

EVALUATION OF THE EFFECTIVENESS OF ADDING DIFFERENT CONCENTRATIONS OF LEAVES ALCOHOLIC EXTRACT OF THE LOCAL SILYBUM PLANT (*SILYBUM MARIANUM*) TO DRINKING WATER ON SOME BIOCHEMICAL CHARACTERISTICS OF BROILER ROSS 308

Sajjad Mohammed Abba, Nihad Abdul-Lateef Ali, Majeed Ajafar

Department of Animal Production, College of Agriculture, Al-Qasim Green University, Iraq
E-mail: dr.nihad@agre.uoqasim.edu.iq

Abstract

This experiment was conducted in the fields of Al-Anwar Poultry Company in Babylon Governorate for the period from 18/2/2023 to 24/3/2023. The study aimed to evaluate the effectiveness of adding different concentrations of leaves alcoholic extract of the local silybum plant (*Silybum marianum*) to Drinking Water on Some Biochemical Characteristics of Broiler, where 300 unsexed Ross 308 broilers equipped with a hatchery were used in the experiment. It was randomly distributed to 15 (cages) with 5 experimental treatments for each 60 bird treatment and each treatment included three replicates per 20 birds. The treatments of the experiment were as follows: the first treatment: a control group free of any addition, the second treatment: the addition of 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 0.5%, the third treatment: the addition of 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 1%, the fourth treatment: the addition of 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 2%, the fifth treatment: Add 10 ml of alcoholic extract of the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 3%.

The results of the experiment showed a significant improvement ($P \leq 0.05$) for the fifth treatment in the concentration of total protein and albumin compared to the other experimental treatments, but with regard to the concentration of globulin and glucose, no significant differences were recorded between all treatments, while a significant decrease ($P \leq 0.05$) is noted in the concentration of the enzyme (Alanine Aminotransferase) ALT for the third, fourth and fifth treatments compared to the first treatment (control), and the treatments of adding alcoholic extract to the third, fourth and fifth leaves of the *Silybum marianum* plant showed a significant decrease ($p \leq 0.05$) in the level of As for the concentration of triglycerides, the second, third, fourth and fifth treatments recorded a significant decrease ($p \leq 0.05$) compared to the first treatment (control), as well as the third, fourth and fifth treatments recorded an increase in the concentration of high-density lipoproteins, and in return recorded a significant decrease ($p \leq 0.05$) in the concentration of low-density lipoproteins in bird blood serum compared to the first treatment (control).

Key words: *Silybum marianum* leaves, alcoholic extract, biochemical characteristics, broiler

Introduction

The production process of international companies specialized in the manufacture of poultry birds is affected by the increase in the population, which encouraged them to increase their

production capacity, especially for broilers from commercial hybrids, which are characterized by rapid growth rates, which are accompanied by a high metabolic rate, which makes them more vulnerable to oxidative stress and free radical formation (Manju et al., 2010), as well as a significant decrease in bird immunity (Deif et al., 2007). Which encouraged researchers to add natural antioxidants as a key factor in poultry nutrition for their role in removing free radicals and thus improving productive performance, maintaining high growth levels and increasing immunity for broilers (Surai, 2020). For its effective role in reducing oxidative reactions, whether inside the body or in meat during storage, being safer and cheaper, and thus being a good alternative to chemical antibiotics, which were banned from being added to poultry diets in 2006 by the European Union countries due to their effects. harmful to consumer safety and health (Salami et al., 2015). One of these plants is the *Silybum marianum* plant or milk thistle (*Silybum marianum*), which is a useful medicinal plant, belonging to the *Carduus marianum* family (Zagari, 1997) and has a wide use in poultry nutrition (Stastnik et al., 2020) and contains many substances with antioxidant effectiveness and has many medicinal and biological activities (Wizard, 2004) and the leaves of *Algalgan* contain protein up to 19% and contain many essential amino acids (Marceddu et al., 2022) as well as About the essential fatty acids predominantly oleic and linoleic acid (Majidi et al., 2021), silymarin is the main active active compound of the plant (Aziz et al., 2021) where silymarin consists of four main components, namely selebinin and is considered the most potent, isosilepinin, seledenin and silycristine (Bijak, 2017) One study indicates that silymarin works in four different pathways: it is an antioxidant, works on the absorption and regulation of intracellular clotathione, anti-inflammatory, reduces cholesterol and blood lipids (Khazaei et al., 2022), as a stabilizing and regulating factor for cell membrane permeability that prevents the introduction of foreign substances into liver cells where it works to completely protect the liver from toxic agents and helps to regenerate it (Alhidary et al., 2017), and also promotes RNA production (Ajay et al., 2009). Sulimarin extracted from the *Silybum marianum* plant has antiviral and anti-inflammatory properties (Bahmani et al., 2015).

Due to the lack of local studies on the addition of alcoholic extract of the leaves of the *Silybum marianum* plant to the drinking water of broilers and to benefit from the *Silybum marianum* plant and spread in large quantities in gardens and agricultural methods, this study aimed to evaluate the effectiveness of adding the alcoholic extract of the leaves of the *Silybum marianum* plant to drinking water on some of the biochemical qualities of broilers.

Materials and methods

This study was conducted in the field of Al-Anwar Poultry Company in Babylon Governorate for the period from 18/2/2023 to 24/3/2023. The experiment was used 300 chicks broiler type Ross 308 age of one day non-naturalized randomly distributed to 15 cages by 5 experimental treatments per treatment 60 birds and each treatment included three repeaters per repeater 20 birds, and feed was provided to birds freely on the basis of the catalog Ross as provided a diet starting from the age of 1 - 21 days, and the growth diet from the age of 22 - 35 days as shown in (Table 1) where the treatments of the experiment as follows: The first treatment: a control group free of any addition, the second treatment: the addition of 10 ml of alcoholic extract to the leaves of the

Silybum marianum plant / liter of drinking water at a concentration of 0.5%, the third treatment: the addition of 10 ml of alcoholic extract to the leaves of the Silybum marianum plant / liter of drinking water at a concentration of 1%, the fourth treatment: the addition of 10 ml of alcoholic extract to the leaves of the Silybum marianum plant / liter of drinking water at a concentration of 2%, the fifth treatment: Add 10 ml of alcoholic extract of the leaves of the Silybum marianum plant / liter of drinking water at a concentration of 3%.

The experiment included the study of the following characteristics: total protein, albumin, globulin, glucose, ALT, AST, cholesterol, triglycerides, high-density lipoproteins, low density lipoproteins, very low density lipoproteins. Completely Randomized Design was used to study the effect of different treatments on the studied traits, and the differences between the averages were compared using the Duncan polynomial test (Duncan, 1995) and the SAS (2012) statistical program was used to analyze the data.

Table 1 Percentages of feed components used in the experiment and their chemical composition

Ingredient	Starter%	Grower%
Corn	38.50	42.29
Soya meal	44.36	40.11
Wheat	10	10
Concentrate	2.5	2.5
Di Calcium phosphate	0.04	0.0
Calcium carbonate	1.26	1.07
Sunflower oil	2.29	3.15
Sodium chloride	0.17	0.17
Supplement vitamin. & mineral	0.5	0.5
DL-Methionine (99%)	0.21	1.2
L-Lysine (99%)	0.09	0.1
Therionin	0.08	0.05
Total	100	100
The calculated chemical analysis		
Me(kcal/Kg)	3000	3.100
C. protein	23	21.5
Ca	0.96	0.78
P	0.58	0.56
Na	0.16	0.16
Dig. Lysine	1.44	1.29
Dig. Methionine	0.77	0.70
Dig. Threonine	0.97	0.88

Dig. Met + Cys	1.08	0.99
DCAB	236.23	220.74
C.F%	89.51	89.56
Fat%	3.10	3.08
Linoleic acid%	1.02	1.09

(1) The protein concentrate was used for broilers produced by the company Provime / Dutch. (2) Represented energy, crude protein, crude fiber, fat, lysine, methionine + cysteine, calcium and biophosphorus were calculated for each feed material according to the catalog of ROSS broiler 2022 using (American UFFDA program).

***The chemical analysis of the diet was calculated on the basis of Ross catalog and UFFD program (2022).**

Results and discussion

Table 2 shows the effect of adding different concentrations of alcoholic extract of *Silybum marianum* leaves to drinking water on some biochemical qualities and liver enzymes of broiler blood serum at the age of 5 weeks, where the results of the statistical analysis regarding the total protein concentration (g / 100 ml) showed a significant improvement ($P \leq 0.05$) where the birds of the fifth treatment recorded the highest concentration of total protein and amounted to 5.30 g / 100 ml, followed by the third treatment, which did not differ significantly from the rest of the experimental treatments and recorded a concentration of total protein. It reached 4.62 g / 100 ml, while the first, second and fourth treatments recorded the lowest concentration of total protein with a significant difference of ($P \leq 0.05$) from the fifth treatment. As for the concentration of albumin g / 100 ml, we note the continuation of the improvement of the fifth treatment significantly ($P \leq 0.05$) on the first, second, third and fourth treatments and recorded the highest concentration of albumin amounted to 2.82 g / 100 ml, as for the concentration of globulin and glucose g / 100 ml, no significant differences were recorded between all the treatment treatments of the experiment, while the first treatment (control) recorded the highest concentration of the enzyme ALT and amounted to 14.79 units / liter with a significant difference ($P \leq 0.05$) for the third, fourth and fifth treatments, which recorded the lowest concentration of the enzyme. While the second treatment did not record any significant difference between it and the rest of the treatments, as for the concentration of the AST enzyme, the results of the statistical analysis showed that there were no significant differences between all the parameters of the experiment.

Table 2 Effect of Addition of Different Concentrations of Alcoholic Extract of *Silybum marianum* Leaves in Drinking Water on Some Biochemical Traits and Liver Enzymes of Broiler Serum at 35 Days of Age (Arithmetic Mean \pm Standard Error)

Treatments	Trial weeks					
	Total protein g / 100 ml	Albumin g / 100ml	Clobulin g / 100 ml	Glucose g / 100 ml	ALT Concentration Unit/L	AST Concentration Unit/L
First Treatment	3.73±0.38 b	1.85±0.27 b	1.88±0.37	281.50±8.58	14.79±1.26 a	22.49±2.63
Second Treatment	3.73±0.33 b	1.90±0.29 b	1.83±0.46	278.99±13.07	12.46±1.54 ab	21.47±0.42
Third Treatment	4.62±0.45 ab	2.12±0.16 b	2.23±0.91	272.32±9.73	10.82±0.70 b	20.10±1.36
Fourth Treatment	4.12±0.31 b	2.18±0.11 b	1.95±0.42	261.05±11.87	11.18±0.15 b	19.99±3.83
Fifth Treatment	5.30±0.23 a	2.82±0.03 a	2.48±0.30	253.99±14.32	10.78±0.41 b	19.17±1.39
Significant level	*	*	NS	NS	*	NS

* Averages with different letters within one column indicate differences at a significant level ($p \leq 0.05$). NS: Non- Significant

First treatment = control treatment (drinking water free of any additive)

Second treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 0.5%.

Third treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 1% .

Fourth treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 2%.

Fifth treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 3%.

The reason for the improvement of the fifth treatment (adding 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 3%) and significantly ($0.05 \geq P$) in the concentration of total protein and albumin and the significant decrease ($0.05 \geq P$) for the third, fourth and fifth treatments in the concentration of the ALT enzyme may be due to the containment of the leaves of the *Silybum marianum* plant on some biologically active compounds such as saponins and glycosides, and these substances have antimicrobial and antioxidant activities as these substances have an important role in reducing the activity of intestinal bacteria through increased secretion Digestive enzymes and improving the immune response as well as working to protect the tissue of the gut (Al-Shahat, 1986; Saad et al., 1988) or perhaps the decrease in the level of ALT and the increase in the concentration of total protein and albumin may be due to the role of silymarin in preserving liver cells from free radicals due to its antioxidant properties (Abenavoli et al., 2011) and the ability to promote the synthesis of liver cells (Muhammed, et al., 2012).

The leaves of the *Silybum marianum* plant also contain the compound quercetin, which has many vital activities within the body, including the ability to protect against oxidative stress by suppressing free radicals, as it regulates the process of apoptosis, especially liver cells, which prevents the leakage of enzymes out of the cells and maintain them, and this explains the reason for the increase in the proportion of total protein and albumin with a decrease in the concentration of the enzyme ALT, which is one of the indicators of liver safety (Romero et al., 2009; Cano-Morales et al., 2014).

Table 3 shows the effect of adding different concentrations of alcoholic extract of the leaves of the *Silybum marianum* plant to drinking water in the forms of fat of broiler blood serum at the age of 5 weeks, where the first treatment (control) recorded the highest concentration of cholesterol and amounted to 217.11 mg / 100 ml and a significant difference ($P \leq 0.05$) for the third, fourth and fifth treatments, which recorded the lowest concentration of cholesterol, followed by the second treatment, which did not differentiate significantly from the rest of the experiment treatments, as for triglycerides (mg 100 / ml) we note the recording of The first treatment has the highest concentration of triglycerides compared to the second, third, fourth and fifth addition factors that recorded the lowest concentration of triglycerides (mg/100 ml), We also note from the same table a significant improvement ($P \leq 0.05$) for birds of the third, fourth and fifth treatments over the birds of the first (control) and second treatments in the characteristic of high-density lipoproteins HDL and the following concentrations were recorded 134.09, 126.79 and 127.83 mg / 100 ml respectively, while the first and second treatments recorded the lowest concentration of high-density lipoproteins HDL and amounted to 91.38 and 91.52 mg / 100 mm (respectively), and we also note the recording of the first treatment (control) the highest concentration of low-density lipoproteins LDL with a significant difference of ($P \leq 0.05$) for The third, fourth and fifth treatments, where the fourth and fifth treatments recorded the lowest concentration of low-density lipoproteins LDL, followed by the third treatment, which did not differ significantly from the second treatment, as for the very low-density lipoproteins VLDL, we note that there are no significant differences between all the experiment treatments.

Table 3 Effect of adding different concentrations of alcoholic extract of *Silybum marianum* leaf to drinking water in the lipid forms of broiler serum at 5 weeks of age (arithmetic mean \pm standard error)

Treatment	Studied traits at the age of 35 days				
	Cholesterol concentration)mg/100ml(Triglycerides concentration)mg/100ml(HDL concentration)mg/100ml(LDL concentration)mg/100ml(Very low density lipoprotein concentration VLDL)mg/100ml(
First Treatment	217.11 \pm 7.23 a	192.44 \pm 6.80 a	91.38 \pm 2.33 b	87.25 \pm 8.05 a	38.48 \pm 5.36

Second Treatment	204.08±10.14 ab	174.67±13.80 b	91.52±10.40 b	77.63±6.00 ab	34.92±3.75
Third Treatment	192.78±14.33 b	176.64±9.11 b	134.09±6.37 a	72.43±2.38 b	35.74±2.12
Fourth Treatment	194.96±3.46 b	176.10±5.10 b	126.79±5.92 a	32.95±4.67 c	35.21±4.02
Fifth Treatment	190.53±6.06 b	173.30±3.99 b	127.83±7.65 a	28.05±4.30 c	34.65±1.80
Significant level	*	*	*	*	NS

* Averages with different letters within one column indicate differences at a significant level ($p \leq 0.05$). NS: Non- Significant

First treatment = control treatment (drinking water free of any additive)

Second treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 0.5%.

Third treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 1% .

Fourth treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 2%.

Fifth treatment = add 10 ml of alcoholic extract to the leaves of the *Silybum marianum* plant / liter of drinking water at a concentration of 3%.

Analyzes of the image of lipids in blood serum are very important for birds as they are an indicator of the animal's physiological, nutritional and health status (Uchegbu et al., 2017) The reason for the low concentration of cholesterol, triglycerides, low-density lipoproteins and high density lipoproteins in the treatments of adding alcoholic extract to the third, fourth and fifth leaves of the *Silybum marianum* plant compared to the first (control) and the second may be due to the role of the *Silybum marianum* plant, which acts as a natural antioxidant as it removes the free radicals formed. naturally within the body before entering the chain reaction and thus reduces the rancidity of fat and enhances the state of antioxidants present in the body (Nowak et al., 2021). Or the reason for the decrease may be due to the presence of phenols in the leaves of the *Silybum marianum* plant, which play an important role as antioxidants, which lead to a reduction in the absorption of cholesterol with an increase in the secretion of bile salts (Leung and Foster, 1996 Dhouibi et al., 2020), in addition to that the leaves of *Algalgan* are characterized by their good content of vitamin C, which plays an important role in reducing the level of cholesterol and triglycerides, by increasing the rate of activity of the thyroid gland and secreting the hormone thyroxine, which in turn works to reduce Cholesterol concentration (Simona et al., 2019). Shehata and 2010 (Yousef) pointed out that the oxidation of low-density lipoproteins comes through high levels of malon dehyde MDA in tissues as a result of stress, and it is known that the low-density lipoprotein molecule is formed when the triglycerides molecule is completely divided by the enzyme Lipoprotein Lipase in tissues outside the liver, where the low-density lipoprotein molecule is the main carrier of cholesterol and triglycerides and this molecule is taken from various

cells of the body for use in cellular construction and manufacturing Products that are included in the construction and composition of fat (Nelson and Cox, 2004).

References

- Abenavoli, L; G. Aviello ; R. Capasso ; N. Milic and F. Capasso .2011.** Milk thistle for treatment of nonalcoholic fatty liver disease. *Hepatitis Monthly*. 11(3):173-177.
- Ajay K. ; Deepa I. ; Purnima A. and Neeraj V.2009.** Silymarin: A Comprehensive Review. *Phcog Rev.* 3 (5) : 126-134.
- Alhaidary, I. A; Rehman, Z; Khan, R. U., and Tahir, M. 2017.** Anti-aflatoxin activities of milk thistle (*Silybum marianum*) in broiler. *World's Poultry Science Journal*, 73(3), 559–566.
- Al-Shahat, Naseer Abu Zeid. 1986.** Medicinal plants and herbs, Dar Al-Bahar, Beirut.
- Aziz, M; Saeed, F; Ahmad, N; Ahmad, A; Afzaal, M; Hussain, S; Mohamed, A. A; Alamri, M. S., and Anjum, F. M. 2021.** Biochemical profile of milk thistle (*Silybum Marianum* L.) with special reference to silymarin content. *Food Science & Nutrition*, 9(1), 244–250.
- Bahmani , M. ; Hedayatollah S. ; Samira R., and Mahmoud R. K.2015.** Silybum marianum: Beyond Hepatoprotection. *Journal of Evidence-Based Complementary & Alternative Medicine* . V. 20(4) : 292-301 .
- Bijak, M. 2017.** Silybin, a major bioactive component of milk thistle (*Silybum marianum* L. Gaernt.)—Chemistry, bioavailability, and metabolism. *Molecules*, 22 (11), 1942.
- Deif, E. A; A., Galal, M. M. Fathi,, and A. Z. El-Dein. 2007.** Immunocompetence of two broiler strains fed marginal and high protein diets. *International Journal of Poultry Science*, 621, 901-911.
- Duncan, D.B .1955.** Multiple range multiple F-test-Biometeics.11:1 – 42.
- <https://www.scribd.com/document/617216364/Ross> BroilerNutritionSpecifications2022-ES**
- Khazaei, R. ; Seidavi, A. and Bouyeh, M. 2022.** A review on the mechanisms of the effect of silymarin in milk thistle (*Silybum marianum*) on some laboratory animals. *Veterinary Medicine and Science*, 8, 289–301 .
- Majidi, M. M; Shafiei-Koij, F; Pirnajmedin, F., Jami, M., and Radan, Z. 2021.** Fatty acid profile, silymarin content and production properties of milk thistle (*Silybum marianum*) germplasm under different water environments. *Crop and Pasture Science*, 72(4), 302–310.
- Manju ,D.K; A. Thangavel; V. Leela and J. Kalatharan .2010.** Effect of dietary supplementation of Amla and Grape Seed on Semen Characterstics of Broiler Breeder Cocks. *Tamilnadu J. Veterinary and Animal Sciences* 6 (2):65-70.
- Marceddu, R; Dinolfo, L;Carrubba, A; Sarno, M. and Di Miceli, G. 2022.** Milk Thistle (*Silybum Marianum* L.) as a Novel Multipurpose Crop for Agriculture in Marginal Environments: A Review. *Agronomy*, 12, 729.
- Morales-Cano; D. C. Menendez; E. Moreno; J. Moral-Sanz; B. Barreira; P. Galindo and F. Perez-Vizcaino. 2014.** The flavonoid quercetin reverses pulmonary hypertension in rats. *PLOS ONE*. 9(12): e114492.

- Muhammad, D; N. Chand; S. Khan; A. Sultan and M. Mushtaq.2012.** Hepatoprotective Role of Milk Thistle (*Silybum marianum*) in Meat Type Chicken Fed Aflatoxin B 1 Contaminated Feed. *Pakistan Veterinary Journal*. 32(3): 443-446.
- Romero, M; R. Jiménez; M.Sánchez; R. López-Sepúlveda; M. J. Zarzuelo; F. O'Valle and J. Duarte .2009.** Quercetin inhibits vascular superoxide production induced by endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. *Atherosclerosis*. 202(1): 58-67.
- Saad, Shukri Ibrahim ; Al-Qadi, Abdullah and Saleh, Abdulkarim Mohamed. 1988.** Medicinal, aromatic and poisonous plants in the Arab world. Arab Organization for Agricultural Development, Khartoum.
- Salami, S. A; M. A. Majoka; S. Saha; A. Garber and J. F. Gabarrou .2015.** Efficacy of dietary antioxidants on broiler oxidative stress, performance and meat quality: science and market. *Avian Biology Research*, 8(2): 65-78.
- SAS. 2012.** Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Stastnik, O; Pavlata, L., and Mrkvicova, E. 2020.** The milk thistle seed cakes and hempseed cakes are potential feed for poultry. *Animals*, 10(8), 1384.
- Surai, P. F. 2020.** Antioxidants in Poultry Nutrition and Reproduction: An Update. *Antioxidants* (Basel, Switzerland), 9(2), 105,1-6.
- Wizard, Mariann. 2004.** Cancer prevention and treatment – the role of phytotherapy. *British Journal of Phytotherapy*, 5(4):199-208.
- Zargari, A. 1997.** Medicinal plants. Tehran University of Medical Sciences.