Iranian Journal of Applied Animal Science

Effect of Milk Thistle (*Silybum marianum*) Seed on Performance, Nutrient Digestibility and Small Intestine Morphology of Broiler Chickens

Research Article

S.A. Khatami[1], M.D. Shakouri[1*] and A. Mojtahedin[2]

[1] Department of Animal Science, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[2] Department of Animal Science, Faculty of Agriculture and Natural Resources (Moghan), University of Mohaghegh Ardabili, Ardabil, Iran

Received on: 2 Jul 2023
Revised on: 26 Oct 2023
Accepted on: 6 Nov 2023
Online Published on: Dec 2023

*Correspondence E-mail: mdshakouri@uma.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

The present study investigated the effect of different levels of milk thistle (*Silybum marianum*) seed on performance, nutrient digestibility and small intestine morphology of broiler chickens. For this purpose, 256 broiler chicks (Ross 308) were randomly assigned to four treatments with four replications and 16 chicks in each replication, by employing a completely randomized design. Treatments were included: 1) control, 2) basal diet + 0.2 % milk thistle seed, 3) basal diet + 0.4 % milk thistle seed and 4) basal diet + 0.6 % milk thistle seed. The results showed that the use of different levels of milk thistle increased feed intake compared with the control, but weight gain decreased with increasing levels of milk thistle (P<0.05). The feed conversion ratio increased by adding milk thistle to the diet (P<0.05). The addition of different levels of milk thistle in the diet reduced the digestibility of dry matter and organic matter compared with the control but did not have a significant effect on the digestibility of ether extract and ash. The concentration of serum LDL-c, VLDL-c and triglyceride was decreased by 0.6% milk thistle (P<0.05). The jejunal villus height and villus height to crypt depth significantly decreased using 0.2% milk thistle seed in the diet of broilers (P<0.05). The villus height to crypt depth in the ileum increased by milk thistle seed (P<0.05). In general, the milk thistle had no positive effect on growth performance, digestibility of dry matter and organic matter, and intestinal morphology. Therefore, according to the results of this study, it is not recommended to use milk thistle seed in broiler chickens diet.

KEY WORDS blood parameters, broilers, digestibility, milk thistle, performance.

INTRODUCTION

Feed additives (especially antibiotics) have been the most cost-effective way to maintain health and feed efficiency status in poultry. However, the problem of drug residues in poultry products and its negative effect on the consumers' health have caused some limitations in usage of antibiotics in the poultry industry since 2006 (Ashraf *et al.* 2019). Nowadays, the specific dietary medicinal plants have been used in poultry as antibiotic alternatives. Natural products like medical plants as an alternative to antibiotics can be used for growth promotion and can effectively improve poultry performance. In addition to herbs' and spices' essential role in human nutrition, these natural additives have been used to improve the health and general well-being of animals, especially in poultry (Puvaca *et al.* 2020).

Milk thistle (*Silybum marianum*) is one of the most important medicinal plants in the world, it has properties of antimicrobial, antioxidant, anticholesterol, anti-inflammatory, cytoprotective, hepatoprotective, scavenging reactive oxygen species (ROS), and inhibiting lipid peroxidation (Surai, 2015). The major biologically active component of the seed of this plant are silymarin which contains silybin (50-60%), isosilybin (5%), silychristin (20%) and silydianin (10%) (Tajmohammadi *et al.* 2018). In poultry production, silymarin have been studied as a tool to reduce aflatoxicosis damage (Alheidary *et al.* 2017). According to Gillessen and Hartmut (2020), milk thistle increase lymphocyte proliferation which are associated with an increase in interferon gamma, interleukin (IL)-4 and IL-10 cytokines. Milk thistle seeds contain 23% ether extract and the oil obtained is a rich source of tocopherols and fatty acids. Chambers *et al.* (2017) reported that milk thistle oil contains 27–64% linoleic acid (C18:2, n-6), 21–50%, oleic acid (C18:1, n-9), 7–14% palmitic acid (C16:0), 2–6% stearic acid (C18:0) and 5% α-linolenic acid (C18:3, n-3). Therefore, by considering the acceptable properties of silymarin as mentioned above, this study was performed to investigate the effect of different levels of milk thistle seed on performance, nutrient digestibility, blood parameters, meat quality, and small intestine morphology of broiler chickens.

MATERIALS AND METHODS

Experimental design and birds husbandry

A total of 256 one-day-old mixed-sex (128 males and 128 females) broiler chicks (Ross 308) were obtained from a local hatchery and then were randomly assigned into four treatments with four replications of 16 birds in each. The treatments consisted of control (without any additives), 0.2% milk thistle seed, 0.4% milk thistle seed and 0.6% milk thistle seed. The trial was carried out in the period of 1-42 days of age. The diets included starter (1 to 10 days of age), grower (11 to 24 days of age) and finisher (25-42 days of age) and were formulated according to Ross 308 (Aviagen, 2019) strain catahoque recimmendations (Table 1). During the experimental period, the feed and water were offered *ad libitum*. The lighting program of 23 h light/1 h darkness was imposed throughout the experimental period. The temperature of the room was set at 32 ˚C for the first 3 days and then reduced until it reached 21 ˚C; this temperature was maintained until the end of the 42-d experiment.

Data collection

Growth performance

The body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined at the end of each period. The mortality of birds in each pen (replicate) was also recorded daily.

Apparent digestibility of nutrients

At 22 to 24 days, fresh fecal samples were collected from 2 birds of each pen (one male and one female). Then the excreta samples were stored in a freezer at -20 ˚C until analysis. Then, the samples were dried at 60 ˚C for 72 h. Chromium oxide (0.3%) was added in bird's diet as an indigestible marker for a period of 5 days prior to fecal collection to determine the apparent digestibility of dry matter (DM), orgnic matter (OM), ash and ether extract (EE). Dry matter, organic matter and lipid contents were measured using the standard procedures (AOAC, 2000). The content of chromic oxide in the samples was measured according to Fenton and Fenton (1979). The apparent digestibility (AD) of nutrients was calculated according to the following equation:

$$\text{AD (\%)} = 100 \times [1 - ((\%\text{ diet } Cr_2O_3 / \%\text{ excreta } Cr_2O_3) \times (\%\text{ excreta nutrient} / \%\text{ diet nutrient}))]$$

Blood parameters

At 42 days, the blood samples were obtained from the wing vein for the determination of blood biochemical parameters. Blood samples were centrifuged to obtain serum for 15 min at 3000 rpm (VISION Model VS-15000 CFN II Made in South Korea). Serum samples were kept in tubes at −20 ˚C until analyzed. Serum biochemical parameters (cholesterol, HDL-cholesterol, triglyceride, and glucose) were measured using commercial kits (Ziest Chem, Iran) by colorimetric methos (Spectrophotometer, UNICO 2100, USA). Also, LDL-cholesterol and VLDL-cholesterol were determined (mg/dl) as follows (Friedewald *et al.* 1972):

$$\text{LDL-c} = \text{TC} - \text{HDL-c} - (\text{TG} / 5)$$
$$\text{VLD-c} = \text{TG} / 5$$

Where:
TC: total cholesterol.
HDL-c: high density lipoprotein cholesterol.
LDL-c: low density lipoprotein cholesterol.
TG: total triglycerides.
VLDL-c: very low density lipoprotein cholesterol.

Small intestine morphology assay

At the end of the feeding trial, the birds were slaughtered and the small intestine was cut as described by Iji *et al.* (2001). Then, the jejunal and ileal tissue samples were then fixed in a formalin solution and were paraffin wax impregnated. Tissue sections with 5 µm thickness were cut by a microtome, stained with hematoxylin and eosin. The slides were examined on light microscope fitted with computer-aided light microscope image analyses. A total of 12 intact well-oriented, crypt-villus units were selected randomly for each sample.

Statistical analysis

The data were analyzed based on a completely randomized design using SAS general linear model (GLM) procedure (SAS, 2001). Duncan's test was used for the comparison differences of means (P<0.05).

$Y_{ij} = \mu + T_i + e_{ij}$

Where:
Y_{ij}: value of each observation.
μ: overall mean of the trait.
T_i: effect of experimental diets.
e_{ij}: residual error.

RESULTS AND DISCUSSION

The results of the effect of different levels of milk thistle in the diet of broiler chickens on feed intake, body weight gain and feed conversion ratio (FCR) are shown in Table 2. In the starter period, the use of 0.2 and 0.4 percent milk thistle in the broiler diet reduced feed consumption compared with the control group (P<0.05). However, during the grower period, broiler chickens' feed consumption was not affected by the experimental treatments. In the finisher and whole experimental periods, milk thistle increased the feed intake compared with the control (P<0.05).

In the starter period, addition of 0.2 and 0.4 levels of milk thistle in the broiler diet reduced body weight gain compared with the control (P<0.05). The addition of different levels of milk thistle could not affect the body weight of chicks during the grower period. During the finisher and whole experimental periods, the body weight gain of broiler chickens fed with 0.2, 0.4 and 0.6 levels of milk thistle seed in the diet had a significant decrease compared with the control group (P<0.05).

During the starter and grower periods, the FCR was not affected by the addition of different levels of milk thistle. However, in the finisher period and the whole period, the use of levels 0.2, 0.4 and 0.6 of milk thistle in the diet caused a significant increase in the FCR compared with the control (P<0.05).

The effects of milk thistle seed on the digestibility of nutrients in broiler chickens are shown in Table 3. The results showed that the use of 0.2, 0.4 and 0.6 levels of milk thistle in the diet of broiler chickens reduced the digestibility of dry matter (DM) compared with the control (P<0.05). Also, the digestibility of organic matter (OM) with the addition of different levels of milk thistle decreased significantly compared with the control (P<0.05). Different levels of milk thistle could not affect on ash and ether extract (EE) digestibility.

The effect of milk thistle seed on the blood parameters of broiler is shown in Table 4. The results showed that supplementing the diet of broiler chickens with different levels of milk thistle did not affect cholesterol and HDL-c concentrations. The concentration of LDL-c, VLDL-c and triglyceride decreased by adding 0.6 level of milk thistle in the broiler diet (P<0.05). The addition of different levels of milk thistle did not affect the glucose concentration.

The results related to jejunum (Table 5) showed that the use of 0.2 and 0.4 levels of milk thistle seed in the diet of broiler chickens reduced the villus height compared with the control (P<0.05). The addition of 0.2 percent milk thistle seed in the diet increased crypt depth compared with the control (P<0.05). The thickness of muscle layer was not affected by the experimental treatments. The adding 0.2 percent milk thistle to the diet decreased the villus height to crypt depth ratio compared with the control (P<0.05). Supplementing the diet with 0.2 and 0.6 percent milk thistle decreased the villus surface area compared with the control (P<0.05). Adding 0.4 and 0.6 percent milk thistle seed to the diet increased ileum villus height (P<0.05). All levels of milk thistle decreased ileal crypt depth and increased villus height to crypt depth ratio and villus surface area (P<0.05).

The results related to the effect of milk thistle seed levels on performance showed that the use of different levels of milk thistle seed in the diet increased feed consumption compared with the control. However, the body weight gain decreased with the addition of different levels of milk thistle seed. Feeding broilers with different levels of milk thistle seed increased the feed conversion ratio compared with the control group. In this regard, Stastnik *et al.* (2016) reported that using 5 and 15% levels of silymarin in the diet decreased the body weight and increased the feed conversion ratio of roosters. Suchy *et al.* (2008) observed that the addition of 0.2% and 1% milk thistle seed cakes caused a decrease in body weight of broilers. Tedesco *et al.* (2004) stated that the use of 600 mg/kg silymarin phytosome in the diet increased the body weight of broiler chickens. In another study Schiavone *et al.* (2007) reported that feeding broiler with 40 and 80 ppm of milk thistle seed reduced feed intake. These researchers stated that feed intake reduction possibly be related to the reduced palatability of the experimental diet which is contrary with the results of the present study. Mousa and Osman (2016) reported that chicks receiving silymarin significantly improved body weight and feed conversion ratio. Ahmad *et al.* (2020) stated that chickens fed with 15 g/kg of dry matter milk thistle improved their body weight and feed conversion ratio in the summer season. Blevins *et al.* (2010) reported no significant differences in feed conversion ratio and growth performance of broiler chicks receiving milk thistle. Also, Muhammad *et al.* (2012) found that milk thistle supplementation (10 g/kg diet) significantly improved broiler chicken body weight gain.

Our study's results showed that using different levels of milk thistle seed in the diet reduced the digestibility of DM and OM, but did not on affect the digestibility of ash and ether extract (EE) (Table 3).

Table 1 Ingredient and nutrient composition of the experimental diets

Ingredient	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	49.12	54.31	58.40
Soybean meal (CP 44%)	42.74	36.92	33.05
Soybean oil	3.84	4.29	4.33
Limestone	1.12	1.00	0.99
Di-calcium phosphate	2.04	1.92	1.84
Common salt	0.47	0.47	0.42
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-methionine	0.33	0.33	0.25
L-lysine HCl	0.29	0.26	0.22
Total	100	100	100
Chemical compassion			
Metabolizable energy (kcal/kg)	2590	3050	3100
Crude protein (%)	23.19	22.07	20.57
Ca (%)	1.05	0.94	0.90
P (%)	0.5	0.47	0.45
Na (%)	0.2	0.2	0.18
Arginine (%)	1.53	1.37	1.26
Lysine (%)	1.51	1.34	1.21
Methionine (%)	0.68	0.66	0.56
Methionine + cysteine (%)	1.05	1.01	0.88

[1] Supplied per kg of diet: vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 18 mg; vitamin K_3: 2 mg; vitamin B_1: 1.75 mg; vitamin B_2: 6.6 mg; vitamin B_3: 9.8 mg; vitamin B_5: 29.65 mg; vitamin B_6: 2.94 mg; vitamin B_9: 1 mg; vitamin B_{12}: 0.015 mg; Coline chloride: 250 mg and Antioxidant: 1 mg.
[2] Supplied per kg of diet: Mn: 99.2 mg; Zn: 84.7 mg; Fe: 50 mg; Cu: 10 mg; I: 0.99 mg and Se: 0.2 mg.

Table 2 Effects of milk thistle seed (MTS, % of diet) on performance of broiler chickens (Ross 308)

MTS	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Starter (1-10 d)			
0	239.03^{a}	171.31^{a}	1.40
0.2 %	225.88^{b}	160.54^{bc}	1.41
0.4 %	218.09^{b}	152.35^{c}	1.43
0.6 %	229.86^{ab}	167.50^{ab}	1.37
SEM	3.90	2.74	0.02
P-value	0.018	0.002	0.29
Grower (11-24 d)			
0	1189.3	745.16	1.60
0.2 %	1206.18	779.23	1.55
0.4 %	1182.26	733.40	1.62
0.6 %	1184.47	770.26	1.54
SEM	20.43	18.15	0.04
P-value	0.83	0.28	0.40
Finisher (25-42 d)			
0	2409.76^{c}	1399.33^{a}	1.72^{b}
0.2 %	2724.77^{ab}	1258.89^{b}	2.16^{a}
0.4 %	2643.87^{b}	1204.50^{b}	2.20^{a}
0.6 %	2773.37^{a}	1238.33^{b}	2.24^{a}
SEM	34.48	21.06	0.04
P-value	0.0001	0.0002	0.0001
Whole experimental period (1-42 d)			
0	3838.04^{c}	2315.81^{a}	1.66^{b}
0.2 %	4156.84^{ab}	2198.70^{b}	1.89^{a}
0.4 %	4044.23^{b}	2090.26^{c}	1.93^{a}
0.6 %	4187.70^{a}	2176.10^{b}	1.92^{a}
SEM	43.24	21.88	0.02
P-value	0.0004	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of milk thistle seed (MTS, % of diet) on digestibility of nutrients in broiler chickens (24 d)

MTS	DM (%)	OM (%)	Ash (%)	EE (%)
0	71.56[a]	74.60[a]	45.67	73.91
0.2 %	69.21[b]	72.56[b]	44.12	73.32
0.4 %	69.08[b]	70.97[c]	44.02	76.37
0.6 %	68.33[b]	72.17[b]	41.11	73.50
SEM	0.52	0.35	1.31	1.80
P-value	0.012	0.0003	0.18	0.53

DM: dry matter; OM: organic matter and EE: ether extract.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of milk thistle seed (MTS, % of diet) on blood parameters of broiler chickens (42 d)

MTS	Blood parameters					
	CHO (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	TG (mg/dL)	GLU (mg/dL)
0	117.48	62.74	46.90[a]	8.84[a]	39.19[a]	158.8
0.2 %	116.24	63.60	44.95[a]	7.67[a]	38.39[a]	157.68
0.4 %	113.42	64.20	41.68[ab]	7.53[a]	37.67[a]	161.45
0.6 %	112.49	66.22	39.26[b]	7.01[b]	35.09[b]	166.42
SEM	1.47	1. 50	1.6702	1.1605	0.8203	6.39
P-value	0.11	0.44	0.03	0.02	0.02	0.77

CHO: cholesterol; HDL-c: high density lipoprotein-cholesterol; LDL: low-density lipoprotein-cholesterol; VLDL-c: very low-density lipoprotein cholesterol TG: triglyceride and GLU: glucose.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In this regard, Sultan *et al.* (2018) showed that the use of silymarin in the diet improved the nutrient digestibility of broiler chickens. Also, Shanmugam *et al.* (2022) showed that the use of 0.04 and 0.06 levels of silymarin in the diet of broilers increased dry matter digestibility compared with the control group.

The results of the present study showed that the use of 0.6% milk thistle seed in the diet of broilers reduced the blood concentration of VLDL-c, LDL-c and triglyceride (Table 4). In this regard, Suchý *et al.* (2008) reported that blood cholesterol concentration was significantly lowered by adding 1% milk thistle seed cakes in the diet of broilers. Metwally *et al.* (2009) demonstrated that the adding a silymarin concentrate significantly decreased the triglyceride, HDL, LDL cholesterol concentrations in rats.
Banaee *et al.* (2011) stated that adding silymarin in fish diet decreases plasma glucose and total cholesterol levels. Hashemi Jabali *et al.* (2017) showed that silymarin increased the secretion of hepatic low-density lipoprotein to reduce cholesterol synthesis in hepatocytes in mice and rabbits. In another research, Schiavone *et al.* (2007) reported that adding 40 and 80 ppm of silymarin in broilers' diet had no significant effect on the cholesterol and triglycerides of broilers. This result was in consistent with the present study. In line with the results of the present study, Hashemi Jabali *et al.* (2017) showed that feeding different amounts of milk thistle flour in laying hens led to a significant reduction in the triglyceride levels in the blood of the animals compared with the control group. They further found that milk thistle flour reduced the concentration of blood cholesterol, and conversely increased the HDL-cholesterol concentration in animals' blood. The Adding 1% milk thistle to the feed mixture significantly decreased blood cholesterol concentration in fattened chickens in a study by Suchy *et al.* (2008). Silymarin can decrease endogenic cholesterol synthesis and total fat concentrations in serum by inhibiting of activity the key enzyme of regulating cholesterol synthesis that is hepatic 3-hydroxy- 3-methylglutaryl (HMG) CoA-reductase (Metwally *et al.* 2009).

In the morphology evaluation, we found that the experimental treatments decreased crypt depth and increased villus height to crypt depth ratio in the jejunum and ileum of broilers.

Low crypt depth and high villus height are the main indicators of intestinal health (Table 5). Deeper crypts and lower villus height to crypt depth ratio are indicators of faster tissue turnover, higher nutrient and energy for gut maintenance and lower animal performance (Xu *et al.* 2003).

Supplemental silymarin by at least 500 ppm increased villus height and width, villus height to crypt depth and apparent villus absorptive area in aflatoxicated broiler chicks. This might be related to the antibacterial activity of silymarin; therefore, it protects villi from endotoxins produced by pathogenic bacteria (Jahanian *et al.* 2017).

Table 5 Effect of milk thistle seed (MTS, % of diet) on intestinal morphology of broiler chickens (Ross 308)

MTS	Villus height (µm)	Crypt depth (µm)	Thickness of muscle layer[1] (µm)	Villus height to crypt depth	Villus surface area (mm^2)
Jejunum					
0	1147.24[a]	146.48[b]	249.76	5.19[a]	0.109[a]
0.2 %	1036.07[c]	167.63[a]	251.76	4.11[b]	0.098[b]
0.4 %	1087.22[bc]	149.40[b]	242.45	4.85[a]	0.111[a]
0.6 %	1096.67[ab]	139.18[b]	229.91	5.21[a]	0.094[b]
SEM	18.179	5.852	6.579	0.188	0.003
P-value	0.008	0.0009	0.13	0.004	0.005
Ileum					
0	775.94[c]	202.84[a]	178.52	2.53[d]	0.074[c]
0.2 %	778.89[c]	186.81[b]	175.86	2.76[c]	0.085[b]
0.4 %	822.17[b]	181.00[b]	179.50	3.00[b]	0.089[a]
0.6 %	845.93[a]	176.16[b]	173.97	3.18[a]	0.068[d]
SEM	0.85	1.89	4.39	1.70	1.46
P-value	0.0001	0.0009	0.9556	0.0001	0.0001

[1] Thick muscle layer: this parameter was defined as the distance between the lamina muscularis mucosae internally and the tunica serosa externally.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Yi *et al*. (2012) showed that dietary supplementation of 100 and 200 mg/kg of silymarin enhanced both villus height and villous height to crypt depth ratio in the jejunum, duodenum and ileum of ducks reared under oxidative stress. Hashemi Jabali *et al*. (2017), reported that the highest villus height values were obtained with 30 and 60 g/kg milk thistle meals incorporated in low-and high-energy diets, respectively, indicating the requirement for higher natural antioxidants inclusion in high-energy diets.

CONCLUSION

In general, it can be concluded that the use of milk thistle in the diet did not have a positive effect on growth performance, digestibility of dry matter and organic matter, intestinal morphology; however, the 0.6 percent milk thistle decreased the concentration of triglycerides, VLDL-c and LDL-c. Therefore, according to the results of this study, it is not recommended to use the milk thistle seed in broiler chickens diet.

ACKNOWLEDGEMENT

We sincerely thank the university of Mohaghegh Ardabili, Iran for financial support of this study.

REFERENCES

Ahmad M., Chand N., Khan R.U., Ahmad N., Khattak I. and Naz S. (2020). Dietary supplementation of milk thistle (*Silybum marianum*): growth performance, oxidative stress, and immune response in natural summer stressed broilers. *Trop. Anim. Health Prod.* **52,** 711-715.

Alheidary A., Rehman Z., Khan R.U. and Tahir M. (2017). anti-aflatoxin activities of milk thistle (*Silybum marianum*) in broiler. *Worlds Poult. Sci. J.* **73,** 559-566.

AOAC. (2000). Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

Ashraf S., Shaukat Bhatti A., Kamran Z. and Ahmed F. (2019). Assessment of refined functional carbohydrates as substitutes of antibiotic growth promoters in broilers: Effects on growth performance, immune responses, intestinal microflora and carcass characteristics. *Pakistan Vet. J.* **39(2),** 157-162.

Aviagen. (2019). Ross 308: Broiler Performance Objectives and Nutrition Specifications. Aviagen Ltd., Newbridge, UK.

Banaee M., Sureda A., Mirvaghefi A.R. and Rafei G.R. (2011). Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **37,** 885-896.

Blevins S., Siegel P.B., Blodgett D.J., Ehrich M., Saunders G.K. and Lewis R.M. (2010). Effects of silymarin on gossypol toxicosis in divergent lines of chickens. *Poult. Sci.* **89,** 1878-1886.

Chambers C.S., Holeˇcková V., Petrásková L., Biedermann D., Valentová K., Buchta M. and Kren V. (2017). The silymarin composition and why does it matter. *Food Res. Int.* **100,** 339-353.

Fenton T.W. and Fenton M. (1979). An improved procedure for the determination of chromic oxide in feed and feces. *J. Anim. Sci.* **59,** 631-634.

Friedewald W.T., Levy R.I. and Fredrickson D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18,** 499-502.

Gillessen A. and Hartmut H.J.S. (2020). Silymarin as supportive treatment in liver diseases: A narrative review. *Adv. Therapy*. **37,** 1279-1301.

Hashemi Jabali N.S., Mahdavi A.H., Ansari Mahyari S., Sedghi M. and Akbari Moghaddam Kakhki R.E. (2017). Effect of milk thistle meal on performance, ileal bacterial enumeration, jejunal morphology and blood lipid peroxidation in laying hens fed diets with different levels of metabolizable energy. *J. Anim. Physiol. Anim. Nutr*. **101,** 1-11.

Iji P.A., Hughes R.J., Choct M. and Tivey D.R. (2001). Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-australasian J. Anim. Sci*. **14,** 54-60.

Jahanian E., Mahdavi A.H., Asgary S. and Jahanian R. (2017). Effects of dietary inclusion of silymarin on performance, intestinal morphology and ileal bacterial count in aflatoxin-challenged broiler chicks. *J. Anim. Physiol. Anim. Nutr*. **101(5),** 43-54.

Metwally M.A.A., El-Gellal A.M. and El-Sawaisi S.M. (2009). Effects of silymarin on lipid metabolism in rats. *World Appl. Sci. J*. **6,** 1634-16377.

Mousa M.A. and Osman A.S. (2016). The implications of l-carnitine and silymarin supplementation on growth performance and some blood parameters of broilers. *Assiut Vet. Med.* **148,** 132-138.

Muhammad D., Chand N., Khan S., Sultan A., Mushtaq M. and Rafiullah M. (2012). Hepatoprotective role of milk thistle (Silybum marianum) in meat type chicken fed Aflatoxin B1 contaminated feed. *Pakistan Vet. J.* **9,** 102-110.

Puvaca N., Lika E., Cocoli S., Shtylla Kika T., Bursic V., Vukovic G., Tomas Simin M., Petrovic A. and Cara M. (2020). Use of tea tree essential oil (*Melaleuca alternifolia*) in laying hen's nutrition on performance and egg fatty acid profile as a promising sustainable organic agricultural tool. *Sustainability*. **12,** 3420-3431.

SAS Institute. (2001). SAS®/STAT Software, Release 8.2. SAS Institute, Inc., Cary, NC. USA.

Schiavone A., Righi F., Quarantelli A., Bruni R., Serventi P. and Fusari A. (2007). Use of Silybum marianum seed extract in broiler chicken nutrition: influence on performance and meat quality. *J. Anim. Physiol. Anim. Nutr*. **91,** 256-262.

Shanmugam S., Hong Park J., Sungbo C., and Kim I. (2022). Silymarin seed extract supplementation enhances the growth performance, meat quality, and nutrients digestibility, and reduces gas emission in broilers. *Anim. Biosci.* **8,** 1215-1222.

Stastník O., Juzl M., Karásek F., Štenclová H., Nedomová S., Pavlata L., Mrkvicová E., Doležal P. and Jarošová A. (2016). The effect of feeding milk thistle seed cakes on quality indicators of broiler chickens meat. *Potravinarstvo*. **10 (1)**, 248-254.

Suchy P., Strakova E., Kummer V., Herzig I. and Blechova E. (2008). Hepatoprotective effects of milk thistle (*Silybum marianum*) seed cakes during the chicken broiler fattening. *Acta Vet. Brno*. **77,** 31-38.

Sultan A., Ahmad S., Khan S., Khan R.U., Chand N., Tahir M. and Shakoor A. (2018). Comparative effect of zinc oxide and silymarin on growth, nutrient utilization and hematological parameters of heat distressed broiler. *Pakistan J. Zool.* **50,** 751-756.

Surai P.F. (2015). Silymarin as a Natural Antioxidant: An overview of the current evidence and perspectives. *Antioxidants*. **4,** 204-247.

Tajmohammadi A., Razavi B.M. and Hosseinzadeh H. (2018). Silybum marianum (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: A review. *Photother. Res*. **32,** 1933-1949.

Tedesco D., Steidler S., Galletti S., Tameni M., Sonzogni O. and Ravarotto L. (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chickens. *Poult. Sci.* **83(11),** 1839-1843.

Xu Z.R., Hu C.H., Xia M.S., Zhan X.A. and Wang M.Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult. Sci*. **82,** 1030-1036.

Yi D., Gu L., Ding B., Li M., Hou Y., Wang L. and Gong J. (2012). Effects of dietary silymarin supplementation on growth performance and oxidative status in *Carassius auratus* gibelio. *J. Anim. Vet. Adv.* **11,** 3399-3404.