Agr. Nat. Resour. 53 (2019) 429–432

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Milk thistle protective effect on liver and kidney of broiler chickens

Shahabodin Gharahveysi

Department of Animal Science, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

Article Info

Article history:
Received 1 December 2017
Revised 29 November 2018
Accepted 11 December 2018
Available online 30 August 2019

Keywords:
Blood metabolite,
Liver,
Medicinal plants,
Silybum marianum

Abstract

Milk thistle, *Silybum marianum* is a useful medicinal plant, whose extract has antioxidant properties. The protective effect of the milk thistle medicinal plant was studied on the liver and kidney of Ross broiler chickens. Milk thistle at three levels (0%, 0.3%, 3%) as well as cotrimoxazole at two levels (0 g/kg, 2 g/kg) were used in five replications. Blood metabolites were measured at age 42 d. Data were analyzed using a general linear model. The effect of milk thistle levels was significant ($p < 0.05$) on blood cholesterol. Cotrimoxazole had a highly significant ($p < 0.01$) effect on blood cholesterol. The effect of milk thistle levels was significant ($p < 0.05$) on the levels of uric acid and blood creatinine. The effect of milk thistle levels was highly significant ($p < 0.01$) on blood bilirubin. The difference in the mean levels of milk thistle and cotrimoxazole was significant ($p < 0.05$) for prothrombin time. The use of different levels of milk thistle reduced liver and kidney problems and modified the undesirable effects of cotrimoxazole. A level of 3% milk thistle had the most protective effect on the liver and kidney of broilers.

Introduction

Feed additives have an important role in increasing the production and profitability of poultry farms (Mousavi Kashani et al., 2017). Medicinal plants are effective in maintaining safety and enhancing the quality of human life and well-being (Tyler, 1993; Radco and Cybulski, 2007). Medicinal plants are beneficial and have no undesirable side effects on animal production (Schiavone et al., 2007). Therefore, they can be used for their various benefits such as therapeutic properties (Alcicek et al., 2003). Medicinal plant extracts due to antioxidant and antibacterial features have been employed traditionally to treat and control some diseases (Hernandez et al., 2004). Milk thistle (*Silybum marianum*) is a useful medicinal plant, belonging to the family Asteraceae (Zargari, 1997). The extract of this plant has a beneficial substance called silymarin (Sobolova et al., 2006; Schiavone et al., 2007). Silybin is the major active constituent of silymarin. This substance is an antioxidant and liver protector. Its concentration in the bile is 60 times higher than in the blood (Tyler, 1993). The most notable effect of milk thistle is related to its level of silymarin (Vogel et al., 1975; Radco and Cybulski, 2007). Silymarin plays a role in liver cell membrane stability and prevents the binding of many toxins and drugs with this membrane. The protective role of silymarin is due to the removal of free radicals and increased superoxide dismutase activity (Tedesco et al., 2004; Kalorey et al., 2005). Neutralization of free radicals and toxins and anti-oxidant properties are some of the effects of milk thistle that act to protect the liver (Vogel et al., 1975).

Sobolova et al. (2006) evaluated the effects of milk thistle powder on the blood metabolites of the broiler chickens and reported that the milk thistle powder exerted protective effects on liver tissue, prevented serious liver problems and caused metabolic changes associated with liver enzymes to modify adverse changes in blood lipids.

E-mail address: S.gharavysi@gmail.com (S. Gharahveysi)

https://doi.org/10.34044/j.anres.2019.53.4.14

The objective of the present study was to study the protective effect of the milk thistle medicinal plant on the liver and kidney of Ross broiler chicken.

Materials and Methods

Experimental design

The study was designed and implemented as a factorial experiment in completely randomized design. The factors were the milk thistle at three levels (0%, 0.3%, 3%) and cotrimoxazole at two levels (0 g/kg, 2 g/kg). Five replications were considered for each treatment, and 30 experimental units (pens) were used. There were 20 Ross broilers (one-day-old) in each experimental unit. At the beginning of the rearing phase and before entering the chickens into the farm, the temperature was fixed at 32°C. The light system was 23:1 light:darkness. The chickens had free access to water and a standard ration based on corn and soybean meal (Table 1). The experimental rations contained no anti-coccidial drugs and antibiotics. As presented in Table 1, the experimental rations for chickens were formulated into starter and grower stages (NRC, 1994). The rearing period of chickens was 42 d.

There are tests to check the liver and kidney function and health status by measuring blood metabolites and the results can be used to diagnose disease or inflammation or damage of the liver and kidney (Guyton and Hall, 2006; Tedesco et al., 2004). Thus, at the end of the experimental period (42 d), eight birds were selected from each treatment, and blood samples were taken from the wing vein to measure blood metabolites. The samples were transferred to a specialized veterinary laboratory. Concentrations of glucose, cholesterol, uric acid, bilirubin, creatinine and prothrombin time (PT) were measured. The PT test measures the time required for blood clotting as well as anticoagulants function (Shalaei and Hosseini, 2016; Fani Maki et al., 2014).

Statistical analysis

Data were analyzed using the general linear model facilitated by SAS statistical software package (version 9.1, SAS Institute, Cary, NC, USA). Duncan's multiple range test was used at a significance level of 0.05 to compare the means of factors and treatments. The statistical model used is shown as:

$$y_{ijk} = m + A_i + B_j + (AB)_{ij} + e_{ijk}$$

where, y_{ijk} is the value of each observation; m is the effect of the mean; A_i is the effect of the milk thistle level; B_j is the effect of the cotrimoxazole level; $(AB)_{ij}$ is the interaction effect of the milk thistle and cotrimoxazole levels and e_{ijk} is the residual effect.

Results and Discussion

The effects of different levels of milk thistle and cotrimoxazole on blood metabolites are shown in Table 2. The interaction of these factors on the traits is presented in Table 3.

Table 1 Feed materials and ration nutrient composition (%)[1]

Ingredient	Starter (1–21 d)	Grower (22–42 d)
Corn	54.30	61.50
Soybean meal	39.00	32.49
Sunflower oil	2.45	2.45
Limestone	1.28	1.39
Di-calcium phosphate	1.84	1.25
Salt	0.47	0.35
Mineral supplement[2]	0.25	0.25
Vitamin supplement[3]	0.25	0.25
DL-methionine	0.16	0.07
Chemical composition		
Metabolizable energy[4]	3020	3110
Crude protein (%)	21.64	19.42
Crude fat (%)	4.83	5.05
Calcium (%)	1.00	0.90
Available P (%)	0.48	0.36
Sodium (%)	0.20	0.15
Lysine (%)	1.37	1.18
Methionine (%)	0.50	0.38

1 = ration containing a minimum concentration of nutrients recommended by the NRC (1994).

2 = 1 kg mineral supplement provides the following items: 50,000 mg of manganese, 25,000 mg of iron, 50,000 mg of zinc, 5,000 mg of copper, 500 mg of iodine, 100 mg of selenium.

3 = 1 kg of vitamin supplement provides the following items: 3,500,000 international units (IU) of vitamin A, 1,000,000 IU of vitamin D3, 9,000 IU of vitamin E, 1,000 mg of vitamin K3, 900 mg of vitamin B1, 3,300 mg of vitamin B2, 5,000 mg of vitamin B3, 1,500 mg of vitamin B5, 150 mg of vitamin B6, 500 mg of vitamin B9, 7.5 mg of vitamin B12, 250,000 mg of vitamin choline and 500 mg of vitamin biotin.

4 = metabolizable energy, in kilocalories per kilogram.

Table 2 Effect of the milk thistle and cotrimoxazole antibiotic on blood metabolites (mean±SD)

Factor	Uric acid (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)	Bilirubin (mg/dL)	PT (s)
MT-0%	5.90^{a}±0.91	0.55^{a}±0.04	265.80±10.21	134.70^{a}±11.45	1.60^{a}±0.12	48.90^{a}±1.76
MT-0.3%	5.00^{ab}±0.98	0.48^{a}±0.05	255.20±11.09	122.80^{b}±12.32	1.10^{b}±0.13	42.10^{ab}±1.82
MT-3%	4.50^{b}±0.93	0.30^{b}±0.05	246.00±10.75	118.20^{b}±11.95	1.00^{b}±0.12	36.50^{b}±1.69
CM-0 g/kg	5.00±0.36	0.58±0.04	241.80^{b}±10.08	118.40^{a}±11.12	1.50±0.12	48.40^{a}±1.34
CM-2 g/kg	5.20±0.41	0.57±0.05	264.20^{a}±10.98	132.10^{b}±11.67	1.90±0.13	41.10^{b}±1.51

MT = milk thistle; CM = Cotrimoxazole; PT = prothrombin time; Means in the same column superscripted with different letters indicate significant difference ($p < 0.05$).

Table 3 Interaction effects of milk thistle and cotrimoxazole antibiotic levels on blood metabolites (mean±SD)

Interaction effects	Uric Acid (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Bilirubin (mg/dl)	PT (Second)
MT-0%×CM-0 g/kg	6.20^{a}±0.41	0.60±0.05	249.30^{ab}±10.34	130.30^{a}±11.32	1.80^{a}±0.13	46.30^{a}±1.70
MT-0%×CM-2 g/kg	5.90^{ab}±0.40	0.58±0.05	266.60^{a}±10.68	139.30^{a}±11.61	1.60^{a}±0.12	47.90^{a}±1.62
MT-0.3%×CM-0 g/kg	5.60^{ab}±0.39	0.57±0.06	235.60^{b}±10.12	115.60^{b}±11.03	1.30^{ab}±0.12	42.60^{ab}±1.43
MT-0.3%×CM-2 g/kg	5.30^{b}±0.39	0.53±0.04	256.30^{a}±10.43	134.30^{a}±11.29	1.10^{b}±0.12	41.30^{ab}±1.36
MT-3%×CM-0 g/kg	5.10^{b}±0.38	0.53±0.04	240.30^{b}10.30	116.30^{b}±11.10	1.00^{b}±0.11	37.10^{b}±1.25
MT-3%×CM-2 g/kg	5.30^{b}±0.38	0.52±0.04	270.20^{a}±10.71	122.20^{ab}±11.27	0.90^{b}±0.10	35.30^{b}±1.20

PT = prothrombin time; MT = milk thistle; CM = Cotrimoxazole; Means in the same column superscripted with different letters indicate significant difference ($p < 0.05$)

The effects of different levels of milk thistle, and the interaction of Cotrimoxazole and milk thistle levels were significant on blood uric acid. With an increase in the levels of milk thistle, kidney function improved because it reduced the concentration of uric acid in the blood. The interaction of the cotrimoxazole and milk thistle levels showed that at the 0% and 0.3% levels of milk thistle, the uric acid concentration in the blood decreased with increased cotrimoxazole. However, at the 3% level of milk thistle, the uric acid concentration increased. In another study, the effect of milk thistle levels was not significant ($p > 0.05$) on uric acid levels in Ross broilers (Schiavone et al., 2007), which was inconsistent with the results of the current study. In another study, 0.8 % silymarin had a significant ($p < 0.05$) effect on uric acid in Ross broilers (Jamshidi et al., 2008), in line with the findings of the present study. According to Table 3, the use of milk thistle resulted in a reduced concentration of blood uric acid; milk thistle modified the additive effect of cotrimoxazole on blood uric acid.

Only the effect of different levels of milk thistle was significant ($p < 0.05$) on blood creatinine. With an increase in the levels of milk thistle, kidney function improved because of the reduced concentration of blood creatinine. Shalaei and Hosseini (2016) reported there was no significant ($p > 0.05$) effect of three levels of milk thistle (0%, 1% and 2%) on the blood creatinine level in laying hens which disagreed with the results of the current study. Two main reasons for the differences could be there were different levels of milk thistle and variation in the breeds of poultry examined.

As can be seen in Table 2, milk thistle has no significant effect on blood glucose but cotrimoxazole did have a significant effect on blood glucose. On the other hand, there was a significant interaction of milk thistle and cotrimoxazole on blood glucose. The interaction of cotrimoxazole and milk thistle levels showed that in a specific amount of milk thistle, the glucose concentration in the blood increased with increased cotrimoxazole. Ebrahimi et al. (2014) conducted an experiment using broilers with different levels of silymarin (0 mg, 100 mg, 200 mg) and reported no significant ($p > 0.05$) effect on blood glucose levels. In another study on broiler chickens, there was no significant ($p > 0.05$) effect of 200 mg/kg of milk thistle on the blood glucose concentration (Fani Maki et al., 2014). The results of both these other studies corresponded with the results of the current study. The level of glucose in the body depends on a variety of mechanisms and the liver can release glucose to the blood when needed to feed cells (Guyton and Hall, 2006). Therefore, hepatic glucose production may be impaired in times of need when there is dysfunction in the liver.

There was a significant effect of milk thistle level on the blood cholesterol concentration and cotrimoxazole had a highly significant ($p < 0.01$) effect on blood cholesterol. Furthermore, the interaction effect of milk thistle and cotrimoxazole was significant on blood cholesterol. The interaction of cotrimoxazole and milk thistle levels showed that in a specific amount of milk thistle, the cholesterol concentration in the blood increased with an increase in the amount of cotrimoxazole. In a study of broiler chickens, different levels of 0 mg, 100 mg and 200 mg of silymarin had no significant ($p > 0.05$) effect on blood cholesterol levels (Ebrahimi et al., 2014). Another study found no significant ($p > 0.05$) effect of different levels of milk thistle on blood cholesterol levels (Schiavone et al., 2007). Fani Maki et al. (2014) reported a significant ($p < 0.05$) effect of 200 mg/kg milk thistle on blood cholesterol in broilers.

The effect of milk thistle levels was highly significant ($p < 0.01$) on blood bilirubin; in addition, the interaction of milk thistle and cotrimoxazole was significant ($p < 0.05$) on blood bilirubin. The interaction of cotrimoxazole and milk thistle levels showed that in a specific amount of milk thistle, the bilirubin concentration in the blood decreased with an increase in the amount of cotrimoxazole. Milk thistle effects were significant ($p < 0.05$) on blood creatinine levels. Bilirubin is a waste product from the breakdown of red blood cells and is processed by the liver (Guyton and Hall, 2006). Therefore, it can be excreted in stools. Furthermore, bilirubin flows in the bile ducts of the liver and is dissolved in the bile (Hernandez et al., 2004; Guyton and Hall, 2006). The serum bilirubin concentration

may increase as a result of impaired bile flow, which can in severe liver disease, gallbladder disease or other conditions of the biliary system (Guyton and Hall, 2006). A high concentration of bilirubin in the blood causes jaundice (Fani Maki et al., 2014; Guyton and Hall, 2006). Thus, assessment of bilirubin is a good test to check liver function (Fani Maki et al., 2014; Guyton and Hall, 2006). The present results indicated that the blood bilirubin level decreased with increasing levels of milk thistle, suggesting a favorable effect of milk thistle herb to improve liver function. On the other hand, the use of antibiotics causes liver damage that can be seen by an increased concentration of bilirubin in the blood (Guyton and Hall, 2006).

The differences in the mean levels of milk thistle and cotrimoxazole, and also the interaction between these two factors were significant ($p < 0.05$) for the PT. The interaction of cotrimoxazole and milk thistle levels showed that with no added milk thistle, the PT increased with an increase in the amount of cotrimoxazole. However, with 0.3% and 3% levels of milk thistle, the PT decreased. The liver secretes some important blood clotting factors and prolongation of the blood coagulation time is a reason for defects and damage to the liver (Fani Maki et al., 2014; Guyton and Hall, 2006). The blood clotting time was reduced by increasing the usage level of milk thistle, which suggested that the plant has a positive effect in improving liver function. It can be concluded that the blood clotting time increases in cases where the liver is damaged or impaired.

The results of the present study indicate that the use of cotrimoxazole caused liver and kidney problems, but the use of different levels of milk thistle reduced these problems because the milk thistle modified the undesirable effects of cotrimoxazole.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are grateful to everyone who participated in this study.

References

Alcicek, A., Bozkurt, M., Cabuk, M. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. S. Afr. J. Anim. Sci. 33: 89–94.

Ebrahimi, E., Mohamad Abadi, T., Sari, M., Zamiri, M.J., Beigi Nasiri, M. 2014. The effect of silymarin on oxidative stress induced by lead in broiler chickens. Iran. J. Anim. Sci. Res. 5: 302–312.

Fani Maki, A., Ebrahimzadeh, A., Ansari Nik, H. 2014. The effect of medicinal plants milk thistle (*Silybum marianum L.*) and thyme (*Thymus vulgaris* L.) on the immune system and some blood parameters in broiler chickens. Iran. J. Vet. Clin. Path. 7: 26–35.

Guyton, C., Hall, E. 2006. Textbook of Medical Physiology. Elsevier Inc. Philadelphia, PA, USA.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., Megias, M.D. 2004. Influence of two plants extracts on broiler performance, digestibility and digestive organ size. Poult. Sci. 83: 169–174.

Jamshidi, A., Ahmadi Ashtiani, H., Gholamhosseini, B., Bakaei, S. 2008. The effects of oral administration of the extract milk thistle (silymarin) on histological and biochemical changes caused by aflatoxin in broilers. Iran. J. Med. Plant. 4: 24–32.

Kalorey, D.R., Kurkure, N.V., Ramgaonkar, I.S., Sakhare, P.S., Warke, S., Nigot, N. 2005. Effect of polyherbal feed supplement "Growell" during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Poult. Sci. 65: 2239–2245.

Mousavi Kashani, S.M., Darvish, S., Gharahveysi, S. 2017. Litter composition comparisons of the four different broiler chicken strains fed with two types of common and specific feeds. Anim. Res. Int. 14: 2785–2792.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry, 9th ed. National Academy Press. Washington D.C., U.S.A.

Radco, L., Cybulski, W. 2007. Application of silymarin in human and animal medicine. Wounds. 1: 22–26.

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

Shalaei, M., Hosseini, S.M. 2016. Effect of purslane and thistle herbs in the diet on enzyme activity, blood metabolites and egg characteristics in laying hens. Iran. J. Anim. Sci. 106: 91–102.

Sobolova, L., Skottova, N., Vecera, R., Urbanek, K. 2006. Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats. Pharmacol. Res. 53: 104–112.

Tedesco, D., Steidler, S., Galletti, S., Tameni, M., Sonzogni, O., Ravarotto, L. 2004. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. Poult. Sci. 83: 1839–1843.

Tyler, V. 1993. Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies, 3rd ed., Haworth Herbal Press. Binghamton, NY, USA.

Vogel, G., Trost, W., Brraatz, R. 1975. Pharmacodynamics, site and mechanism of action *Silybum marianum* (L.) Gaertn. 1. Acute toxicology or tolerance, general and specific (liver-) pharmacology. Arzneimittel-Forsch. 25: 82–89.

Zargari, E. 1997. The Medicinal Plants. Tehran University Publication. Tehran, Iran.