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Immune Status and Liver Function for Laying Hens Supplied with Milk Thistle (*Silybum marianum*) as Food Additive under Heat Stress of Iraqi Summer

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Abstract:

Background

Many previous studies proved the ability of Milk thistle (*Silybum marianum*) to treat and immune enhance against many diseases and hepatic dysfunctions with antioxidant activities to support cells protection in heat stress conditions.

Aims

To assess the effects of graded dietary supplementation by milk thistle (*Silybum marianum*) extract on immune responses, hepatic biomarkers and Hematology parameters in pre-laying hens under natural Iraqi summer heat stress.

Methods

One hundred twenty Isa-brown pre-laying hens (10 weeks old) were randomly allocated in four groups of 30 birds per group and housed till 18 weeks of age in a closed system using floor pens. The diets included a basal diet without extract (control, G1), or basal diet supplemented with 0.5 gm/kg hens ration (G2), 1 gm/kg hens ration (G3), or 2 gm /kg hens ration (G4) of the extracted material. The experimental period was 8 weeks, from August 24, 2025, to October 26, 2025. Measured variables were alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, triglycerides (TG), total cholesterol (TC), heterophil ratio and lymphocyte ratio and antibody titers against Newcastle disease vaccine.

Results

Silybum marianum supplementation resulted in a significant increase in liver function compared to the control group. In particular, G3 and G4 had considerably lower ALT and AST levels compared to G1 and G2. Moreover, compared with control, there were significant reductions in glucose, TG and TC due to the extract. G4 showed significant improvement in immunity measured as Newcastle disease antibody titers compared to all the other groups.

Conclusions

Silybum marianum extract (2 gm/kg) modulates glucose, TG, TC, ALT and AST and the blood immune status as well in pre-laying hens under summer heat stress.

Key words: laying, Milk thistle, Immune status, Liver function, Hematology

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Introduction:

A significant increase in global average temperatures has also negatively affected agricultural systems (Kennedy *et al.*, 2022) in recent decades. When ambient temperatures are above 32°C poultry show decreased feed intake, poor conversion efficiency, reduced growth along with lesser meat and egg production, higher broiler mortality due to heat pressure (Rostagno, 2020) while reproductive performance is also impaired. Moreover, heat stress adversely affects production metrics, digestive health, core body temperature, immune function, control of appetite-related hormones and oxidative status in birds (Goel, 2021); it also impairs regular physiological circumstances and causes mobile injury (Abbas *et al.*, 2021). Dietary strategies are thus the main focus of intervention because cooling livestock facilities is not only prohibitively expensive, its implementation presents practical challenges (Mahasneh et al., 2024). The usage of phytobiotics is on the rise due to their pharmacological characteristics that stimulate feed intake, act as antioxidants (Kim *et al.*, 2011), health-promoting agent, modulating membrane fluidity and permeability and improve absorption of nutrients (Vicente Martínez *et al.*, 2022). One of the medicinal plants that can be used in animal feeding is milk thistle (*Silybum marianum*), which belongs to the Asteraceae family (Rivas Caceres *et al.*, 2024). Its seeds have been used as a natural remedy for hepatic and biliary ailments for over 2000 years ago (Guo *et al.*, 2024). The active functional substance of milk thistle is silymarin, a mixture of flavonolignan isomers (silibinin, isosilibinin, silychristin, and silydianin) and a flavonoid; its unified impact includes antioxidant, anti-inflammatory, antifibrotic, anti-lipid peroxidative cell membrane stabilization and liver-regenerative properties (Gousias *et al.*, 2025).
Based on these findings, this study aims to assess the impact of graded additions of milk thistle (*Silybum marianum*) extract on serum oxidative indicators, immune status and laying performance in pre-laying hens undergoing natural summer heat stress.

Materials and Methods:

Study Site and Environmental Conditions

The study was conducted in the rearing facility of Experimental Farm, Agricultural Researchs Department, Poultry Research Station, the Iraqi Ministry of Agriculture; Baghdad. All tests were carried out within 8 weeks between the dates of 24 August, 2025 to 26 October, 2025 in which natural summer heat stress conditions occurred with ambient temperatures >40 °C.

Animals and Housing Arrangements

A total of one hundred twenty Isa Brown pre-laying hens (10 weeks old) were randomly separated into four treatment groups at 30 birds per group (the chickens used in the experiment raised from one day old). Hens were housed in a closed system on 4 floor pens/group. Each pen had dimensions of 2.5 meter (m)

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by 2m, creating an area of 5m². The photoperiod during the trial consisted of 10 hours (h) of light and 14 h. of dark. All pens were equipped with removable trough feeders and drinkers to allow 24 h. access to feed and water (Lohmann Tierzucht GmbH, 2020) Lohmann Brown Management Guide for laying hens.

Experimental Protocol and Dietary Groups:

The four dietary regimens were as follows:

- T1 (control): Basal diet without any milk thistle extract supplementation.
- T2: Basal diet supplemented with 0.5 gram (gm) of milk thistle extract per kilogram (kg) of feed.
- T3: Basal diet supplemented with 1 gm of milk thistle extract per kg of feed.
- T4: Basal diet supplemented with 2 gm of milk thistle extract per kg of feed.

Feed Composition

The basal diet as in table (1) was prepared in accordance with the nutritional guidelines specified by the Lohmann Tierzucht GmbH (2020) Lohmann Brown Management Guide for laying hens.

Blood Sampling and Laboratory Procedures

Blood samples were collected from birds in each treatment group in14 weeks of ages and at the end of the trial (at 18 weeks of age). Serum was obtained for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, triglycerides (TG), total cholesterol (TC) and antibody titers against Newcastle disease vaccine by standard laboratory procedures.

Origin and Preparation Protocol of Milk Thistle Extract (*Silybum marianum*)

The milk thistle extract utilized in this study was a commercially available product, extracted from seeds of *Silybum marianum* (4:1 such that 1000 mg of milk thistle is equivalent), acquired from Nutricost (USA). Dietary inclusion rates were determined based on silymarin concentrations found in previous studies (250 to 1000 milligram per kilogram) and extrapolated from investigations in broilers, quail and late-stage laying hens published in PMC and BioMed Central publications (Guo *et al*., 2024).

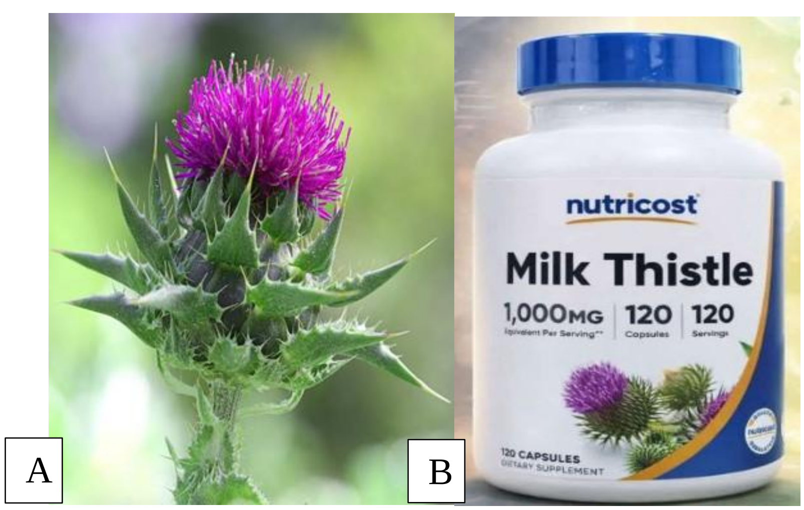

Figure 1: (A) The natural planet of the Milk Thistle and (B) The commercially available product of Milk Thistle extract

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Vaccination Protocol

A common vaccination schedule was followed for all experimental birds. They were vaccinated the day after hatching against Marek's disease virus with 0.2 ml/chick subcutaneously (S/C). An oil-adjuvanted bivalent vaccine include NDV and AIV (0.2ml/bird S/C) was given on day 3 of life. A NDV booster was administered via drinking water at 7, 17, 27, and 56 days of age. A last booster oil-adjuvanted trivalent vaccine (ND+IB+EDS) 0.5ml/bird (S/C) was administered, 4 weeks after the second vaccination, when hens were 16 weeks old. Moreover, the birds were vaccinated with an intermediate strain vaccine against infectious bursal disease virus (IBDV) at 14 day of age.

Table (1): Ingredient composition and nutrient level of the basal diets (dry basis)

Ingredients	Content, %	Content, Kg	Calculated chemical analysis	
Corn	8	74.4	Energy (kcal/kg diet)	2760
Soybean meal	15	139.5	Crude protein (%)	16
Wheat	60	558	Calcium %	1.5
Barley	7.1	66.03	Lysine %	0.65
Wheat bran	5	46.5	Methionine %	0.34
Limestone	4	37.2	Available phosphorus %	0.44
Premix[1]	0.8	7.44		
Salt	0.1	0.93		
Total	100.00	930 Kg		

The vitamin-mineral premix supplied the following nutrients per kilogram of the diet: 10,000 IU of vitamin A; 2,500 IU of vitamin D_3; 27.0 IU of vitamin E; 3.0 mg of vitamin K_3; 1.0 mg of thiamine (vitamin B_1); 4 mg of riboflavin (vitamin B_2); 32 mg of niacin (nicotinic acid); 11 mg of pantothenic acid; 3.0 mg of pyridoxine (vitamin B_6); 0.5 mg of folic acid; 25 mg of cobalamin (vitamin B_{12}); 50.0 mg of biotin; 60.0 mg of iron (Fe); 60.0 mg of zinc (Zn); 100.0 mg of manganese (Mn); 5.0 mg of copper (Cu); 0.5 mg of iodine (I); and 0.2 mg of selenium (Se).

Hematology:

Blood Collection

Blood samples (5 mL per bird) were collected from five hens in each experimental group at two different time points: first at 14 weeks of age and second at 18 weeks of age. They were collected by puncturing the wing vein. Two kinds of tubes were used for each sample: one with heparin for plasma separation and one without heparin for serum isolation.

Serum Separation

The blood was centrifuged for 10 minutes (min) at 3,000 round per minute (rpm) and 5 centigrade (°C) after which the separated serum was collected into Eppendorf tubes and stored at −20°C until analysis.

Liver Function Assessment

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Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase were spectrophotometrically determined with the use of commercial kits from AGAPPE DIAGNOSTIC SWITZERLAND GmbH (Knonauerstrasse 54, 6330 Cham, Switzerland), following manufacturer's instructions.

Plasma Biochemical Parameters
Plasma was separated immediately after centrifugation (3,000 rpm for 10 minutes). Commercial biochemistry kits were utilized to measure plasma total lipid concentration, and concentrations of triglycerides and total cholesterol (expressed in mg/dL). Serum glucose concentration was determined using the commercial kits from Glucose Liquicolor GOD-PAP enzymatic colorimetric assay (HUMAN Gesellschaft fur Biochemische Diagnostica mbH, Wiesbaden, Germany; REF 10260), according to the manufacturer's instructions.

Immune Response Assays

Hematological Parameters (RBC and WBC):
Red blood cells (RBCs) and white blood cells (WBCs) were counted in fresh blood samples. Differential white blood cell counts were performed as previously described (Hawkey and Dennett 1989).
Heterophil and Lymphocyte
The immune-related indicators, such as leukocyte profiles, heterophils (H) and lymphocytes (L) were examined by a semi-automated cell counter using the method described previously by Khazaei *et al.*, (2021).

Newcastle Disease Virus (NDV) Antibody Titers (ELISA)
Serum samples were obtained at 14 and 18 weeks of age by the withdrawal of blood from five birds per test group in plain tubes, with subsequent clot formation (no anticoagulant was used). The serum was obtained by centrifugation at 3,000 rpm for 10 min. Antibody titers against NDV were estimated with the use of commercial enzyme-linked immunosorbent assay (ELISA) kits (iD. vet, Innovative Diagnostic (310 rue Louis Pasteur, Grabels, France), and values were mean ± SE expressed in picograms per milliliter (pg/ml) (Ahmad *et al.*, 2020)

Temperature-Humidity Index
Inside temperature (°C) and relative humidity (%) were measured over the summer period (August 24, 2025, to October 26, 2025). It also provides an explanation of how the temperature-humidity index (THI) was calculated according to the method described by Yayli and Kiliç (2024) as well as the Weather Safety Index (LCI, 1970).

Statistical Analyses
All data were expressed as mean values with their corresponding standard errors. Statistical evaluations were performed using the Statistical Package for Social Sciences (SPSS, version 18.0). Comparisons between two groups were conducted using the t-test, while comparisons among all vaccinated groups were carried out by one-way analysis of variance (ANOVA). A probability value of less than 0.05 was considered statistically significant (SPSS Inc., Chicago, IL, USA).

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Ethical Approval:

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (245) (October, 2025 A, S, S and A).

Results:

Temperature humidity index (THI) estimation

Table (2) illustrates the averages in the indoor temperature (ºC) and RH% during summer months of experimental period. Temperatures were 34.3, 33.2 and 31.7 for August, September and October, respectively. Maine had the average RH% of 65.3, and Vener & NH included a RH% of 61.8% and 61.5%, respectively, also over the same experimental months. Estimation of THI according to Yayli and Kiliç (2024) who stated that, the chickens exposed to danger degree of heat during August and emergency degrees during rest of experimental months.

Table (2): The temperature degrees ºC, humidity percentages during the summer of 2025

Months	Months Temperature degrees ºC			Humidity %			THI
	Maximum	Minimum	Average	Maximum	Minimum	Average	
August	34.6±1.2	32.8±1.4	34.3±0.6	80.8±6.9	38.0±5.8	65.3±3.5	86
September	34.5±1.2	31.8±1.9	33.2±1.0	81.3±3.7	35.2±7.6	61.8±3.3	85
October	31.8±1.7	29.6±1.7	31.7±3.0	79.9±6.5	42.2±7.4	61.5±3.4	84

Temperature-Humidity Index (THI), Associated Livestock Weather Safety Index (LWSI) categories are also shown.

Liver function profile at 14 weeks of ages:

The means values of all the liver functional tests indicated significant difference ($P<0.05$) which found numerically lower in birds received milk thistle (MTh) extract from treatment groups through milk thistle supplemented compared to control in (Table 3). The level of glucose in the serum of different layers fed diets are significantly ($P<0.05$) lower than control group. The concentrations of total lipids (triglycerides and cholesterol) among the experimental groups varied significantly, with G3 and G4 showing a decrease. Additionally, serum lipid profile was significantly ameliorated for birds supplemented with MTh levels; the concentrations of serum triglycerides (TG) and total cholesterol (TC) birds were significantly lowered by increasing dosages of MTh extract compared to that attained in control group. Moreover, there was a dose-dependent reduction in serum ALT and AST activity upon administration of MTh extract. The liver

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functions of the chicken fed MTh supplemented diet were significantly better as indicated by lower levels of AST and ALT activity (P<0.05) when compared to level for control group.

Table (3): Effect of MTh extract on Liver function profile of pre-laying chickens under summer condition at 14 weeks of ages

Liver function profile	Experimental group				ANOVA p value
	G1	G2	G3	G4	
Glucose, (mg/dl)	95.62±1.15 a	95.16±2.62 ab	86.68 ±3.88 c	86.52±3.05 d	0.053
Triglycerides, (mg/dl)	86.06 ±1.60 a	81.98±0.74 b	79.36± 0.56 c	71.42± 0.63 d	0.00001
Cholesterol, (mg/dl)	155.2±0.2 a	152.2±0.73 b	136± 3.67 c	130.6± 1.16 d	0.000001
Alanine Aminotransferase (ALT), IU/L	23±1.88 a	19.56± 0.67 b	19.34±0.97 bc	17.04 ± 1.16 d	0.0512
Aspartate Aminotransferase (AST), IU/L	33.02±2.34 a	27.4 ± 1.148 b	27.16 ± 1.92 cd	25.64±0.71 d	0.1132

(ALT)-Alanine amino transferase, (AST)-Aspartate aminotransferase, Glucose, Triglycerides and Cholesterol Note: ^{abc} Mean values with different superscripts within a row differ significantly.

Liver function profile at 18 weeks of ages:

At week 18, serum AST and ALT activity was significantly lower (P<0.05) in all the supplement groups containing increasing doses of MTh extract when compared to control group as illustrated in (Table 4) and a decline with respect to their levels at 14 weeks was also observed for AST and ALT activity particularly in the fourth group. Also, there was a quadratic decrease (P < 0.05) for TC and TG of serum lipid profile, and decreases in the means of glucose were obtained compared to control by increasing MTh extract additions.

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Table (4): Effect of MTh extract on Liver function profile of pre-laying chickens under summer condition at 18 weeks of ages

Liver function profile	Experimental group				ANOVA p value
	G1	G2	G3	G4	
Glucose, (mg/dl)	88.94±0.52 a	83.74±1.10 b	81.60±1.01 c	80.16 ±0.09d	0.0003
Triglycerides, (mg/dl)	87.4±1.54 a	85.12±0.95 b	81.84± 0.43 c	80.4±0.20 d	0.0040
Cholesterol, (mg/dl)	155±0.83 a	150.6±0.4 b	144± 4 c	138 ± 4.06 d	0.0096
Alanine Aminotransferae (ALT) IU/L	23.04± 1.65 a	19.58±0.51 b	18.78 ±0.84 c	18.04±0.54 d	0.0189
Aspartate Aminotransferase (AST) IU/L	31.02± 1.14 a	27.28 ±0.73 b	27±0.36 c	24.36±0.49 d	0.00354

(ALT)-Alanine amino transferase, (AST)-Aspartate aminotransferase, Glucose, Triglycerides and Cholesterol Note: ^{ABCD} Mean values with different superscripts within a row differ significantly.

Immune response assays:

Hematological constituents:

It was observed from Table (5) and Table (6), Hematological parameters among the groups during hot summer condition at 14 and 18 weeks of age respectively as being significant (P <0.05) The values for RBCs, WBCs and platelets gave higher significant results in groups receiving supplement at the 2000 mg MTh. per kg diet versus. In contrast, lymphocyte and heterophile percentages differed significantly between the experimental groups.

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Table (5): Effect of MTh extract on blood hematology of pre-laying chickens under summer condition at 14 weeks of ages

Blood parameters	G1	G2	G3	G4	ANOVA p value
WBC ($x10^3/\mu l$)	16.00±1.87 a	20.80±1.80 b	22.00±3.00 c	26.00±1.87 d	0.06878
RBC ($x10^{6/}\mu l$)	2.30±0.20 a	2.50±0.15 b	2.80±0.12 c	3.30±0.12 d	0.0006*
L%	34 ± 1.87 a	43 ±3.39 b	48 ±3.39 c	54 ±1.87 d	0.01153*
H%	20 ± 1.58 a	18 ±1.22 b	17 ± 1.22 c	14 ±1.87 d	0.026839*
Pletelet×10^5	220±12.24 a	240± 29.15 b	290±18.70 c	318±13.19 d	0.00078*

^{a,b,c} Means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means, P value: probability level.

Table (6): Effect of MTh extract on blood hematology of pre-laying chickens under summer condition at 18 weeks of ages

Blood parameters	G1	G2	G3	G4	ANOVA p value
WBC ($x10^3.\mu l$)	19.00±0.63 a	21.80±1.31 b	26.00±1.00 c	27.00±1.22 d	0.0029*
RBC ($x10^6$.μl)	2.30±0.12 a	2.50±0.00 b	2.50±0.15 ab	2.80±0.12 c	0.11872
L%	42 ±2.00 a	46 ±2.44 b	48 ± 3.74 c	54 ±1.87 d	0.011068*
H%	19 ±1.87 a	18 ±1.22 b	17 ±1.22 c	16 ±1.00 d	0.51740
Pletelet×10^5	220±12.24 a	270±12.24 b	271±12.24 ab	290±10 c	0.036255*

^{a,b,c} Means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means, P value: probability level.

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Newcastle Disease (ELISA) antibody titer at 14 and 18 weeks:

Mean ELISA antibody titers against Newcastle disease (ND) for all treatment groups were significantly different ($p < 0.05$) at 14 weeks of age (four weeks post initiation of milk thistle extract supplementation) (Table 7). The highest mean titers were observed in birds of group G4 (2000 mg.kg extract), whilst the intermediate values pertained to those from group G3 (1000 mg.kg). Group G2 (500 mg.kg); had the lowest mean titers followed by control group (G1) values.
Moreover, at the age of 18 weeks (8 weeks after the initiation of supplementation), significant differences ($p < 0.05$) were still observed among the groups as shown in Table 8. The high mean ND antibody titers of G4 again statistically outperformed all other groups. G3 and G2 titers were intermediate between control group G1. Remarkably, the mean ND antibody titers were higher at 18 weeks of age in all groups compared to their own values at 14 weeks of age.

Table (7): Differences in Antibody NDV titer among vaccinated chicken groups at 14 weeks of ages.

Experimental group	N	Minimum NDV titer	Maximum NDV titer	Mean ± SE Antibody NDV titer	ANOVA P Value
G1	5	1.22	7.54	4.2274 ± 1.19473 a	0.164862
G2	5	1.82	15.65	6.9452± 2.56474 b	
G3	5	5.35	13.13	8.3938 ± 1.34038 c	
G4	5	4.43	15.78	10.3360± 1.97250 d	

[a,b,c] Means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means, P value: probability level.

Table (8): Differences in Antibody NDV titer among vaccinated chicken groups at 18 weeks of ages.

Experimental group	N	Minimum NDV titer	Maximum NDV titer	Mean ± SE Antibody NDV titer	ANOVA P Value
G1	5	2.87	19.61	12.3150 ± 3.46101 a	0.007*
G2	5	1.88	19.15	13.5798 ± 3.43725 b	
G3	5	2.67	19.15	14.5168 ± 3.09099 c	
G4	5	16.62	20.37	19.0240 ± .66828 d	

[a,b,c] means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means, P value: probability level.

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DISCUSSION:

The phytochemical screening for Milk thistle extract showed that the total polyphenols content and antioxidant activity. In this respect, Kim *et al.*, (2011) reported that phenolic substances have been shown to be responsible for the antioxidant activity of plant materials. In addition, higher antioxidant activity has been positively correlated with the concentration of phenolic compounds in extracts, these agreed with the current data of oral administration of *Silybum marianum* (milk thistle) extract at dietary concentrations of 500, 1000 and 2000mg/kg for a period of 8 weeks was found to significantly improve hepatic biomarkers (serum AST and ALT), as well as serum glucose, triglyceride and total cholesterol levels in pre-laying hens fed under summer heat stress conditions.

The Temperature-Humidity Index (THI) was computed in the present study in order to assess heat stress exposure. The results showed that during August, the chickens reached a level of danger from heat stress and emergency levels were reached in the remaining experimental period. Specifically, the total THI values for August (86), September (85), and October (84) based on Weather Safety Index (LCI, 1970). These findings are largely consistent with the observations of North and Bell (1990), who indicated that optimum temperature for laying hens is around 20°C, while heat stress begins at ambient temperatures above about 25 °C and manifests clearly when temperatures surpassing 30 °C (Konca *et al.*, 2009).
Thermal stress leads to oxidative damage to the intestinal epithelium and modifies gut microbiota population/structure, leading to increased permeability of the intestine, and higher susceptibility to infection/inflammation (Cao et al., 2021).
Silymarin, the primary bioactive constituent accountable for candidates' main effect of action, demonstrates hepatoprotective activity and is marketed to reduce multiple hepatic diseases (Tighe *et al.*, 2020). It has been well-established by many studies that silymarin possesses potent antioxidant properties and inhibits lipid peroxidation in liver toxicity model induced by various agents (Abd Eldaim *et al.*, 2021).
Nutritional status in bird species is not only determined by feed consumption but also affected by liver function and metabolic pathways (Elnesr *et al.*, 2023). The liver is a multifunctional organ that has important physiological roles in poultry (Attia *et al.*, 2019).
According to one study from Italy, a daily 240mg silymarin/silybin dose was associated with significant reductions in serum liver enzyme levels (ALT, AST, GGT and ALP), which indicate hepatic injury/stress biomarkers (Faryadi *et al.*, 2021).
The work of Tavakolinasab *et al.*, (2020) showed that incorporating 250 mg/kg of milk thistle extract into broiler feed significantly reduced ($p < 0.05$) glucose, AST, and ALT concentrations relative to the control group. These outcomes are consistent with those of the present study, in which dietary supplementation with milk thistle extract across all treated groups, particularly at the 2 gm/kg level, lowered serum ALT and AST levels at both 14 and 18 weeks of age. This consistency extends to earlier investigations reporting reductions in serum ALT and AST in broilers exposed to mycotoxins (Armanini et al., 2021) and in experimental models of chemically induced liver damage (Baradaran *et al.*, 2019).
Excessive lipid accumulation within hepatocytes can induce structural changes in the liver. Serum triglyceride (TG) and total cholesterol (TC) levels are important indicators of an animal's lipid metabolic capacity. In the present experiment, treatment with milk thistle (MTh) extract significantly decreased serum TG and TC concentrations. These results are in agreement with the findings of Ghazaghi et al (2024), who

observed a significant reduction in serum TC in quails treated with silymarin, as well as with a recent study reporting decreased serum TC and LDL-C in broilers (Zhou *et al.*, 2022). Earlier research (Zaker *et al.*, 2021) suggests that silymarin may enhance production performance in late-stage laying hens by mitigating hepatic steatosis and improving the synthesis of lipoproteins and yolk precursors. In that context, a silymarin dose of 500 mg/kg was most effective in regulating lipid metabolism in those hens.

Khatami *et al.*, (2023) reported a very-low-density lipoprotein (VLDL) value in *Silybum marianum* treated and control broiler chicks values significantly decreased, which confirms the significant effect upon total cholesterol concentration observed in this study. VLDL is the bad cholesterol when we talk about cholesterol. The circulating cell population is altered by heat stress (Goel, 2021).

In addition, the increased numbers of RBCs, WBCs, and percentage of lymphocytes as well as TAC shown in this study demonstrated that MTh supplementation in diet had a beneficial immunological effect on poultry raised under heat stress during summer.

The current data of higher erythrocytic indices in MTh extract supplemented pre-laying hens are comparable to those reported previously by El-Damrawy *et al.*, (2023) reported that the *Silybum marianum* could mitigate aflatoxin B1-sustained effects in broiler chickens, tended to increase red blood cell concentrations. That finding implies that S. marianum encourages the process of erythropoiesis, or red blood cell production.

The main pathways through which the body’s immune response can be regulated are mediated through the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenal medulla (SAM) axis (Goel, 2021). Both axes' neuroendocrine products, particularly cortisol and catecholamines, have documented receptors on monocytes, lymphocytes, and granulocytes that can affect proliferation, cytokine production, cell trafficking, cytolytic activity and antibody synthesis (Bohler *et al.*, 2021).

It has been demonstrated that broilers subjected to heat stress exhibit decreased concentration of circulating free antibodies, and decreased levels of IgM and specific IgG to IL-6 together with lesser general and humoral reactivity (Van Goor *et al.*, 2017). They were also noticed sharply reduced weights of bursa, thymus, liver and spleen.

Due to lower amounts of circulating lymphocytes and higher concentrations of heterophils, heat stress induces a marked increase in the heterophil-to-lymphocyte (H/L) ratio, an index of chronic stress. Hence, during summer in tropical environment, communicable and infectious poultry diseases like Newcastle disease and infectious bursal disease become more common (Saelao *et al.*, 2021).

According to Wang *et al.*, (2020), The active compounds of *Silybum marianum* have anti-inflammatory and antioxidant effects, which can eventually lead to the improvement of gut microbiota and intestinal function integrity (Valková *et al.*, 2020).

Furthermore, Ahmad et al. (2020) reported that supplementation of dried milk thistle seeds at doses of 10g.kg and 15g.kg improved ($p \leq 0.05$) antibody titers against Newcastle disease. This finding correlates with the apparent major influence on Newcastle disease vaccination antibody titers documented in this study.

In summary, the supplementation of milk thistle extract has beneficial effects on pre-laying hens and can increase poultry profitability based on individual farms. The economic return provided with groups supplemented with milk thistle extract may be higher than that of other groups.

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CONCLUSIONS:

Oral administration of *Silybum marianum* (milk thistle) extract at dietary concentrations of 500, 1000 and 2000mg/kg for a period of 8 weeks was found to significantly improve hepatic biomarkers (serum aminotransferase such as aspartate and alanine aminotransferase), as well as serum glucose, triglyceride and total cholesterol levels in pre-laying hens fed under summer heat stress conditions when monitored at the two periods of age: first at fourteen weeks old then again an hour later eighteen weeks old. In addition, the extract increased cell-mediated and humoral immune responses. These findings indicate that milk thistle extract have properties of hepatoprotective, antioxidative and immunomodulatory. Based on the dose-dependent effects and/or 2gm/kg feed supplementation is recommended. Since *Silybum marianum* is a worldwide plant that grows well without special care, it can be considered as an effective natural feed additive that may replace synthetic feed additives while reducing the risk of health problems in both animals and consumers.

Recommendations:

Further research is warranted to determine optimal dosing strategies and evaluate long-term effects on poultry performance and health.

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Conflict of Interest:

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The authors were solely responsible for the design, sample collection, laboratory experiments, data analysis, and manuscript preparation of this study.

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