and meat taste was significant and was as follows:
Meat perfume: $-0.1407x^2 + 0.8111x + 7.4$
Meat taste: $y = -0.2074x^2 + 1.2444x + 7$

In Table 10, the reports were limited to the fatty acid profile values and health-related indices of broiler breast meat for each treatment, and statistical analysis and Duncan comparisons were not included. The fatty acid content and attention to the ratios and indices reported for meat in future studies could be an important item in the future perspective of healthy nutrition through the poultry industry.

DISCUSSION

Milk thistle (*Silybum marianum*) is becoming popular in animal nutrition and has a significant positive and promising effect on production performance, carcass yield and digestibility in broilers, quails and even growing rabbits (Stastnik et al., 2020; Attia et al., 2025). So, hypothesis of the study was evaluation of its effect on broiler productivity.

Regarding the reduction in performance (FCR) and improvement in health-related parameters (biochemical status and blood antioxidant) and preservation of carcass characteristics and meat quality in the present study and comparing the results with the report of Gharahveysi (2018) which showed that increasing the amount of *Silybum marianum* in chicken nutrition leads to a decrease in body weight and feed consumption. Also, in the study of Suchý et al. (2008), feeding at levels of 0.2 and 1% *Silybum marianum* on broiler chickens resulted in a noticeable decrease in weight and an increase in FCR. Kalantar et al. (2014) found a decrease in weight and an increase in FCR with the addition of 0.5% *Silybum marianum* in the diet of broiler chickens. The results of the aforementioned studies were consistent and in agreement with the results of the present study.

In the report of Khatami et al. (2023), feeding levels of 0, 0.2, 0.4 and 0.6% *Silybum marianum* seeds in broilers was associated with a decrease in performance and LDL and blood triglycerides, which was in line with and in agreement with the present study.

The decrease in carcass yield after adding *Silybum marianum* to the diet of broilers reported by Schiavone et al. (2007) and Ondrej et al. (2015) was in contrast to the results of the present study. Ondrej et al. (2015) and Rashidi et al. (2014) did not observe any negative effects on breast muscle and abdominal fat up to a 2% *Silybum marianum* intake, which was in line with the results of the present study. In the study of Šťastník et al. (2016) the intake of high levels of *Silybum marianum* was

Table 9. Results of sensory and taste traits of breast meat of broilers fed with *Silybum marianum*

Treatments	Meat perfume	Meat taste	Meat smell	Meat crispy	Meat color	Desirability and acceptance of meat
%0	7.40	7.00^{b}	8.60	7.20	7.40	7.20
%4.5	8.20	8.40^{a}	7.80	8.00	8.00	8.40
%6	7.20	7.00^{b}	8.20	8.00	7.80	8.00
SEM	0.337	0.294	0.337	0.383	0.408	0.316
P value	0.126	0.008	0.281	0.272	0.585	0.055
Control Vs. *Silybum marianum*	0.481	0.076	0.171	0.114	0.337	0.024
Linear	0.682	0.990	0.417	0.165	0.502	0.099
Quadratic	0.050	0.002	0.171	0.411	0.439	0.061

Means with the same letter for each column are not significantly different

Table 10. Report on fatty acid profile values and indices in broiler chickens fed with *Silybum marianum*

Items	Treatments		
	0%	4.5%	6%
Caprylic acid (C8:0)	0.02	0.15	0.14
Capric acid (C10:0)	0.02	0.11	0.11
Lauric acid (C12:0)	0.10	1.05	0.91
Myristic acid (C14:0)	0.47	1.11	1.06
Pentadecanoic acid (C15:0)	0.10	0.12	0.09
Palmitic acid (C16:0)	23.05	27.65	29.19
Margaric acid (C17:0)	0.15	0.17	0.14
Stearic acid (C18:0)	6.96	8.45	7.83
Arachidic acid (C20:0)	0.25	0.12	0.11
Behenic acid (C22:0)	0.50	0.40	0.17
Total SFA	31.62	39.33	39.75
Myristoleic acid (C14:1)	0.47	1.11	1.06
Palmitoleic acid (C16:1)	3.25	3.40	4.09
Elaidic acid (C18:1t)	0.12	0.16	0.15
Oleic acid (C18:1c)	33.62	32.65	35.22
Gondoic acid (C20:1)	-	-	-
Erucic acid (C22:1)	-	-	-
Total MUFA	71.08	69.97	75.74
Linoleic acid (C18:2c)	25.56	20.31	18.51
Linolelaidic acid (C18:2c)	25.56	20.31	18.51
Cis-11,14-Eicosadienoic acid (C20:2)	0.17	0.18	-
Arachidonic acid (C20:4)	0.18	0.09	0.13
Cervonic acid (22:6)	-	-	-
Total PUFA, n-6	51.30	40.89	37.15
Dihomo-γ-linolenic acid (C20:3)	2.66	2.16	0.73
Linolenic acid (18:3)	1.93	1.24	1.01
Total PUFA, n-3	4.59	3.40	1.74
Total PUFA, n-6/Total PUFA, n-3	11.17	12.02	21.35
Other	0.19	0.22	0.10
UFA	122.57	111.08	112.99
UFA/SFA	3.87	2.82	2.84
Atherogenic index (AI)	2.06	2.68	4.49
Thrombogenic index (TI)	4372.54	5237.89	5792.20
Hypocholesterolemia index (HI)	2.71	1.96	1.83
Hypocholesterolemic / Hypercholesterolemic	5.21	3.86	3.73

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

associated with an increase in liver weight, which was not observed in the present study. Grela et al. (2020) observed a decrease in the fat content of the muscle of chickens fed diets containing 3 and 6% *Silybum marianum* seeds, and a parallel increase in the proportion of stearic and linoleic acids was noticeable, which was in contrast to the results of the present study. The atherogenic, thrombogenic index and the ratio of hypocholesterolemia and hypercholesterolemia in breast meat after feeding *Silybum marianum* in the studies of Janocha et al. (2021), Grela et al. (2020), Kralik et al. (2015) and Šťastník et al. (2016) had positive effects on breast meat in chickens, which in the present study these parameters were only measured without statistical evaluation and the achievements of the available results were promising. The positive effect of diets containing *Silybum marianum* on the sensory and taste characteristics of meat was evaluated positively by Janocha et al. (2021), but Ondrej et al. (2015), with the addition of 5 to 15% *Silybum marianum*, the taste of meat was reduced, the output of the aforementioned results was largely in line with and in agreement with the results of the present study and there was only a contradiction at high levels.

Improved liver metabolism and synthesis of protein, fat, and carbohydrate nutrients, and increased glycosidase enzyme activity as a result of increased aminopeptidases after feeding *Silybum marianum*, were the reasons for the relatively positive effectiveness of the studied ingredient test on health-related parameters and meat quality (Bendowski et al., 2022). β-glucosidase has been shown to play an important role in the removal of non-reducing glycosyl residues from saccharides and glycosides and has an important contribution to glycolipid metabolism and subsequently blood health (Cairns and Esen, 2010; Bendowski et al., 2022). Guerrini and Tedesco (2023) in their review report, by examining the reductive activity of *Silybum marianum* on serum biochemical parameters, oxidative status, immunity and function, stated that *Silybum marianum* is more effective in combating mycotoxin toxicity on organs, biochemical and immunological functions and its use for hepatoprotection and antioxidant and reducing organ damage caused by poisoning is significant and promising. Guerrini and Tedesco (2022) reported that feeding *Silybum marianum* in damaged liver leads to an increase in liver enzymes such as ALT, AST and ALT, and in healthy liver this effect will be reversed. The process of liver regeneration is associated with the synthesis of ribosomal RNA and the transformation of stellate hepatocytes into myofibroblasts, which together lead to the improvement of normal liver functions and the production of enzymes in normal amounts (Adetuyi et al., 2021; Guerrini and Tedesco, 2023). A review of the scientific literature shows that silymarin in *Silybum marianum* acts as an immunostimulant and can increase lymphocytes, affect the production of cytokines interferon gamma, interleukin IL-4 and IL-10 (Wilasrusmee et al., 2002). In fact, silymarin in liver cells can stabilize the homeostasis of cell membranes and stimulate the secretion of histamine, lipoxygenase and prostaglandin synthetase and leukotriene synthesis. Amiridumari et al. (2014) found that supplementation of aflatoxin-contaminated diets with *Silybum marianum* seeds had no significant effect on iron, phosphorus, cholesterol, triglycerides, uric acid, and LDL, but the capacity of *Silybum marianum* as inhibitors of free radicals and lipid peroxidation (i.e., MDA) and suppression of proinflammatory signals derived from nuclear factor-B (NF-B) activation, which play an effective role in inducing the synthesis of cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-1, IL-6), and granulocyte-macrophage colony-stimulating factor (GM-CSF), could ensure consumer health and safety (Esmaeil et al., 2017; Juráňová et al., 2018; Guerrini and Tedesco, 2023). In summary, reviewing the results of the aforementioned reports on important biochemical and antioxidant parameters of the blood, as well as the status of the immune system, and comparing them with the results of the present study, it can be confidently stated that the effect of dietary supplementation with different forms and levels of *Silybum marianum* on blood, liver, and immune health parameters was completely positive, and the results were completely consistent and in agreement with the mechanisms and results.

An important point in this study was the introduction of the possibility of using this emerging plant in feeding broiler chickens. This plant, with its unique properties, can be a key element in the advancement of poultry nutrition knowledge. As the limitations of this study, we can mention the lack of measurement of microbiological study of the digestive tract and the lack of examination of the histological characteristics of the chicken intestines, which should be addressed in future research. Also, as future directions for research, it is suggested that the effect of other levels of this plant on the performance of the

broiler flock be investigated, and the effect of this plant under stress conditions and microbial contamination of the flock be studied.

CONCLUSION

Increasing levels of *Silybum marianum* seed powder consumption in the experimental groups were associated with a decrease in performance, especially an increase in FCR. Of course, the positive effects on improving the status of glucose, cholesterol, HDL, LDL, atherogenic index, triglyceride, albumin, protein, phosphorus, iron, ALT, AST, MDA and TAC in the groups fed this source were quite noticeable. In addition, the effectiveness on immune system items and meat sensory traits was also quite noticeable. It seems that the role of *Silybum marianum* as a food source in improving health and maintaining the quality of meat and carcass products can be confirmed and indicates the positive effects of consuming this supplement, but its use to increase performance requires more detailed studies to select the optimum levels to create conditions for practical use in poultry nutrition.

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