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# Cardioprotective and Hepatoprotective Activity of Silymarin in Broiler Chickens Fed on Mash and Pellet Diets

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### **Abstract**

**BACKGROUND:** The liver and heart are two main damaged organs in ascites syndrome in fast-growing broilers. Using silymarin with a protective effect on the liver and heart may be a beneficial strategy to decrease ascites-induced mortality.

**OBJECTIVES:** The present study assessed the cardiohepatic effects of silymarin in broilers fed on mash and pellet diets by assessing electrocardiographic (ECG) indices and some serum biochemical parameters.

**METHODS:** A total of 120 *Arbor Acres* chicks were allocated to 6 groups and treated as follows: basal mash diet (CM); basal pellet diet (CP); silymarin at 500 ppm of mash (M500) and pellet diets (P500); and silymarin at 2500 ppm of mash (M2500) and pellet diets (P2500).

**RESULTS:** CP had higher serum activities of aspartate aminotransferase, alanine aminotransferase, and creatine kinase MB (CK-MB) enzymes compared to CM (P<0.05). P2500 had a higher total protein and lower aspartate aminotransferase, alanine aminotransferase, and CK-MB compared to CP (P<0.05). T-duration, ST-segment, and R-R intervals were longer in CP compared to CM and were shorter in P2500 than in CP and P500 (P<0.05).

**CONCLUSIONS:** The pellet diet led to changes in some biochemical and ECG indices in broilers, and silymarin at the 2500 ppm dose can be used as a hepatoprotective and cardioprotective compound to modulate cardiohepatic failure in susceptible broilers.

**KEYWORDS:** Cardiac evaluation, Hepatic evaluation, Mash diet, Pellet diet, Silymarin

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# Introduction

The genetic selection has led to a fast growth rate in modern strains of broiler chickens (Ahmadipour *et al.*, 2015). In fast-growing broilers, the metabolic rate and the requirement of oxygen are increased, and the heart and lungs are not capable of providing enough oxygen, which causes metabolic disorders (Wideman *et al.*, 2013). Right ventricular failure (RVF) and ascites syndrome are the most prominent metabolic disorders, commonly seen in broiler chickens with a high-growth rate (Ahmadipour *et al.*, 2019).

Diet form (pellet, mash, and crumble) is one of the most important effective factors on the incidence of RVF and ascites in broilers (Sahraei, 2014). Broilers fed on the pellet diet present higher weight gain and better feed conversion ratio compared with those fed the mash diet (Amerah et al., 2008). Several studies have stated that susceptibility to RVF and ascites was higher in birds fed pelleted diets than in birds received mash diets (Jafarnejad et al., 2010; Sahraei, 2014). The heart and liver are two major damaged organs in RVF and ascites syndrome (Jafarnejad et al., 2010). Improving the health of the heart and liver using herbal extracts is a benefit and can be a clinical strategy to control RVF lesions and decrease RVF-induced mortality. Milk thistle is one of the more common medicinal herbs with protective effects on the liver and heart.

Milk thistle (Silybum marianum L., Asteraceae) is one of the most common herbs for the treatment of liver diseases (Schrieber et al., 2008). The seed and fruit of milk thistle contain silymarin that shows a variety of pharmacological activities, as well as antioxidant, anti-inflammatory, antibacterial, and antiviral properties in many experimental and clinical studies (Koçarslan et al., 2016).

Some studies have evaluated the positive effects of silymarin extract on growth performance and hepatic disorders in broilers

(Schiavone *et al.*, 2007; Kralik *et al.*, 2015; Hosseinian *et al.*, 2020). Further, various researches have established that silymarin had cardioprotective activity due to its antioxidant content in laboratory animals (Rao & Viswanath, 2007; Al-Rasheed *et al.*, 2014; Koçarslan *et al.*, 2016). However, there is a lack of knowledge about the positive effects of silymarin on broiler's heart health. Evaluating heart electrical activity by electrocardiogram (ECG) is a simple method to assess heart health in birds (Yogeshpriya *et al.*, 2018).

In veterinary medicine, there are several diagnostic tools for the evaluation of cardiac abnormalities, but ECG is one of the most useful and non-invasive techniques, which gives significant information about heart rate (HR), cardiac arrhythmias, and electrical conductance abnormalities (Cushing *et al.*, 2013; Yogeshpriya *et al.*, 2018).

In avian medicine, ECG is a useful tool utilized to measure HR and detect cardiac arrhythmias and cardiac chamber enlargement (Sharifi et al., 2015; Hosseinian et al., 2019). Several studies have indicated that RVF and ascites syndrome induce a change in the morphology of ECG waves in broilers, and they used ECG to detect and diagnosis the ascites syndrome (Hassanpour et al., 2009; Yousefi et al., 2013). In this study, broilers were fed with two different forms of diet (mash and pellet), and heart and liver health was evaluated by assessing some serum biochemical and ECG induces. Also, the effects of two different doses of dietary silymarin (500 and 2500 ppm) were assessed on some serum parameters and ECG. In this research, this hypothesis was assessed that pellet diet can be led to heart and liver dysfunction in broilers, and silymarin could attenuate the cardiac and hepatic damages.

The results of the present study may clarify the protective effects of silymarin on the liver and heart in fast-growing broilers, and silymarin can be used as a cardioprotective and hepatoprotective compound during the rearing period of susceptible broilers to ascites syndrome.

# **Materials and Methods**

# **Birds and Treatment Groups**

This experiment was performed at the Veterinary College of Shiraz University. A total of 120 1-day-old *Arbor Acres* broiler chicks were used for the experiment and reared for 42 days. During study, broilers were raised in environmentally controlled rooms under standard environmental conditions suggested by commercial recommendations of the chick producer company (Aviagen, 2018).

On day 14 of the experiment, birds were weighed and randomly assigned into 6 equal groups (n=20) with five replicates. The treatment groups consisted of supplementation with silymarin extract in the mash and pelleted diets as follows: the control mash group (basal mash

diet [CM], fed on a mash diet without silymarin), control pellet (basal pellet diet [CP], fed on a pellet diet without silymarin), two doses of silymarin in mash feed (silymarin at 500 ppm of mash [M500] and silymarin at 2500 ppm of mash [M2500]), and two doses of silymarin in pellet feed (silymarin at 500 ppm of pellet [P500] and silymarin at 2500 ppm of pellet [P2500]).

In the present study, the basal diet was cornsoybean meal-based and formulated to meet or exceed the minimum National Research Council (NRC; 1994) Standards. The starter (0-10 days), grower (10-25 days), and finisher (25-42 days) feeds were used during the experiment. All birds were fed on a pellet diet during the first and second weeks of the experiment, and then two forms of diet (mash and pellet) with a similar composition of ingredients (see <u>Table 1</u>) were used from the third to sixth weeks of the experiment.

Table 1. Ingredients and composition of mixture feed (gkg<sup>-1</sup>)

Component		Diets	
	Starter	Grower	Finisher
Ingredients, %			
Yellow Corn	53.4	58.0	61.3
Soybean meal (44%)	39.0	35.0	31.0
Dicalcium phosphate	1.7	1.5	1.3
CaCO3 (38%)	1.7	1.4	1.3
Sunflower oil	3.0	3.0	4.0
Sodium chloride	0.3	0.3	0.3
Methionine	0.2	0.15	0.15
Lysine	0.2	0.15	0.15
Premix*	0.5	0.5	0.5
Total	100	100	100
Nutrient levels			
ME, kcal/kg	2900	3000	3100
Crude protein, %	22	20.5	19

Component		Diets	
Calcium, %	1.05	0.9	0.8
Available Phosphorus, %	0.5	0.45	0.4
Sodium, %	0.19	0.19	0.19
Lysine, %	1.31	1.25	1.14
Methionine, %	0.54	0.48	0.44
Threonine, %	0.85	0.81	0.75
Tryptophan, %	0.25	0.22	0.2

<sup>\*</sup> Vitamin and mineral content per kilogram of premix: vitamin A: 3,600,000 IU; vitamin D3: 800,000 IU; vitamin E: 7,200 IU; vitamin K3: 0.8 g; vitamin B1: 0.71 g; vitamin B2: 2.64 g; vitamin B3: 3.92 g, vitamin B5: 11.88 g; vitamin B6: 1.176 g; vitamin B12: 6 mg; folic acid: 0.4 g; biotin: 40 mg; choline chloride: 100 g; selenium: 80 mg; cobalt: 100 mg; iodine: 396 mg; copper:4 g; zinc: 33.88 g; iron: 20 g; manganese: 39.68 g.

The used diets in this study was prepared in the livestock affairs of the Faculty of Veterinary Medicine, Shiraz University. Firstly, the feed was supplied based on NRC Standards for broilers. Then, a part of the feed was finely grounded, mixed, and used as a mash form in mash treatments during the entire the experiment, and the other part of the feed was pelleted in a steam pellet mill. After pelleting, the pelleted feed was dried and cooled to an average temperature of 37°C and used as a pellet form in pellet treatments during the entire the experiment. Feed and freshwater were supplied *ad libitum* during the entire experiment.

Silymarin powder was purchased from BarijEsans Company (Iran), containing 43.87% of silibinin and 96.76% dry matter and was added to mash and pellet diets from days 14 to 42 of the experiment. All diets contained neither anticoccidials nor any other medications.

The temperature was maintained at 32°C during the first week and reduced gradually until a constant temperature of 24°C was achieved. A 24-hour lighting schedule was used during the first week and then reduced gradually until 20 h light: 4 h darkness (20L:4D).

# **Blood Sampling**

Ten apparently healthy birds of each group were selected, and blood sampling (5 mL/birds) was done from the brachial vein on days 21, 28, 35, and 42. Blood samples were kept at room temperature for 30 minutes, and then the clotted blood samples were centrifuged at 3000g for 10 minutes to separate sera. The clear sera were collected and stored at -20°C until the biochemical analysis.

# **Evaluating Hepatic Parameters**

The hepatic parameters, including total protein, albumin, globulin, uric acid, triglyceride, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), were measured by an automated analyzer (Alpha Classic, Sanjesh Company, Iran) using commercial clinical investigation kits (Pars Azmoon, Tehran, Iran). Moreover, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were evaluated using a commercial kit (Pars Azmoon, Tehran, Iran).

## **Evaluating Cardiac Parameters**

Biochemical Assays

Calcium, magnesium, phosphorous, chloride, and creatine kinase MB (CK-MB) were measured using commercial kits (Pars Azmoon,

Iran) and biochemical auto analyzer (Alpha Classic, Sanjesh Company, Iran). Sodium and potassium were determined using a flame photometer (Clinical flame photometer, Fater Company, Iran).

# **Electrocardiographic Studies**

From each group, 7 apparently healthy chicks were randomly selected on 21, 28, 35, and 42 days, and ECGs were recorded by a single channel ECG machine (Kenz-line EKG 110, Suzuken Company, Japan). The ECG measurements were obtained from unanesthetized and restrained birds in a standing position. Before ECG recording, the chickens rested for about 5 minutes to calm down. ECG gel was applied to the skin, and then alligator clip electrodes were positioned at the base of the right and left wings and gastrocnemius muscle of the right and left limbs (Yogeshpriya *et al.*, 2018).

All ECGs were recorded using a calibration of 10 mm/mV and a paper speed of 50 mm/sec (Reddy *et al.*, 2016). ECGs were recorded by different leads, including I, II, III, and aVR, aVL, and aVF for every chicken. In birds, lead II is commonly used to evaluate waves' morphology in ECG (Reddy *et al.*, 2016). For this reason, the amplitude and duration of P, T, R, and S waves, as well as the duration of QT, R-

R, PR, and ST intervals, in each bird of the present experiment were measured from lead II as a standard lead (Reddy *et al.*, 2016). Finally, HR was measured in each bird.

# Statistical Analysis

The statistical analysis was performed using SPSS 22 (SPSS Inc., Chicago, Ill., USA). In the present study, the data from various serum parameters and ECG indices were analyzed by 1-way analysis of variance (ANOVA). The data were presented as mean  $\pm$  SE. The significance level was set as P-value<0.05.

#### Results

The results of serum hepatic parameters are presented in <u>Tables 2</u> and <u>3</u>.

As seen in <u>Table 2</u>, total protein and albumin significantly decreased in CP compared with CM at some times of the experiment (P<0.05). P500 and P2500 significantly had a higher total protein compared with CP on days 28, 35, and 42. Circulating uric acid increased significantly in CP and P500 compared with CM. In this experiment, P2500 had a lower level of uric acid compared to CP and P500. The serum activity of ALT and AST enzymes in the CP group was significantly higher than in CM, and the activity levels of ALT and AST decreased significantly in P2500 compared to CP on day 42 (P<0.05) (<u>Table 2</u>).

**Table 2.** Circulating hepatic parameters (mean±SE) of broiler chickens (n=20) following dietary supplementation of pellet and mash diets by silymarin extract.

D	D	Groups (mean ±SE)					
Parameters	Days -	CM	M500	M2500	CP	P500	P2500
	21	2.43±0.071 <sup>a</sup>	2.92±0.022ª	2.13±0.040a	2.80±0.041ª	3.12±0.080 <sup>b</sup>	2.72±0.101 <sup>a</sup>
Total protein	28	3.47±0.092ª	3.35±0.073ª	2.89±0.033b	2.25±0.072b	3.46±0.052ª	3.70±0.081 <sup>a</sup>
(gr/L)	35	2.80±0.051ª	2.94±0.072a	2.94±0.022ª	2.59±0.074a	3.09±0.084b	3.01±0.017 <sup>b</sup>
	42	3.72±0.091ª	3.98±0.020a	3.87±0.080a	2.28±0.038b	3.36±0.063ª	3.92±0.043a
	21	1.37±0.031ª	1.34±0.040a	1.21±0.062a	1.42±0.035a	1.52±0.023a	1.42±0.044a
Albumin (gr/L)	28	1.40±0.064ª	1.46±0.016 <sup>a</sup>	1.39±0.074a	1.57±0.051ª	1.19±0.065ª	1.42±0.065a
(8 : -)	35	1.37±0.087a	1.38±0.055a	1.39±0.020a	1.22±0.050a	1.28±0.066ª	1.32±0.022ª

Parameters	D	Groups (mean ±SE)					
	Days	CM	M500	M2500	СР	P500	P2500
	42	2.43±0.034 <sup>a</sup>	2.47±0.054a	2.44±0.020a	1.26±0.120b	1.36±0.074 <sup>b</sup>	1.92±0.060 <sup>b</sup>
	21	1.08±0.170a	1.54±0.068a	0.97±0.114a	1.39±0.081a	1.68±0.123 <sup>a</sup>	1.37±0.205 <sup>a</sup>
Globulin	28	1.42±0.080a	1.74±0.125ª	1.48±0.065a	1.68±0.112ª	1.05±0.115ª	1.60±0.144 <sup>a</sup>
(gr/L)	35	1.53±0.111 <sup>a</sup>	1.61±0.164a	1.25±0.176 <sup>a</sup>	1.69±0.142a	1.63±0.121ª	1.68±0.135 <sup>a</sup>
	42	1.27±0.201 <sup>a</sup>	1.58±0.097 <sup>a</sup>	1.36±0.078 <sup>a</sup>	1.40±0.163 <sup>a</sup>	1.73±0.130 <sup>a</sup>	1.39±0.084 <sup>a</sup>
	21	2.83±0.225 <sup>a</sup>	3.58±0.189b	2.85±0.197 <sup>a</sup>	3.23±0.305 <sup>b</sup>	4.60±0.404°	3.98±0.513 <sup>b</sup>
Uric acid	28	3.22±0.297 <sup>a</sup>	3.95±0.348a	3.85±0.135a	4.51±0.434 <sup>b</sup>	2.71±0.175°	3.80±0.305 <sup>a</sup>
(mg/dL)	35	3.94±0.271a	3.92±0.313a	4.09±0.491a	3.72±0.300a	4.55±0.424b	3.71±0.294 <sup>a</sup>
	42	3.90±0.519a	4.21±0.190b	3.28±0.294a	4.26±0.455b	4.08±0.313b	3.20±0.315 <sup>a</sup>
	21	126.84±9.110 <sup>a</sup>	122.60±9.158a	99.45±3.780 <sup>b</sup>	125.39±8.937 <sup>a</sup>	121.44±4.844ª	128.55±4.385a
Alanine aminotransferase	28	127.88±7.910 <sup>a</sup>	129.29±7.856 <sup>a</sup>	110.88±6.125 <sup>b</sup>	132.79±3.809ª	129.98±4.496ª	125.01±6.147 <sup>a</sup>
(IU/L)	35	128.04±9.747a	124.55±6.144a	97.58±3.751 <sup>b</sup>	132.88±6.568a	119.57±4.728a	109.29±6.446ª
	42	97.88±5.887 <sup>a</sup>	112.45±6.783 <sup>b</sup>	109.11±5.360 <sup>b</sup>	128.19±4.018 <sup>b</sup>	130.01±3.950 <sup>b</sup>	95.48±5.010 <sup>a</sup>
	21	253.70±10.317 <sup>a</sup>	245.60±10.958a	199.90±7.888 <sup>b</sup>	258.30±8.178ª	242.90±9.981ª	248.30±6.480ª
Aspartate	28	252.80±8.015 <sup>a</sup>	257.20±8.154ª	240.00±12.125 <sup>b</sup>	265.70±6.906ª	259.90±7.410 <sup>a</sup>	249.70±10.744a
aminotransferase (IU/L)	35	240.30±10.846a	242.40±11.541ª	193.20±6.785 <sup>b</sup>	263.50±11.765 <sup>a</sup>	229.70±7.027 <sup>a</sup>	203.20±12.033 <sup>b</sup>
	42	199.50±6.812a	219.50±7.683b	207.90±8.664a	241.30±6.112 <sup>b</sup>	247.90±8.255 <sup>b</sup>	198.60±9.050°a

CM: control mash diet (0 ppm silymarin); CP: control pellet diet (0 ppm silymarin); M500: mash diet + 500 ppm silymarin; M2500: mash diet + 2500 ppm silymarin; P500: pellet diet + 500 ppm silymarin; P2500: pellet diet + 2500 ppm silymarin; a,b,c Different letters in the superscripts of the same row indicate significant differences (P < 0.05).

As seen in <u>Table 3</u>, the serum levels of total triglyceride, cholesterol, and LDL in supplemented groups (i.e., M500, M2500,

P500, and P2500) decreased significantly compared to CM and CP groups (*P*<0.05). The CP group had lower concentrations of VLDL and LDL compared to the CM group.

**Table 3** Circulating hepatic indices (mean±SE) of broiler chickens (n=20) following dietary supplementation of pellet and mash diets by silymarin extract.

Parameters	Days	Groups (mean ±SE)						
		CM	M500	M2500	СР	P500	P2500	
	21	85.70±1.820 <sup>a</sup>	87.70±3.285 <sup>a</sup>	62.80±1.655 <sup>b</sup>	83.30±3.850a	77.50±1.22 <sup>b</sup>	63.00±2.35 <sup>b</sup>	
Triglycerid	28	85.70±3.180 <sup>a</sup>	71.50±1.980 <sup>b</sup>	79.00±3.996 <sup>b</sup>	72.50±1.544 <sup>b</sup>	55.80±1.96°	66.90±1.49b	
e (mg/dL)	35	76.40±1.431 <sup>a</sup>	64.70±1.321 <sup>b</sup>	66.20±2.027 <sup>b</sup>	84.90±1.242°	65.40±1.65 <sup>b</sup>	63.40±3.91 <sup>b</sup>	
	42	83.80±1.262 <sup>a</sup>	73.50±1.825 <sup>b</sup>	62.40±3.221°	89.10±2.19 <sup>a</sup>	72.50±1.65 <sup>b</sup>	60.40±3.91°	

<b>D</b>	D.	Groups (mean ±SE)						
Parameters	Days	CM	M500	M2500	СР	P500	P2500	
	21	148.50±2.39a	100.80±6.32 <sup>b</sup>	98.50±2.551 <sup>b</sup>	145.60±2.16a	101.50±2.54 <sup>b</sup>	103.20±3.1 <sup>b</sup>	
Cholesterol	28	148.50±2.71ª	122.80±2.51 <sup>b</sup>	122.40±2.03 <sup>b</sup>	144.30±3.74a	96.10±2.640°	122.50±3.8 <sup>b</sup>	
(mg/dL)	35	139.40±2.64ª	110.70±3.48 <sup>b</sup>	107.60±4.62 <sup>b</sup>	139.50±3.09ª	100.70±3.49 <sup>b</sup>	97.20±3.87 <sup>b</sup>	
	42	135.20±1.24 <sup>a</sup>	116.00±2.13 <sup>b</sup>	105.40±2.24°	143.20±3.20 <sup>a</sup>	113.90±1.71 <sup>b</sup>	93.30±2.58°	
	21	70.60±1.07a	78.20±0.284a	71.10±1.425a	70.90±0.704 <sup>a</sup>	71.70±1.094a	74.20±0.520	
HDL	28	70.90±0.92ª	72.60±0.921ª	76.00±0.285a	71.40±1.175 <sup>a</sup>	70.30±0.975a	77.30±0.634	
(mg/dL)	35	75.30±0.25 <sup>a</sup>	70.30±0.908 <sup>a</sup>	70.80±1.034 <sup>a</sup>	75.00±0.870 <sup>a</sup>	76.00±0.891ª	70.90±0.910°	
	42	67.10±1.09a	74.20±0.169a	71.30±0.363ª	70.00±1.954 <sup>a</sup>	73.10±1.208 <sup>a</sup>	71.40±1.097	
	21	93.00±2.23ª	58.70±2.41 <sup>b</sup>	54.80±1.27 <sup>b</sup>	81.20±1.944°	55.90±1.50 <sup>b</sup>	51.00±1.74 <sup>b</sup>	
LDL	28	85.60±0.64a	54.00±1.38 <sup>b</sup>	59.60±1.34 <sup>b</sup>	77.70±1.485 <sup>a</sup>	53.40±0.21 <sup>b</sup>	60.50±1.47 <sup>b</sup>	
(mg/dL)	35	89.80±0.45ª	53.20±2.43 <sup>b</sup>	49.50±2.20 <sup>b</sup>	71.70±1.853°	56.70±1.79 <sup>b</sup>	52.70±1.30 <sup>b</sup>	
	42	91.50±1.40a	55.80±2.187°	49.90±0.372°	75.60±1.05 <sup>b</sup>	50.50±1.660°	57.70±1.950	
	21	17.06±0.43ª	18.13±0.588a	12.54±0.638°	15.52±0.95 <sup>b</sup>	15.68±0.25 <sup>b</sup>	12.65±0.594	
VLDL	28	17.19±0.67ª	14.29±0.18 <sup>b</sup>	16.58±0.780 <sup>a</sup>	14.99±0.36 <sup>b</sup>	11.05±0.966°	13.28±0.49 <sup>b</sup>	
(mg/dL)	35	15.24±0.48a	12.98±0.52b	13.24±0.45 <sup>b</sup>	16.90±0.818a	13.80±0.95 <sup>b</sup>	12.65±0.73 <sup>b</sup>	
	42	16.80±0.36a	14.72±0.37 <sup>b</sup>	12.40±0.676°	17.80±0.890a	14.54±0.190 <sup>b</sup>	12.31±0.760°	
	21	0.46±0.024°	0.51±0.014b,c	0.42±0.011a,c	0.50±0.074b	0.55±0.104b	0.48±0.081a,q	
Total	28	0.48±0.078 <sup>b</sup>	0.51±0.076 <sup>a,b</sup>	0.51±0.050a,b	0.56±0.090a	0.44±0.054°	0.49±0.041 <sup>b,c</sup>	
billirubin (mg/dL)	35	0.51±0.017 <sup>a</sup>	0.51±0.074 <sup>a</sup>	0.50±0.104 <sup>a</sup>	0.50±0.012a	0.54±0.031a	0.50±0.032a	
	42	0.46±0.019a	0.58±0.020b	0.50±0.024a	0.51±0.090a,b	0.53±0.080a,b	0.49±0.071a,l	
	21	0.03±0.011 <sup>a,b</sup>	0.02±0.003 <sup>b</sup>	0.03±0.011 <sup>a,b</sup>	0.03±0.004 <sup>a,b</sup>	0.02±0.006 <sup>a,b</sup>	0.04±0.001ª	
Direct	28	0.04±0.020a	0.04±0.003ª	0.03±0.021a	0.03±0.007a	0.03±0.003a	0.03±0.002a	
billirubin (mg/dL)	35	0.03±0.000a	0.04±0.001a	0.03±0.002ª	0.03±0.005a	0.04±0.004a	0.04±0.003ª	
	42	0.04±0.002ª	0.03±0.011ª	0.03±0.004 <sup>a</sup>	0.03±0.004 <sup>a</sup>	0.03±0.003a	0.03±0.001a	
-								

CM: control mash diet (0 ppm silymarin); CP: control pellet diet (0 ppm silymarin); M500: mash diet + 500 ppm silymarin; M2500: mash diet + 2500 ppm silymarin; P500: pellet diet + 500 ppm silymarin; P2500: pellet diet + 2500 ppm silymarin; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; VLDL: very low-density lipoprotein cholesterol; Different letters in the superscripts of the same row indicate significant differences (P < 0.05).

### **Serum Cardiac Parameter Analysis**

The results of serum cardiac parameters are presented in <u>Table 4</u>.

The circulating levels of calcium and phosphorus were significantly lower in the CP group than in other groups on 28, 35, and 42 days (P<0.05). P500 and P2500 had significantly

higher concentrations of calcium and phosphorus compared with CP (P<0.05). CP had a higher activity of CK enzyme compared with

CM on day 42, and P500 and P2500 had a lower serum activity of CK compared with CP (P<0.05) (Table 4).

**Table 4** Circulating electrolytes and cardiac parameters (mean±SE) of broiler chickens (n=20) following dietary supplementation of pellet and mash diets by silymarin extract.

		Groups (mean ±SE )							
Parameters	Days	CM	M500			D500	D2500		
		CM	M500	M2500	СР	P500	P2500		
	21	5.71±0.180a	5.09±0.354a	3.99±0.354 <sup>b</sup>	5.28±0.091 <sup>a</sup>	6.66±1.771°	5.7±0.721a		
Calcium	28	7.28±0.020a	7.39±0.092a	6.94±0.101ª	5.45±0.011 <sup>b</sup>	6.88±0.082a	7.09±0.062a		
( mg/dL)	35	6.31±0.116 <sup>a</sup>	6.03±0.093 <sup>a</sup>	6.57±0.230a	4.28±0.081 <sup>b</sup>	6.36±0.482a	6.32±0.240 <sup>a</sup>		
	42	$7.79{\pm}0.064^a$	$7.79\pm0.151^a$	$7.05\pm0.192^a$	$5.14\pm0.060^{b}$	$7.22 \pm 0.033^a$	$7.84 \pm 0.120^a$		
	21	4.90±0.351 <sup>a</sup>	$4.79{\pm}0.350^{a}$	4.56±0.231a	$5.15{\pm}0.304^a$	$5.23{\pm}0.304^a$	4.79±0.323a		
Phosphorus	28	$6.09\pm0.101^{a}$	5.32±0.111a	5.63±0.122a	$4.63\pm0.151^{b}$	$4.61{\pm}0.082^{b}$	5.80±0.062a		
(mg/dL)	35	$5.59\pm0.102^{a}$	$5.35{\pm}0.082^a$	5.77±0.113a	$3.17 \pm 0.052^{b}$	$5.05{\pm}0.044^{a}$	5.25±0.060a		
	42	$6.72 \pm 1.130^{a}$	5.99±0.203 <sup>a</sup>	5.12±0.314 <sup>b</sup>	$4.78{\pm}0.343^{\rm b}$	$5.67 \pm 0.332^a$	6.89±0.330a		
	21	125.53±3.880 <sup>a</sup>	122.33±5.540 <sup>a</sup>	129.76±5.34a	119.98±8.981ª	111.28±3.990a	113.98±6.184a		
Sodium	28	130.45±4.431a	129.87±7.011ª	138.65±9.89a	112.23±6.941 <sup>b</sup>	119.63±5.844 <sup>b</sup>	115.23±6.44 <sup>b</sup>		
(mmol/L)	35	132.98±5.211ª	132.13±8.902ª	128.51±7.09a	119.98±7.862 <sup>b</sup>	129.47±7.012a	142.78±6.961°		
	42	$140.89{\pm}10.09^a$	132.44±5.985a	130.87±9.89a	123.67±9.321 <sup>b</sup>	119.17±8.330 <sup>b</sup>	143.97±7.920a		
	21	$4.87{\pm}1.06^{a}$	4.56±2.032a	5.01±1.234 <sup>a</sup>	4.88±0.985a	4.77±1.094a	4.61±0.285a		
Potassium	28	4.78±0.984ª	3.99±1.014 <sup>a</sup>	4.66±2.135a	4.35±0.691ª	4.98±1.971a	4.09±0.325a		
(mg/dL)	35	$4.90{\pm}0.214^{a}$	4.52±1.124a	4.87±3.095a	$5.01 \pm 0.274^a$	$4.81{\pm}0.542^{a}$	4.62±0.477a		
	42	$4.98{\pm}0.792^{a}$	5.06±3.095a	4.72±2.017 <sup>a</sup>	$4.55{\pm}0.936^a$	$4.76{\pm}0.437^{a}$	4.72±0.732a		
	21	1.88±0.091a	$2.15{\pm}0.067^{a}$	1.84±0.065a	2.02±0.071a	$2.25{\pm}0.134^{a}$	2.06±0.134a		
Magnesium	28	$2.07{\pm}0.084^{a}$	$2.31{\pm}0.085^{a}$	$2.22 \pm 0.046^a$	$2.23{\pm}0.060^{a}$	$1.85{\pm}0.062^a$	2.29±0.085a		
(mg/dL)	35	$2.41\pm0.072^{a}$	$2.38{\pm}0.076^a$	2.40±0.057a	2.26±0.074a	2.22±0.045a	2.39±0.066a		
	42	2.52±0.033a	$2.55{\pm}0.044^a$	2.62±0.070a	$2.16\pm0.052^{a}$	$2.29{\pm}0.080^{a}$	2.54±0.070 <sup>a</sup>		
	21	105.30±0.225a	103.90±0.455a	107.50±0.445a	105.70±0.512a	107.50±0.664a	106.80±0.53ª		
Chloride	28	106.70±0.650a	$108.70\pm0.346^a$	105.80±0.323a	$104.60\pm0.380^a$	110.30±0.415a	106.30±0.47ª		
(mmol/L)	35	107.80±0.642ª	109.40±0.854ª	103.90±0.327ª	104.50±0.514 <sup>a</sup>	108.50±0.865ª	108.30±0.44ª		
	42	104.80±0.337ª	106.20±0.473ª	105.10±0.610a	103.80±0.515a	107.40±0.534a	108.30±0.49a		
	21	1481.80±12.890a	1474.50±10.081a	1235.22±13.06 <sup>b</sup>	1307.17±6.181°	1348.80±9.990°	1344.80±3.361°		
CK-MB (IU/L)	28	1482.50±17.780a	1303.90±14.632 <sup>b</sup>	1482.10±19.43a	1328.60±12.710 <sup>b</sup>	1201.40±12.114°	1377.60±22.96 <sup>b</sup>		
	35	1491.10±27.841ª	1289.00±15.462 <sup>b</sup>	1260.80±18.7 <sup>b</sup>	1485.00±12.227a	1447.60±17.893a	1214.00±19.28 <sup>b</sup>		

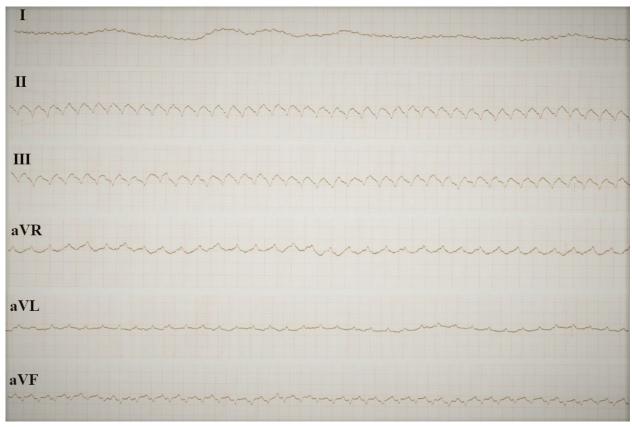
Parameters Day	Dave	Groups (mean ±SE )					
	Days	CM	M500	M2500	CP	P500	P2500
	42	1288.80±14.021a	1126.50±19.803b	1108±21.130b	1644.30±20.360°	1443.80±11.870 <sup>d</sup>	1332.95±10.25a

CM: control mash diet (0 ppm silymarin); CP: control pellet diet (0 ppm silymarin); M500: mash diet + 500 ppm silymarin; M2500: mash diet + 2500 ppm silymarin; P500: pellet diet + 500 ppm silymarin; P2500: pellet diet + 2500 ppm silymarin; CK-MB: creatine kinase MB; a,b,c Different letters in the superscripts of the same row indicate significant differences (P < 0.05).

# **Electrocardiographic Analysis**

All ECG leads (6 leads) of the broilers in various groups seen in Figure 1. The durations and

amplitudes of all waves in lead II are presented in Table 5.



**Figure 1.** Samples of different electrocardiograms in experimental groups (CM, CP, M500, M2500, P500 and P2500) with six simultaneously recorded leads.

T duration in CP was significantly higher than in CM, M500, and M2500 (P<0.05). The duration of the T wave significantly decreased in P500 and P2500 compared to CP (P<0.05). The amplitude of the S wave significantly increased in CP compared to M500 and CM. The S-wave amplitude was significantly lower in P2500 than in CP on days 28 and 42. The amplitude of

the T wave was higher in CP than in CM on day 42.

Receiving the supplemented pellet diet with 500 ppm of silymarin in P500 group decreased T-wave amplitude compared to CP group on days 35 and 42. The R-R interval in CP significantly decreased compared with CM. Birds in the P2500 group had a higher R-R interval

compared with the CP group on day 42. The ST segment increased in CP compared to CM, M500, and M2500. In P500 and P2500, the ST

segment was lower compared with CP. HR increased significantly in CP compared to CM, and P2500 had lower HR compared with CP on day 42 (<u>Table 5</u>).

**Table 5** Electrocardiographic parameters (mean±SE) of broiler chickens (n=20) following dietary supplementation of pellet and mash diets by silymarin extract.

Parameters	D	Groups						
	Days	CM	M500	M2500	СР	P500	P2500	
	21	0.02±0.010a	0.02±0.010a	0.02±0.010a	0.02±0.010a	0.02±0.011a	0.02±0.010a	
P-duration	28	0.02±0.011ª	0.02±0.010 <sup>a</sup>	0.04±0.011 <sup>b</sup>	0.02±0.010a	0.02±0.011a	0.02±0.010a	
<b>(S)</b>	35	0.02±0.010a	0.04±0.011b	0.02±0.010a	0.03±0.011°	0.02±0.010a	0.02±0.011ª	
	42	0.02±0.012a	0.02±0.010 <sup>a</sup>	0.02±0.011ª	0.02±0.012a	0.02±0.010 <sup>a</sup>	0.02±0.011ª	
	21	0.03±0.011a	0.02±0.010 <sup>b</sup>	0.03±0.010a	0.02±0.010 <sup>b</sup>	0.02±0.011 <sup>b</sup>	0.02±0.010 <sup>b</sup>	
S-duration	28	0.03±0.012a	0.03±0.010 <sup>a</sup>	0.03±0.010 <sup>a</sup>	0.02±0.010 <sup>b</sup>	0.02±0.010 <sup>b</sup>	0.03±0.010a	
<b>(S)</b>	35	0.03±0.010 <sup>a</sup>	0.04±0.012b	0.04±0.010 <sup>b</sup>	0.03±0.010 <sup>a</sup>	0.04±0.011 <sup>b</sup>	0.04±0.010 <sup>b</sup>	
	42	0.04±0.010a	0.04±0.011ª	0.04±0.010a	0.04±0.010a	0.04±0.010a	0.04±0.010a	
	21	0.04±0.010 <sup>b</sup>	0.03±0.011ª	0.03±0.011ª	0.05±0.011°	0.04±0.010 <sup>b</sup>	0.04±0.011 <sup>b</sup>	
T-duration	28	0.05±0.010°	0.04±0.011a	0.04±0.010a	0.07±0.010 <sup>d</sup>	0.06±0.011 <sup>b</sup>	0.05±0.011°	
<b>(S)</b>	35	0.06±0.010 <sup>d</sup>	0.05±0.010 <sup>a</sup>	0.05±0.010 <sup>a</sup>	0.07±0.010 <sup>b</sup>	$0.07 \pm 0.010^{b}$	0.05±0.010°	
	42	0.06±0.010°	0.05±0.010 <sup>a</sup>	0.05±0.010a	0.08±0.010 <sup>d</sup>	0.07±0.010 <sup>b</sup>	0.05±0.010a	
	21	0.05±0.011a	0.05±0.010 <sup>a</sup>	0.04±0.011 <sup>b</sup>	0.04±0.010 <sup>b</sup>	0.03±0.010°	0.05±0.011ª	
P-	28	0.05±0.010 <sup>a</sup>	0.05±0.010 <sup>a</sup>	0.05±0.011 <sup>a</sup>	0.05±0.011 <sup>a</sup>	0.05±0.010 <sup>a</sup>	0.05±0.011ª	
amplitude (mV)	35	0.05±0.010a	0.05±0.011ª	0.05±0.011ª	0.05±0.010a	0.05±0.010a	0.05±0.010a	
	42	0.05±0.010a	0.05±0.011a	0.05±0.011a	0.05±0.010a	0.05±0.010a	0.05±0.010 <sup>a</sup>	
	21	0.07±0.030°	0.05±0.011a	0.08±0.020b	0.08±0.020b	0.07±0.021°	0.08±0.021 <sup>b</sup>	
S-	28	0.05±0.010 <sup>b</sup>	0.07±0.020 <sup>a</sup>	0.05±0.010 <sup>b</sup>	0.12±0.020 <sup>d</sup>	0.10±0.010°	0.05±0.010 <sup>b</sup>	
amplitude (mV)	35	0.08±0.040a	0.08±0.040a	0.05±0.010 <sup>b</sup>	0.07±0.020d	0.10±0.010°	0.10±0.011°	
	42	0.08±0.020°	0.07±0.020a	0.10±0.010 <sup>b</sup>	0.10±0.010 <sup>b</sup>	0.10±0.010 <sup>b</sup>	0.07±0.021ª	
	21	0.08±0.021°	0.06±0.030 <sup>a</sup>	0.06±0.011ª	0.06±0.031a	0.10±0.010 <sup>b</sup>	0.10±0.041 <sup>b</sup>	
T-	28	0.09±0.010 <sup>d</sup>	0.08±0.021ª	0.06±0.021 <sup>b</sup>	0.05±0.011°	0.06±0.010 <sup>b</sup>	0.06±0.011 <sup>b</sup>	
amplitude (mV)	35	0.08±0.021°	0.13±0.021a	0.05±0.011b	0.08±0.021°	0.07±0.020 <sup>d</sup>	0.09±0.040e	
	42	0.07±0.020d	0.07±0.020a	0.07±0.021ª	0.08±0.021b	0.05±0.000°	0.07±0.021 <sup>d</sup>	
QT-	21	0.10±0.010 <sup>a</sup>	0.10±0.011ª	0.10±0.011ª	0.10±0.010 <sup>a</sup>	0.10±0.011ª	0.10±0.010 <sup>a</sup>	
Interval (S)	28	0.10±0.010a	0.09±0.011ª	0.10±0.011a	0.11±0.010a	0.11±0.011a	0.10±0.001ª	

D	Groups						
Days	CM	M500	M2500	СР	P500	P2500	
35	0.11±0.010a	0.11±0.011a	0.10±0.010a	0.12±0.010a	0.11±0.010 <sup>a</sup>	0.11±0.001a	
42	0.11±0.010a	0.10±0.011ª	0.11±0.011a	0.12±0.001ª	0.12±0.010 <sup>a</sup>	0.11±0.001a	
21	0.12±0.011 <sup>b</sup>	0.13±0.011 <sup>a</sup>	0.13±0.010 <sup>a</sup>	0.13±0.012 <sup>a</sup>	0.13±0.011ª	0.12±0.011 <sup>b</sup>	
28	0.13±0.011 <sup>b</sup>	0.14±0.010 <sup>a</sup>	0.14±0.011ª	0.14±0.012 <sup>a</sup>	0.14±0.012 <sup>a</sup>	0.14±0.012a	
35	0.14±0.011°	0.15±0.010 <sup>a</sup>	0.15±0.012a	0.14±0.012°	0.16±0.012 <sup>b</sup>	0.14±0.013°	
42	0.15±0.011 <sup>b</sup>	0.14±0.010a	0.14±0.011a	0.14±0.013ª	0.14±0.011ª	0.15±0.011 <sup>b</sup>	
21	0.02±0.012a	0.02±0.011ª	0.02±0.010 <sup>a</sup>	0.03±0.010 <sup>b</sup>	0.03±0.010 <sup>b</sup>	0.03±0.011 <sup>b</sup>	
28	0.03±0.012a	0.03±0.011a	0.02±0.010 <sup>b</sup>	0.04±0.011°	0.04±0.010°	0.03±0.010a	
35	0.02±0.011 <sup>d</sup>	0.03±0.011ª	0.03±0.010 <sup>a</sup>	0.07±0.011e	0.06±0.021b	0.05±0.011°	
42	0.04±0.011 <sup>d</sup>	0.03±0.010a	0.02±0.010 <sup>b</sup>	0.06±0.010e	0.05±0.010°	0.04±0.011 <sup>d</sup>	
21	0.03±0.011°	0.02±0.011ª	0.04±0.011 <sup>b</sup>	0.02±0.011ª	0.02±0.010 <sup>a</sup>	0.02±0.010 <sup>a</sup>	
28	0.03±0.012a	0.03±0.011ª	0.03±0.011ª	0.03±0.010 <sup>a</sup>	0.02±0.010 <sup>b</sup>	0.02±0.010 <sup>b</sup>	
35	0.02±0.012°	0.03±0.011ª	0.04±0.010 <sup>b</sup>	0.03±0.010 <sup>a</sup>	0.04±0.010 <sup>b</sup>	0.02±0.010°	
42	0.02±0.010 <sup>a</sup>	0.02±0.010 <sup>a</sup>	0.02±0.010 <sup>a</sup>	0.02±0.010 <sup>a</sup>	0.02±0.011ª	0.02±0.011ª	
21	500±31.650b	468.75±32.562 <sup>a,b</sup>	447.76±22.986 <sup>a</sup>	458.01±20.342a	468.75±20.451 <sup>a,b</sup>	500.00±19.781 <sup>b</sup>	
28	458.01±20.450 <sup>a</sup>	428.57±19.872 b	428.57± 16.870 <sup>b</sup>	428.57±18.092 <sup>b</sup>	428.57±18.761 <sup>b</sup>	428.57±20.181 <sup>b</sup>	
35	413.76±18.721 <sup>b</sup>	397.35±18.234 <sup>a,c</sup>	405.40±13.541 <sup>b,c</sup>	413.76±17.102 <sup>b</sup>	375.00±12.091 <sup>a</sup>	428.57±19.319 <sup>b</sup>	
42	389.61±13.661°	428.57±16.626 <sup>a,b</sup>	428.57±10.982 <sup>a,b</sup>	413.76±19.110 <sup>a</sup>	437.95±11.541 <sup>b</sup>	387.76±13.090 <sup>b</sup>	
	42 21 28 35 42 21 28 35 42 21 28 35 42 21 28 35	CM  35  0.11±0.010 <sup>a</sup> 42  0.11±0.010 <sup>a</sup> 21  0.12±0.011 <sup>b</sup> 28  0.13±0.011 <sup>b</sup> 35  0.14±0.011 <sup>c</sup> 42  0.15±0.011 <sup>b</sup> 21  0.02±0.012 <sup>a</sup> 28  0.03±0.012 <sup>a</sup> 35  0.02±0.011 <sup>d</sup> 42  0.04±0.011 <sup>d</sup> 21  0.03±0.012 <sup>c</sup> 28  0.03±0.012 <sup>a</sup> 35  0.02±0.012 <sup>a</sup> 35  0.02±0.010 <sup>a</sup> 21  500±31.650 <sup>b</sup> 28  458.01±20.450 <sup>a</sup> 35  413.76±18.721 <sup>b</sup>	CM M500  35 0.11±0.010 <sup>a</sup> 0.11±0.011 <sup>a</sup> 42 0.11±0.010 <sup>a</sup> 0.10±0.011 <sup>a</sup> 21 0.12±0.011 <sup>b</sup> 0.13±0.011 <sup>a</sup> 28 0.13±0.011 <sup>b</sup> 0.14±0.010 <sup>a</sup> 35 0.14±0.011 <sup>c</sup> 0.15±0.010 <sup>a</sup> 42 0.15±0.011 <sup>b</sup> 0.14±0.010 <sup>a</sup> 21 0.02±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 28 0.03±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 35 0.02±0.011 <sup>d</sup> 0.03±0.011 <sup>a</sup> 42 0.04±0.011 <sup>d</sup> 0.03±0.010 <sup>a</sup> 21 0.03±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 42 0.04±0.011 <sup>d</sup> 0.03±0.010 <sup>a</sup> 21 0.03±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 28 0.03±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 28 0.03±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 28 0.03±0.012 <sup>a</sup> 0.02±0.011 <sup>a</sup> 35 0.02±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 42 0.02±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 35 0.02±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 42 0.02±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 35 0.02±0.012 <sup>a</sup> 0.03±0.011 <sup>a</sup> 42 0.02±0.010 <sup>a</sup> 0.02±0.010 <sup>a</sup> 35 458.01±20.450 <sup>a</sup> 428.57±19.872 <sup>b</sup> 36 413.76±18.721 <sup>b</sup> 397.35±18.234 <sup>a,c</sup>	CM         M500         M2500           35         0.11±0.010a         0.11±0.011a         0.10±0.010a           42         0.11±0.010b         0.10±0.011a         0.11±0.011a           21         0.12±0.011b         0.13±0.011a         0.13±0.010a           28         0.13±0.011b         0.14±0.010a         0.14±0.011a           35         0.14±0.011c         0.15±0.010a         0.15±0.012a           42         0.15±0.011b         0.14±0.010a         0.14±0.011a           21         0.02±0.012a         0.02±0.011a         0.02±0.010a           28         0.03±0.012a         0.03±0.011a         0.02±0.010b           35         0.02±0.011d         0.03±0.011a         0.02±0.010b           42         0.04±0.011d         0.03±0.011a         0.04±0.011b           21         0.03±0.011c         0.02±0.011a         0.04±0.011b           28         0.03±0.012a         0.03±0.011a         0.04±0.011b           35         0.02±0.010a         0.02±0.010a         0.02±0.010a           42         0.02±0.010a         0.02±0.010a         0.02±0.010a           35         458.01±20.450a         468.75±32.562ab         447.76±22.986a           28<	CM         M500         M2500         CP           35        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0.02±0.011a           42         0.04±0.011c         0.02±0.011a         0.04±0.011b         0.02±0.010a           28         0.03±0.012a         0.03±0.011	CM         M500         M2500         CP         P500           35         0.11±0.010a         0.11±0.011a         0.10±0.010a         0.12±0.001a         0.11±0.010a           42         0.11±0.010b         0.10±0.011a         0.11±0.011a         0.12±0.001a         0.12±0.010a           21         0.12±0.011b         0.13±0.011a         0.13±0.011a         0.13±0.011a         0.13±0.012a         0.13±0.011a           28         0.13±0.011b         0.14±0.010a         0.14±0.011a         0.14±0.012a         0.14±0.012a           35         0.14±0.011c         0.15±0.010a         0.15±0.012a         0.14±0.012c         0.16±0.012b           42         0.15±0.011b         0.14±0.010a         0.14±0.011a         0.14±0.013a         0.14±0.011a           21         0.02±0.012a         0.02±0.011a         0.02±0.010a         0.03±0.010b         0.03±0.010b         0.03±0.010b         0.03±0.010c           28         0.03±0.011d         0.03±0.010a         0.02±0.010b         0.06±0.010c         0.05±0.010c           21         0.03±0.011c         0.02±0.011b         0.02±0.011c         0.06±0.010c         0.05±0.010c           22         0.03±0.011c         0.02±0.011a         0.03±0.011a         0.03±0.011a         0.03±0	

CM: control mash diet (0 ppm silymarin); CP: control pellet diet (0 ppm silymarin); M500: mash diet + 500 ppm silymarin; M2500: mash diet + 2500 ppm silymarin; P500: pellet diet + 500 ppm silymarin; P2500: pellet diet + 2500 ppm silymarin; a,b,c Different letters in the superscripts of the same row indicate significant differences (P < 0.05).

# **Discussion**

Silymarin has potent antioxidant activity, and the inhibition of oxidative damage by silymarin could be responsible for the protective effect on various organ disorders, such as the liver and heart (Razavi & Karimi, 2016). Main damages in broilers with RVF and ascites syndrome were seen in the liver and heart (Ahmadipour *et al.*, 2019). The present study was designed to assess the positive effect of silymarin extract on liver and heart health in broilers fed on mash and pellet diets.

In this study, circulating total protein was significantly lower in the CP group than in the CM

group, and silymarin at two doses (500 and 2500 ppm) in P500 and P2500 increased the serum level of total protein compared to the unsupplemented pellet diet (CP) on days 28, 35, and 42. In the present study, CP had significantly lower circulating albumin compared to CM on day 42, and supplementing with silymarin increased the serum level of albumin in P500 and P2500, but it was not statistically significant (*P*>0.05).

Albumin is the most abundant plasma protein, which is synthesized by the liver and has an important role in maintaining the plasma oncotic

pressure within the vascular compartments, preventing the leaking of fluids into the extravascular spaces (Soeters *et al.*, 2018). Hypoproteinemia and hypoalbuminemia were seen in various conditions, such as severe hepatic impairment, heart failure, nephrotic syndrome, malnutrition, and inflammation (Walayat *et al.*, 2017; Soeters *et al.*, 2018). Several previous researchers have stated that hepatic disorders in poultry led to hypoproteinemia and hypoalbuminemia, which these conditions result in fluid leakage into the extravascular spaces and the incidence of ascites (Lutensko *et al.*, 2008; Milsavljevic, 2014).

Further, in agreement with our finding, Wang et al. (2018) showed that silymarin increased serum total protein in animal models with liver disorders. Also, Lutensko et al. (2008) and Baradaran et al. (2019) reported that silymarin increased the serum level of total protein in broiler chicks with liver disorders. It is established that silymarin stimulates protein synthesis through activating the RNA synthesis of ribosomes and improves body protein metabolism (Vargas-Mendoza et al., 2014). Based on these reports, a decrease in serum levels of protein and albumin in the CP group of the present study may be due to liver dysfunction, and silymarin improved protein metabolism due to its hepatoprotective action.

In the present study, the serum concentration of uric acid was significantly higher in CP than in CM on days 21, 28, and 42. Uric acid is one of the serum parameters, which increases in hepatocellular damages (Zaefarian *et al.*, 2019). In the current research, circulating uric acid significantly decreased in P500 and P2500 groups compared with the CP group that showed the protective effects of silymarin on liver health in broilers fed on a pellet diet.

In the current experiment, the activities of AST and ALT enzymes in serum significantly increased in CP compared to CM on day 42, and

supplementary silymarin at dose 2500 ppm significantly decreased the serum activities of these enzymes in the P2500 group. The serum activity levels of AST and ALT are an indicator of liver damages in birds (Senanayake *et al.*, 2015). Several studies have shown that ALT and AST increase in ascetic birds due to cardiac and hepatic damages (Arab *et al.*, 2006; Fathi *et al.*, 2015).

In the current study, supplementation with silymarin in birds fed on a pellet diet decreased the serum activities of ALT and AST enzymes, but this decrease was more significant in P2500. In agreement with these results, several studies have shown that silymarin has a hepatoprotective effect and decreases the serum AST and ALT activities in broilers with damaged liver (Fani Makki *et al.*, 2014, Wang *et al.*, 2018).

The protective effect of silymarin on the liver has been more related to antioxidant effects, which eliminates free radicals. In addition, this substance leads to increased RNA polymerase activity in the nucleus of hepatocytes and elevated ribosomal protein synthesis, which improves hepatic cell regeneration and can inhibit lipid peroxidation, reduce glutathione oxidation, and enhance liver detoxification (Moayedi Esfahani *et al.*, 2015). Therefore, silymarin as a free radical chelating agent may be effective in hepatic disorders of RVF and ascites syndrome.

In this experiment, silymarin decreased serum concentrations of triglyceride, total cholesterol, LDL, and VLDL in M500, M2500, P500, and P2500 groups compared to control groups (CM and CP). Gobalakrishnan *et al.* (2016) and Wang *et al.* (2018) established that silymarin has a hypolipidemic effect.

Silymarin enhances hepatic bile acid synthesis and stimulates the excretion of bile acids to the intestine. Silymarin increases endogenous cholesterol conversion to bile acids and stimulates the production of hepatic LDL receptors, leading to increased clearance of plasma LDL, HDL, and indirectly that of VLDL (Gobala-krishnan *et al.*, 2016). Also, silymarin was able to inhibit the biosynthesis of cholesterol in the liver and reduce LDL cholesterol oxidation (Wang *et al.*, 2018).

Various studies have been performed on the hepatoprotective action of silymarin in birds, but there are few studies on the protective effect of silymarin on heart health. However, the cardioprotective activity of silymarin has been established on laboratory animals in several experiments (Rašković *et al.*, 2011; Razavi & Karimi, 2016; Vilahur *et al.*, 2018).

In the present research, serum CK activity increased significantly in the CP group compared to the CM group, and the activity of CK-MB decreased significantly in birds treated with 2500 ppm silymarin on day 42. The high serum activity levels of AST and CK are an indication of cardiac injuries in birds (Bodor, 2016). Several studies have shown that CK increased in ascetic birds due to cardiac damages (Arab *et al.*, 2006; Fathi *et al.*, 2015).

In the present study, the simultaneous increase in AST and CK in the CP group may be due to heart injury, which a high level of CK attenuated in the supplemented pellet group with silymarin. In the current research, circulating levels of calcium, phosphorus, and sodium were significantly lower in the CP group than in other groups on 28, 35, and 42 days. Also, birds in the CP group had lower circulating potassium and magnesium compared to the CM group, but it was not statistically significant on day 42.

Heart failure usually leads to acid-base and electrolyte disturbance due to the activation of several neurohumoral mechanisms. The most common electrolyte abnormalities in heart failure are hyponatremia, hypokalemia, and hypomagnesemia (Hanton *et al.*, 2007). Silymarin at doses 500 and 2500 ppm increases

the concentrations of calcium and phosphorus in P500 and P2500 groups compared to CP group. In the present experiment, silymarin at different doses had no improvement effect on electrolyte abnormalities in birds fed on a pellet diet. In agreement with obtained results, Lutensko *et al.* (2008) reported that silymarin increased serum levels of calcium and phosphorus.

In this study, T amplitude and T duration in the CM group were significantly lower compared to the CP group on day 42, and silymarin at dose 2500 ppm decreased significantly the duration and amplitude of the T wave in P2500 compared to CP and P500 groups. In the present study, supplementing a pelleted diet by silymarin at two different doses P500 and P2500 decreased traits of the T wave, but this effect was greater and significant in dose 2500 ppm (P2500 group). Several studies have shown that RVF in broilers changes the ECG waves' pattern, especially in R, S, and T waves (Hassanpour et al., 2009; Yousefi et al., 2013; Sharifi et al., 2015). Hassanpour et al. (2005) stated that the duration of the T wave was enhanced in the broilers susceptible to pulmonary hypertension syndrome PHS and ascites.

In this study, the amplitude of the S wave significantly increased in CP compared with M500 and CM. The S-wave amplitude was significantly lower in P2500 than in CP on days 28 and 42. Previous studies have reported that an increase in the S amplitude reflected the hypertrophy of RVF and ascites (Hassanpour *et al.*, 2009; Yousefi *et al.*, 2013; Sharifi *et al.*, 2015). In this research, S duration on days 21 and 28 was shorter in the CP group than in CM, 500 and M2500 groups, and P22500had a significantly longer duration of the S wave 500, and M2500 on different days of the trial. In P500 and P2500, the ST segment was lower than in CP on all days.

In contrast to our research, Hassanpour *et al*. (2009) reported that the ST interval had no

changes in broilers with RVF. In this research, the R-R interval in CP significantly decreased compared to CM. Also, P2 had a higher R-R interval than CP on day 42. The obtained results showed that HR significantly increased in CP compared to CM, and P2 had a lower HR than CP on day 42. Contrary to our results, Olkowski (2007) stated that HR decreased in broiler chickens susceptible to pulmonary hypertension and ascites syndrome. They showed that progressive bradycardia was seen in these birds due to circulatory insufficiency.

Our finding showed that feeding with a pellet diet changed some ECG parameters and serum activity of CK-MB enzyme in broilers, which can indicate heart disorders in birds fed on a pellet diet, and silymarin extract could modulate these changes. The main property of silymarin is the antioxidant activity of its flavonolignans and other polyphenolic constituents, which is attributable to free radical scavenging. Silymarin leads to replenish endogenous antioxidant enzymes, suppress neutrophil infiltration, and reduce serum malondialdehyde as an end product of myocardial lipid peroxides (Moayedi Esfahani et al., 2015). Previous studhave stated that silymarin cardioprotective effects in animal models due to lipid peroxidation inhibition and an increase in antioxidant enzymes production in heart tissue (Gabrielová et al., 2019).

Based on our results, silymarin had a protective effect on heart function and reversed ECG

indices and serum biochemical changes in serum in fast-growing broilers. Thus, silymarin can be a clinical strategy to decrease the incidence of heart failure and ascites-induced mortality and has a financial benefit in the poultry industry.

# **Conclusion**

In conclusion, it seems that using a pelleted diet in fast-growing broiler chickens could increase the susceptibility of broilers to liver and heart disorders and change some blood biochemical parameters and ECG values. Also, the present findings confirmed that silymarin had a dose-dependent protective effect on heart and liver health in broilers and attenuated induced ,negative changes using a pellet diet. Thus silymarin can be used as a cardioprotective and hepatoprotective compound in broilers susceptible to ascites syndrome for decreasing ascites-induced mortality.

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# **Conflict of Interest**

The authors reported no conflict of interest.

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# فعالیت محافظت کننده قلب و محافظت کننده کبد عصاره سیلی مارین در جوجههای گوشتی تغذیهشده با جیرهٔ آردی و پلت

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زمینه مطالعه: کبد و قلب دو عضو اصلی آسیب دیده در سندرم آسیت در جوجههای گوشتی است. استفاده از سیلیمارین با اثر محافظتی بر کبد و قلب ممکن است یک راهکار مفید برای کاهش مرگ و میر ناشی از آسیت باشد.

هدف: مطالعه حاضر با بررسی شاخصهای ECG و برخی از پارامترهای بیوشیمیایی سرم، اثرات قلبی-کبدی سیلیمارین را در جوجههای گوشتی تغذیه شده با رژیم غذایی آردی و پلت ارزیابی کرد.

روش کار: تعداد ۱۲۰ قطعه جوجه نژاد آربراکرز به شش گروه تقسیم شدند: رژیم غذایی پایه آردی (CM)، رژیم غذایی پایه پلت (CP)، سیلیمارین در دوز P500) و پلت (P2500) و پلت (P2500). سیلیمارین در دوز P500 جیرههای آردی (M2500) و پلت (P2500).

نتیجه گیری نهایی: جیره پلت در جوجههای گوشتی منجر به تغییر برخی از شاخصهای بیوشیمیایی و الکتروکاردیوگرافی می شود و سیلی مارین با دوز ppm می تواند به عنوان یک ترکیب محافظت کنندهٔ کبد و قلب جهت تعدیل نارسایی قلبی - کبدی در جوجههای گوشتی حساس استفاده شود. واژههای کلیدی: ارزیابی قلبی، ارزیابی کبدی، جیره آردی، جیره پلت، سیلی مارین