

# Evaluation of blood chemical, lipids profile and immune response on broiler chicks fed with milk thistle (*Silybum marianum* L.) and thyme (*Thymus vulgaris* L.) seeds in south-eastern Iran

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## Abstract

This study was conducted to investigate the effects of supplementation of milk thistle seeds (MTS) and thyme seeds (TS) on blood chemical, lipid profile and immune response in broiler chicks. In this study, 160 one-day-old chicks (Ross 308) were allocated to four treatments with four replicates based on a completely randomized design in a 2x2 factorial arrangement. The treatment groups were (A) basal control diet, (B) basal diet with 0.2 g/kg of MTS, (C) basal diet with 0.2 g/kg of TS and (D) basal diet with 0.2 g/kg of MTS and 0.2 g/kg of TS. Birds fed the (D) supplemented treatment (MTS plus TS) had the greatest levels of total protein, 4.26±0.27 g/L; albumin, 2.21±0.02 g/L; globulin 2.28±0.23 g/L; and aspartate aminotransferase (AST) activity, 152.18±4.46 U/L than the control birds [group (A)] (P<0.05). MTS alone or in combination with TS reduced the cholesterol in the serum of the broilers (P<0.05), and this effect was more pronounced for the (C) treatment (TS alone) (P<0.05). Treatment consumption with MTS plus TS [group (D)] significantly increased the concentration of high-density lipoprotein cholesterol (HDL-C), 90.32±2.28 mmol/L, but low-density lipoprotein cholesterol (LDL-C), 10.44±0.07 mmol/L, and triglyceride concentrations, 60.75±2.65 mmol/L, were decreased compared to control (P<0.05). However, none of the immunity parameters and liver enzymes differed significantly in MTS or TS groups. The present research indicated that supplements of MTS and TS have a

protective influence on the lipids profile, total protein, globulin, albumin and AST levels in broiler chicks.

## Introduction

Milk thistle (*Silybum marianum* L.) is easy to identify due to the presence of white veins in the leaves.<sup>1</sup> Silymarin is a polyphenolic compound extract from milk thistle seeds (MTS).<sup>1,2</sup> Its active constituents, the flavonolignans silybin, isosilybin, silydianin and silycristin, are well-known for their hepatoprotectivity.<sup>2,3</sup> In fact, silybin is the most bio-active compound of silymarin and it has been demonstrated that silymarin acts as an antioxidant, reducing free radical mediated damage in tissues and inhibiting lipid peroxidation.<sup>3,4</sup> In recent years, some authors have reported that silybin and silymarin can stimulate protein synthesis and tissue regeneration in the liver.<sup>5</sup> Also, silymarin has alternative activities on metabolic parameters such as cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and glucose.<sup>6-8</sup> Thyme (*Thymus vulgaris* L.) is known in Iran as shirazi-thyme. The major components of thyme seeds (TS) are thymol and carvacrol.<sup>9</sup> Also, these components have been shown to possess potent antioxidant properties.<sup>10,11</sup> These plants are grown in all areas of Iran. MTS is typically administered as an encapsulated standardized extract and commonly used for treatment of chronic liver diseases.<sup>1,2,3</sup> TS is a valuable medicinal and condimental plant. It has several traditional uses as an anti-spasmodic, carminative, anti-fungal, antiseptic, and anti-rheumatic.<sup>9,10,11</sup> The *in vivo* reports on the effect of thyme and thistle seeds on blood parameters and immunity are very limited. Therefore, the objectives of this study were to investigate the effects of milk thistle and thyme seeds on blood chemical, lipids profile and immune response in broiler chicks.

## Materials and Methods

### Ethical approval

All animals received humane care in compliance with the guidelines of Research Center of Special Domestic Animal at Zabol University, Iran.

### Plants collection

*Silybum marianum* L. and *Thymus vulgaris* L. seeds were collected from Kashmar-Kohsorkh district (16.35° N latitude, 18.58° E longitude, about 1052 meters asl) in Khorasan-Razavi province, in the north-east of Iran, dur-

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Key words: Broiler chicks; immune response; lipids profile; milk thistle; thyme.

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Contributions: the authors contributed equally.

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ing autumn 2011 (Figure 1). Also, the samples were identified by Research Center of Special Domestic Animal, Zabol University, Iran.

### Animals and dietary treatments

Field work was conducted from 10 May to 10 July 2012 at Zabol University (Sistan and Baluchestan province in 61°29' 52" east longitude and 31°1' 47" north latitude, south-eastern Iran). A total of 160 1-day-old (Ross 308) male broiler chicks obtained from a commercial hatchery were used in this experiment to compare four dietary of MTS and TS. The chicks were randomly distributed into four treatment groups consisting of four replicate pens of 10 chicks each. Feed and water were provided *ad libitum*. The chicks were raised for a total of 35 days in metal batteries consisting of four cages each as a group (LxWxH=147x90x50 cm). The treatment groups were: (A) received the diet rather than the NRC (1994)<sup>12</sup> recommendation in starter and grower diets as a control (Table 1), (B) control diet +0.2 g/kg of MTS, (C) control diet +0.2 g/kg of TS, and (D) control diet +0.2 g/kg of MTS and 0.2 g/kg of TS.

### Sample collection and procedures

On day 35, after 8 h of fasting, blood samples

were collected in non-heparinized tubes of two birds in each group by puncturing the brachial vein. Serum was separated by centrifuging in a cooling centrifuge (Sigma 1-15K, Osterode am Harz, Germany) at 750 g for 10 min at room temperature and the plasma was stored at  $-20^{\circ}\text{C}$  until analysis. Serum biochemical assays like total protein, globulin, albumin, glucose, cholesterol, HDL-C, LDL-C, and triglycerides were measured using commercial kits (Accurex Biomedical Pvt. Ltd., Thane, India), whereas aspartate transaminase (AST), alanine aminotransferase (ALT) and  $\gamma$ -glutamyl transferase (GGT) were measured using kit (Agappe Diagnostics Ltd, Ernakulam, Kerala, India) on an auto-analyzer (Micro lab 300).<sup>13</sup> Also, antibody titer against newcastle and influenza viruses were measured by the hemagglutination inhibition test (HI).<sup>14</sup> Antibody titer against gambaro and bronchitis viruses plus immunoglobulin G (IgG) and immunoglobulin M (IgM) were evaluated by the enzyme-linked immunosorbent assay (ELISA) as described by Houghton *et al.*<sup>15</sup> In addition, one ml of collected blood sample was taken and transferred to tubes with ethylenediaminetetra-acetic acid (EDTA) for determination of heterophil and lymphocyte blood cell counts. A total of 100 leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope. The heterophil-to-lymphocyte ratio (H/L) was calculated and recorded.<sup>16</sup>

### Statistical analysis

The data were analyzed in a completely randomized design with a  $2 \times 2$  factorial arrangement using SAS software (SAS, 1999).<sup>17</sup> The treatments means were compared by Tukey-Kramer and least squares with their means  $\pm$  standard errors reported. Statistical significance for all data was considered to be  $P \leq 0.05$ . A P value between 0.05 and 0.01 was also considered to indicate trends.



Figure 1. Milk thistle (*Silybum marianum* L.) seeds and dry leaves of thyme (*Thymus vulgaris* L.).

Table 1. Composition of the diets fed to broilers during the starter and grower periods (as fed).

Ingredients	Starter (1-14 day)	Grower (15-28 day)
Maize	54.43	50.42
Wheat	-	10.00
Soybean meal (44% CP)	35.00	30.29
Fish meal (60% CP)	3.070	2.040
Soybean fat	3.290	3.570
Dicalcium phosphate	1.730	1.470
Oyster shell	1.160	1.040
Mineral premix <sup>a</sup>	0.500	0.500
Vitamin premix <sup>b</sup>	0.500	0.500
sodium chloride	0.200	0.200
DL-methionine	0.350	0.280
L-lysine	0.240	0.190
ME <sub>n</sub> (Kcal/kg)	2980	3050
CP (%)	22.00	20.00
Lys (%)	1.430	1.240
Met + Cys (%)	1.070	0.950
Thr (%)	0.840	0.740
Ca (%)	1.050	0.900
P (%)	0.520	0.450

<sup>a</sup>Provided at the following rates per kilogram of diet: Mn (from  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ), 0.63 mg; Zn (from ZnO), 0.52 mg; Fe (from  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), 22 mg; Cu (from  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 3 mg; I (from  $\text{Ca}(\text{IO}_3)_2 \cdot \text{H}_2\text{O}$ ), 0.63 mg; Se, 0.08 mg (from sodium selenite). <sup>b</sup>Provided at the following rates per kilogram of diet: 3400 IU vitamin A, 800 IU vitamin D<sub>3</sub>, 11 IU vitamin E, 0.74 mg vitamin B<sub>1</sub>, 4.3 mg vitamin B<sub>2</sub>, 0.4 mg vitamin B<sub>3</sub>, 1.6 mg vitamin B<sub>6</sub>, 0.41 mg vitamin B<sub>12</sub>, 1.8 mg vitamin K<sub>3</sub>, 0.6 mg folic acid, 1.8 mg H<sub>2</sub>, 200 mg Choline chloride. CP, crude protein; ME, metabolisable energy.

Table 2. Effect of different dietary treatments (g/kg) on blood chemical factors and lipids profile at the end of period (day 35).

Item	Unit	Control	MTS diet	TS diet	MTS+TS diet	P
Total protein	g/L	3.21 $\pm$ 0.29 <sup>b</sup>	3.44 $\pm$ 0.31 <sup>b</sup>	3.57 $\pm$ 0.27 <sup>ab</sup>	4.26 $\pm$ 0.27 <sup>a</sup>	<0.05
Glucose	g/L	161.47 $\pm$ 8.12	160.43 $\pm$ 8.07	164.16 $\pm$ 8.33	158.65 $\pm$ 8.42	0.42
Globulin	g/L	1.91 $\pm$ 0.23 <sup>b</sup>	1.95 $\pm$ 0.26 <sup>b</sup>	2.06 $\pm$ 0.24 <sup>ab</sup>	2.28 $\pm$ 0.23 <sup>a</sup>	<0.05
Albumin	g/L	1.91 $\pm$ 0.02 <sup>b</sup>	1.92 $\pm$ 0.02 <sup>b</sup>	1.94 $\pm$ 0.02 <sup>b</sup>	2.21 $\pm$ 0.02 <sup>a</sup>	<0.05
AST	U/L	109.44 $\pm$ 3.45 <sup>b</sup>	104.55 $\pm$ 3.41 <sup>b</sup>	131.03 $\pm$ 4.52 <sup>ab</sup>	152.18 $\pm$ 4.46 <sup>a</sup>	<0.05
ALT	U/L	45.37 $\pm$ 2.54	42.35 $\pm$ 2.54	46.27 $\pm$ 2.57	47.38 $\pm$ 2.54	0.28
GGT	U/L	35.21 $\pm$ 1.24	34.13 $\pm$ 1.26	33.28 $\pm$ 1.24	33.35 $\pm$ 1.25	0.34
Cholesterol	mmol/L	177.08 $\pm$ 3.35 <sup>a</sup>	153.77 $\pm$ 3.42 <sup>ab</sup>	142.06 $\pm$ 3.51 <sup>b</sup>	153.15 $\pm$ 3.44 <sup>ab</sup>	<0.05
Triglyceride	mmol/L	69.56 $\pm$ 2.69 <sup>a</sup>	68.39 $\pm$ 2.66 <sup>ab</sup>	66.07 $\pm$ 2.56 <sup>ab</sup>	60.75 $\pm$ 2.65 <sup>b</sup>	<0.05
LDL cholesterol	mmol/L	13.37 $\pm$ 0.07 <sup>a</sup>	12.17 $\pm$ 0.07 <sup>ab</sup>	11.41 $\pm$ 0.07 <sup>ab</sup>	10.44 $\pm$ 0.07 <sup>b</sup>	<0.05
HDL cholesterol	mmol/L	82.14 $\pm$ 2.19 <sup>b</sup>	85.68 $\pm$ 2.22 <sup>b</sup>	98.43 $\pm$ 2.21 <sup>a</sup>	90.32 $\pm$ 2.28 <sup>ab</sup>	<0.05

<sup>a,b</sup> Means within a row with different superscripts differ significantly ( $P < 0.05$ ). MTS, Milk thistle seed; TS, thyme seed; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT,  $\gamma$ -glutamyl transferase; LDL, very low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol.

## Results

### Blood chemical factors

The blood chemical factors and lipids profile of experimental diets are summarized in Table 2. In broilers fed with MTS and TS, the concentrations of serum protein, globulin, albumin and AST ( $4.26 \pm 0.27$  g/L,  $2.28 \pm 0.23$  g/L,  $2.21 \pm 0.02$  g/L and  $152.18 \pm 4.46$  U/L, respectively), in group (D) were greater than those in groups (A) and (B). Despite this evident effect of TS plus MTS [Group (D)], there was no difference in the protein and globulin levels when the birds received only MTS or TS in their standard diet [groups (B) and (C), respectively]. Also, dietary MTS or TS did not affect the concentration of glucose and enzyme activities such as ALT and GGT in any of the experimental groups.

### Lipids profile

Consumption of TS alone in their standard diet [group (C)] resulted in the chickens having a significantly reduced level of serum cholesterol ( $142 \pm 3.51$  mmol/L) and increased level of HDL-c ( $98.43 \pm 20.21$  mmol/L) compared to the control [group (A)]. The cholesterol levels were found the same in groups (B), (C) and (D) compared to the control [group (A)]. The total concentrations of LDL-c ( $10.44 \pm 0.07$  mmol/L) and triglycerides ( $60.75 \pm 2.65$  mmol/L) in the group (D) (feed supplemented by MTS and TS) were significantly less than those of group (A) ( $P < 0.05$ ). However, the concentration of serum HDL-c ( $98.43 \pm 2.21$  mmol/L) was significantly increased in group (C) over that of control ( $P < 0.05$ ).

### Humoral immune responses

Table 3 shows the effect of different levels of MTS and TS on humoral immune responses in broilers at the end of the period (day 35). The treatment groups had no statistical effect on antibody titers against Newcastle, influenza,

gambaro and bronchitis disease virus on day 35 of the period. Immuno cells, consisting of heterophil, lymphocyte, and the ration of the former to the latter did not differ significantly among the different treatments. Also, the immunoglobulin antibody titer showed no significant change in either IgM or IgG in any of the groups that were treated. The present study was unable to answer questions concerning the mechanisms of the immune effect provided by MTS or TS alone, and further studies are required in this area.

## Discussion and Conclusions

Main pharmacological effects of thyme are attributed to thymol and carvacrol, these are the main bioactive components of thyme.<sup>9</sup> Recently, a few studies were conducted to interaction effects of MTS and TS on serum biochemical factors in broiler chicks. Similar to our results, Fani makki *et al.*<sup>18</sup> reported that with combination of 200 mg/kg of MTS plus TS in poultry diets at 21 days of age, some variables in serum such as, total protein, globulin and albumin were increased compared to control diet ( $P < 0.05$ ). Silymarin mechanism of action is still poorly understood. It may act as an antioxidant.<sup>19,20</sup> The results of this study indicate that the dietary treatments did not influence the serum glucose concentrations. Other authors reported that, the dietary supplementation of this plant's extracts such as silymarin, thymol and carvacrol may have increased the serum protein and globulin concentrations by increasing the absorption of albumin in the lower intestine.<sup>19,21</sup> Moreover, the complete identification effect of MTSs and TSs on hematological activity in broilers needs more research in this field. Similar to the results obtained in this study, Fani makki *et al.*<sup>18</sup> showed a significant decrease in triglycerides, total cholesterol and LDL in the serum of broilers treated with MTS and TS. Similarly, Radwan *et al.*<sup>22</sup> reported that the addition of

1% thyme to broiler diet resulted in a marked decrease in plasma total lipids. Milk thistle also exerts antioxidant and membrane stabilizing activity and has important attributes for the livers secretion and uptake of plasma lipoproteins, thereby altering lipid metabolism.<sup>7</sup> These findings of the current study are consistent with those reported by Ali *et al.*<sup>23</sup> who found that the HDL-C concentration in laying hens was higher (36 mmol/L) than the control diet. Also, the reduction of triglycerides and cholesterol noticed with thyme in animal studies was attributed to the lowering effect of thymol or carvacrol on HMG-Co A reductase, the rate-limiting enzyme of cholesterol synthesis.<sup>16</sup> Chand *et al.*<sup>24</sup> reported that milk thistle supports the immune system through its powerful antioxidant, free-radical-scavenging action, its ability to preserve the supply of another important antioxidant, glutathione, as well as its direct effects on immune cells. Silymarin protects the immune cells and organs against oxidative damages that cause immunosuppression. Also, thyme has been reported to have anti-bacterial and fungicidal activities and the major components of thyme, *i.e.*, the essential oils thymol and carvacrol, have been suggested to possess potent antioxidant properties, hence an increase in immune responses of the chicks was anticipated.<sup>16,25</sup> Although the dietary treatments did not induce any significant effect on the immune-related parameters measured in this study, neither was any deleterious impact detected as a result of the addition of thistle and/or thyme seeds to the broilers diet. This was probably due to the low levels of the additives that were used in our study. Unfortunately, to the best of our knowledge, no reports are available on the impact of thyme plus thistle seeds on bird's immune responses. In conclusion, our results indicated that supplements of thistle seeds plus thyme seeds could improve total protein, globulin, albumin, HDL-c, while simultaneously decreasing cholesterol, triglycerides and LDL-C by affecting the metabolism of fat in broilers.

**Table 3. Effect of the experimental diets (gr/kg) on antibody titers viruses, heterophil & lymphocyte ratio and immunoglobulins at day 35.**

Item	Unit	Control	MTS diet	TS diet	MTS+TS diet	P
Newcastle	Log <sub>2</sub>	10.51±0.08	10.45±0.08	10.48±0.08	10.38±0.08	0.99
Influenza	Log <sub>2</sub>	6.63±0.07	6.25±0.07	6.54±0.07	6.42±0.07	0.47
Gambaro	-	2341.24±101.1	2415.35±98.21	2430.21±110.1	2483.32±124.5	0.24
Bronchitis	-	2104.17±127.7	2052.21±134.5	2084.45±124.6	2021.13±133.3	0.33
Heterophil	%	27.84±1.34	28.58±1.35	28.41±1.37	28.09±1.32	0.18
Lymphocyte	%	60.16±1.66	61.23±1.61	61.64±1.59	61.27±1.53	0.65
H/L	%	0.469±0.24	0.475±0.27	0.461±0.21	0.458±0.26	0.38
IgG	mg/dL	486.72±25.35	444.11±25.44	441.15±25.62	452.25±25.49	0.84
IgM	mg/dL	146.06±7.47	141.35±7.44	142.39±7.28	144.34±7.64	0.14

MTS, Milk thistle seed; TS, thyme seed; H/L, heterophil to lymphocyte ratio; IgG, immunoglobulin G; IgM, immunoglobulin M.

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