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# EFFECT OF SUPPLEMENTING MILK THISTLE (*SILYBUM MARIANUM*) SEEDS OR ITS EXTRACT ON SOME PHYSIOLOGICAL TRAITS OF BROILER CHICKENS REARED UNDER HIGH ENVIRONMENTAL TEMPERATURE

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## Abstract

This experiment was conducted to study the effect of adding milk thistle seeds to the diet or its extract to drinking water for six hours/day on blood cellular and biochemical parameters, body temperature and liver glutathione of broiler chickens reared under high environmental temperature (30.5-36.5 ºC). A total of 150 unsexed, day old, Ross 308 broiler chicks were used and randomly distributed into five treatments with three replicates per each (10 chick/replicate) as follows: T1 control group without any addition, T2,T3 adding 250 or 500 mg milk thistle extract/liter drinking water respectively, T4,T5 adding 8 or 16 g milk thistle seeds/kg diet respectively. Results showed a significant ($p \leq 0.05$) decrease in body temperature of T4 group compared with control. Blood cellular traits showed significant ($p \leq 0.05$) decrease in heterophil% and in heterophil/lymphocyte in T4 group compared with control. Blood biochemical traits indicated a significant ($p \leq 0.05$) decrease in creatinine in T2 group compared with control while AST was decreased significantly ($p \leq 0.05$) and total protein was increased in T4 compared with control. Serum ALT, glucose and uric acid didn't differ significantly between all treatments. Liver glutathione increased significantly ($p \leq 0.05$) in all supplemented groups compared with control. It can conclude from these results that adding 8g milk thistle seeds/kg diet of broiler chickens reared under high environmental temperature could alleviate the deleterious effect of heat stress.

Key words: Milk thistle seeds, heat stress, physiological traits, liver glutathione

## Introduction

The factors called stressors include various environmental, chemical, physical and infectious. Environmental stress results from several factors, including temperature and humidity, which leads to damage to the physiology of birds and thus to a reduction in production efficiency and the safety of food produced. Heat stress represents a severe threat to balance and has negative effects on health. General for poultry, and the period to which birds are exposed determines the effect of the stressful factor, and stress can be determined by describing the adaptive response of the animal in general to everything that threatens its natural balance (Nawab et al., 2018).

Many researches have shown that there is a relationship between heat stress and oxidative stress. It lies in the fact that heat stress reduces the efficiency of energy production in mitochondria and causes a defect in electron transport chain and increases the production of free radicals, which causes a defect in the mitochondria and reduces the production of enzymatic antioxidants with the depletion of antioxidants In the body, free radicals accumulate and the oxidative balance is broken,

causing oxidative stress (Emami et al., 2021). Medicinal plants and herbs differ from other plants in their effect because they contain clear medicinal value, and it has been possible to limit the plants used in folk medicine and extract and purify their effective components, whether these effective components are in the leaves, stems, roots, flowers or buds (Qandil and Ibrahim, 2007). It is also characterized by its positive effect productive and physiological characteristics, and raising on body's immunity against pathogenic microbes, in addition to its stimulating effects on the digestive system (Cifftic et al., 2005).

There has been an interest by poultry production researchers towards experimenting with the possibility of benefiting from the effective compounds found in some medicinal plants in alleviating the types of stress for the purpose of increasing economic income, especially wild plants that are available in the nature of the country in which they live, which can be Obtaining it for free and easily, and one of these plants that has been tried to be used in the field of poultry production is milk thistle, ( *Silybum marianum* ). It is considered a medicinal plant, belongs to the Asteraceae family, and contains several compounds, including flavonoids, which are the main active compounds in the plant, the most important of which are Silymarin, Silibin, Taxifolin and Quercetin (Fraschini et al., 2002) It also contains fats, sugars, mucous substances and vitamins, including A, B12, E, C, K, as well as contains Apigenin, Histamine and Tyramine and contains 20-30% fixed oils, including Palmitic, Linolic and Oleic acid. It also contains some elements such as zinc, selenium, calcium, and iron, and magnesium (Duke, 2004).

For the purpose of knowing the extent to which milk thistle is rich in effective compounds and cheap, and does not have negative effects on the health of birds, and does not leave harmful residues affecting human health, as a consumer of broiler meat, and due to the lack of a sufficient study that dealt with the addition of milk thistle seeds in their raw form or extracted. Under uncontrolled high environmental temperatures, this study was conducted.

## MATERIALS AND METHODS

This experiment was conducted in poultry field/Department of Animal Production / College of Agricultural Engineering Sciences / University of Baghdad during the period 9/29/2021 to 11/11/2021. A total of 150, unsexed Ross308 broiler chicks, day old were brought from National Society Hatchery, In Abu Ghraib / Baghdad. The average weight was 42 ch.ck, the chicks were reared together on the ground on wood shave litter with a thickness of 3 cm. Water and feed were provided add libitum lighting system was 24 hours throughout the experiment period. from 7 days the chicks were distributed randomly in to five treatments with three replicates per each (10 chick / replicate) and were weighed. The experiment included and the treatments were as follows (T1) control without any addition, T2 adding 250 mg milk thistle seed extract / liter of drinking water, T3 adding 500 mg milk thistle seed extract Milk / liter of drinking water, T4 adding 8 gm of milk thistle seeds / kg of diet, T5 adding 16 gm of milk thistle seeds / kg of diet. The birds were raised under high environmental temperatures, which ranged 30.5-36 C throughout the experiment period. Table (1) shows the average temperatures and relative humidity during the experiment weeks, Table 2 showed the components of diet and its calculated chemical com position.

**Table 1. House temperatures (C°) and relative humidity (%)during the weeks of the experiment**

| Weeks of experience | time (hour) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | eight in the morning (8) | | am (10) | | twelve noon (12) | | )p.m. (15 | | Six in the afternoon (18) | | nine o'clock in the evening (21) | | twelve o'clock in the evening (24) | | AM 4 | |
| | D(cº)[1] | H(%)[2] | D(cº) | H(%) | D(cº) | H(%) | D(cº) | H(%) | D(cº) | H(%) | D(cº) | H(%) | D(cº) | H(%) | D(cº) | H(%) |
| 2 | 31` | 29 | 33 | 28 | 34 | 25 | 36 | 25 | 31 | 27 | 31 | 27 | 30.7 | 28 | 31.2 | 27 |
| 3 | 31.9 | 35 | 34 | 30 | 35.3 | 29 | 33 | 30 | 31.6 | 32 | 31.7 | 32 | 31 | 33 | 30.5 | 33 |
| 4 | 31.3 | 28 | 32.3 | 27 | 32.6 | 26 | 33.4 | 26 | 31.8 | 26 | 31.5 | 28 | 30.4 | 29 | 30.7 | 30` |
| 5 | 31.9 | 37 | 33 | 36 | 33.5 | 34 | 33.8 | 36 | 31.5 | 38 | 31.3 | 37 | 30.8 | 38 | 30.5 | 37 |
| 6 | 30.5 | 40 | 31.5 | 34 | 31.8 | 35 | 32.6 | 38 | 31 | 37 | 30.7 | 40 | 30.6 | 30.8 | 30.3 | 40 |

**(1) The temperature inside the room**
**(2) The relative humidity.**

**2-3- The studied measurmets**

**2-3-1 Body temperature (Cº):**

Rectal body temperature was measured in weeks 4,5,6 of the experiment at two o'clock in the afternoon using a digital thermometer sensitive to one decimal place, where the probe of the thermometer is inserted into the manhole at a distance of 5 cm and wait for two minutes and then record the reading when it becomes stable.

**2-3-2 Blood tests:**

Blood was collected at the age of 42 days through brachial vein. A syringe of 3 ml with a needle was used. Blood is drawn from a vein with a syringe and then transferred into two tubes, one of which contains an EDTA anticoagulant, to conduct cellular tests, which included measuring each of the percentages of different types of white blood cells, include (Heterophil , Lymphocyte, Eiosinophil , Basophil and Monocyte ) heterophil to lymphocytes ratio. The second tube did not contain an anticoagulant for the purpose of obtaining blood serum (after centrifuging it) to estimate biochemical tests included measuring the level of glucose, total protein, total cholesterol, uric acid, creatinine, and liver function enzymes ALT and AST. These measurements were taken in a laboratory of a medical complex in Abu Ghraib, using a Japanese-made FUJIFILM DRI-CHEM device.

**2 . The components of the diet and its calculated chemical composition**

| material | starter diet % (1-11) days | % grower diet ( 12-24)days | | Finisher % ( 24-42) days |
|---|---|---|---|---|
| yellow corn | 45.8 | 47.2 | | 49.7 |
| Wheat | 10 | 11.3 | | 11.1 |
| Soybean meal | 34.3 | 30.6 | | 27.1 |
| Concentrated protein)[1] ) | 5 | 5 | | 5 |
| Oil | 2.6 | 3.7 | | 4.9 |
| Limestone | 1.1 | 1.1 | | 1.1 |
| Di-calcium phosphate | 0.7 | 0.6 | | 0.6 |
| Salt | 0.3 | 0.3 | | 0.3 |
| ( Vitamin and Mineral Mixture)[2] | 0.2 | 0.2 | | 0.2 |
| Total | 100 | 100 | | 100 |
| (3) The calculated chemical composition | | | | |
| Crude protein (%) | 23.46 | | 21.9 | 20 |
| Wet a bolizable energy Kcal/Kg | 3031.4 | | 3132 | 3200.5 |
| methionine(%) | 0.512 | | 0.49 | 0.47 |
| Lysine (%) | 1.35 | | 1.25 | 1.16 |
| Calcium(%) | 1.02 | | 0.99 | 0.98 |
| Available Phosphorus (%) | 0.5 | | 0.48 | 0.47 |
| methionine+cysteine(%) | 0.885 | | 0.84 | 0.8 |

**(1) Protein concentrate type Brocon – 5 Jebur. Each kg contains 30% crude protein, 3.50%, 4.86% fiber, 7.46% moisture, 31.33 ash, 6.81% calcium, 2.65% phosphorus, 5.34% available phosphorus, 3.85% lysine. 3.70% methionine, 4.06% methionine + cysteine, 0.30% tryptophan, 1.80% threonine, 1.07% valine, 1.52% arginine, 1740.68 kilocalories / kg of energy, 2.40% sodium, 3.74% chloride. (2) It also contains a group of vitamins, including 200,000 IU vitamin A, 80,000 IU vitamin D3, 600 mg vitamin E, 50 mg vitamin K3, 60 mg vitamin B1, 140 mg vitamin B2, 80 mg vitamin B6, 700 mg vitamin B12, 800 mg niacin, 2 mg biotin, 20 mg folic acid, 7000 mg choline chloride, and also contains 200 mg copper, 1600 mg manganese, 1200 mg zinc, 1000 mg iron, 20 mg iodine, 5 mg selenium. (3) According to NRC (1994).**

**2-4-1- Liver glutathione**

At the end of the experiment and after the process of slaughtering the birds, samples were taken from the liver tissue, where a part with a length of (2 cm) was taken and washed well with water and then placed in a tightly closed plastic package and frozen until measurements were made on it. The measurement was made in the Nutrition Contamination Laboratory of the Ministry of Science and technology. Statistical analysis of the studied traits was conducted using statistical program SAS (2001), as the effect of different coefficients on the studied traits was determined using the complete random design CRD. To test the significance of the differences between the studied averages, the Duncan multiple test (1955) was used

## Results and discussion

### 3-1-1- Body temperature (C)

The results are shown in Table 3. In general, it was found that there was a significant decrease ($P \leq 0.05$) in body temperature of birds treated with the addition of 8 g seeds / kg diet (T4) in comparison with control sometimes and with the rest of the treatments at other times. In the fourth week, T4 led to a significant decrease in body temperature ($P \leq 0.05$) compared to the control (T1) and with each of the two treatments of adding seed extract to drinking water (T2 and T3), while there were no significant differences between (T4) and treatment of adding 16 gm seeds / kg diet (T5).

The significant decrease in body temperature in T4 treatment continued in the fifth week, but compared to the treatment of adding 500 mg seed extract / liter of drinking water ( T3), and there was no significant difference between it and the control and T2 and T5 group on the other hand, the T1, T2 did not differ, T3 and T5 were significantly different from each other.

In the last week of the experiment (sixth), body temperature of the birds decreased significantly ($P \leq 0.05$) in (T4) compared to control and with the treatment of adding 250 mg seed extract / liter of drinking water (T2) as well. There was a significant decrease ($P \leq 0.05$) in body temperature in (T3) compared to control.

The decrease in body temperature in T4 group ( 8 gm seeds / kg diet) may be due to the benefit of this level of addition, as it contains taxifolin, which works to expand Blood vessels (Seong et al., 2022), which increases the chance of cooling excess heat from the body through means of convection, conduction, and radiation along with its vitamin C content and a cycle in getting rid of excess heat through blood vessels.

### 3-1-2- Diffrential count of white blood cells

A significant decrease ($p \leq 0.05$) was observed in the percentage of lymphocytes inT4 compared to T5 and T3, but it did not differ significantly from T2 and control (T1).. As for heterophil % T3, recorded a significant difference ($p \leq 0.05$) with control, T1, but it did not differ significantly with T5, and T2, and, T4, which did not differ with control, which recorded higher percentage of heterophil , while there were no significant differences between the treatments in terms of the number of eosinophilic and basophil%, there was a significant difference ($p<0.05$) in mononcye% between T2 and T4, but the T2 did not differ from T1, T3 and T5 and the previous treatments did not differ significantly with T4. Concerning the ratio between heterophil and

lymphocytes, There was a significant difference ($p<0.05$) between T4 and control, but it did not differ from T3, T2, T5, while there was no significant difference between T5 and T2 and T1, as shown in Table (4)

**Table 3. The effect of adding milk thistle seeds to the diet or its extract to drinking water on body temperature of broilers subjected to heat stress (mean ± SE)**

| Treatments | body temperature °C | | |
|---|---|---|---|
| | fourth week | fifth week | sixth week |
| T1 | 42.61±0.18 [a] | 43.21±0.11 [a] | 43.46±0.07 [a] |
| T2 | 42.72±0.15 [a] | 42.93±0.05 [ab] | 42.67±0.17 [bc] |
| T3 | 42.86±0.22 [a] | [ab]0.21±43.11 | 42.96±0.03 [b] |
| T4 | 42.06±0.07 [b] | 42.76±0.09 [b] | 42.56 ±0.04 [c] |
| T5 | 42.46±0.13 [ab] | 43±0.05 [ab] | 42.75±0.14 [bc] |
| LSD | * | * | * |

**T1: control, T2 and T3, including the addition of 250 or 500 mg of seed extract / liter of drinking water, respectively. T4 and T5, including the addition of 8 or 16 gm of seeds / kg diet, respectively.**
*** The means that bear different letters within one column indicate that there are significant differences between the mean of the coefficients at the level (P≤0.05).**

Through the health indicators of the birds, it is possible to infer the extent of the birds' exposure to heat stress, as Shini et al. (2008) indicated that exposure to stress increases the percentage of immature heterophil cells, and this condition results from stimulation of bone marrow cells as a response to stress by the influence of the adrenal cortex hormone, Corticosterone, Also, the high H/L is considered one of the indicators that the birds are exposed to chronic stress (Tukyilmaz, 2008). It is noted from the results in general that the third and fourth treatments were distinguished in increasing the number of lymphocytes and reducing the number of heterophil cells, which reflected positively on the health of the birds and increased their resistance to the harmful effects of the high environmental temperature.

The reason for improving the health of birds may be due to the role of the active compounds in milk thistle seeds or their extract in reducing stress and fighting pathogens that may affect birds, as Ibrahimi et al. (2015) indicated in his study to evaluate the effect of flavonoids present in milk thistle that they act as antibacterial and causative bacteria For diseases (*Staphylococcus saprophyticus, Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia*). Silymarin may penetrate the cell wall and destroy enzymes necessary for vital activities within the bacterial

cell. Chemical analysis of the seeds showed the presence of many compounds that have antibacterial activity, including terpenoids, flavonoids, and tannins.

Also, the seeds contain the compound quercetin, which is considered an anti-inflammatory, as it supports anti-inflammatory by removing free radicals (Bureau et al., 2008).

**3-1-3- Serum biochemical indicators**

The results of the statistical analysis of the levels of liver function enzymes (ALT and AST), blood glucose, total protein, uric acid, and creatinine in the serum of broiler chickens subjected to heat stress and treated with the addition of milk thistle seeds or its extract are shown in Table 5. It is clear from the table that the addition of 8 g seeds / kg diet led to a significant decrease ($P \leq 0.05$) in the level of the liver function enzyme AST compared with control, while there was no significant difference between it and each of the other addition treatments (T2, T3 and T5). ) which in turn did not differ significantly with control.

With regard to the liver function enzyme ALT, all addition treatments did not lead to a significant difference in its value in blood serum of birds exposed to heat stress, and this non-significant result also occurred with the values of glucose and uric acid. As for total protein, it increased significantly ($P \leq 0.05$). In T4 compared control (T1), the fourth treatment did not differ significantly with T2, T3, and T5, which in turn did not differ significantly with control group.

With regard to creatinine, its level was significantly decreased ($P \leq 0.05$) in T2 compared to control (T1) and T3 and T4, but it did not differ significantly with T5, which in turn did not differ significantly with T3 and T4 ($P \leq 0.05$) and there was no significant difference between T3 and control group.

**Table 4. Effect of adding milk thistle seeds to the diet or its extract to drinking water on the ratios of different types of white blood cells and the ratio of heterophil to lymphocytes (H/L) of broilers subjected to heat stress (mean ± SE)**

| treatment | Lymphocyte | Heterophil | Eiosinophil | Basophil | Monocyte | H/L |
|---|---|---|---|---|---|---|
| T1 | 51.8± 4.17 ab | 23.31 ±1.20 a | 8.30±1.33 | 4.51±0.57 | 12 ±2.08 ab | 0.45 ±0.05 a |
| T2 | 54.1± 3.05 ab | 23.3 ±2.64 ab | 11.30±0.66 | 3.30±0.33 | 8.00±1.15 b | 0.43 ±0.07 ab |
| T3 | 62.80 ±4.09 a | 16.33±1.20 b | 8.66±1.45 | 3.21±0.33 | 9.00 ±4.09 ab | 0.26±0.008 b |
| T4 | 60.39 ±1.45 a | 16.31±0.88 b | 7.20±0.88 | 4.10±0.33 | 12.00±0.66 ab | 0.27 ±0.02 b |
| T5 | 48.11 ±1.15 b | 20.21±3.05 ab | 12.39±4.09 | 3.29±0.33 | 16.00±1.52 a | 0.42±0.07 ab |
| LSD | * | * | N.S | N.S | * | * |

**T1: control, T2 and T3, including the addition of 250 or 500 mg of seed extract / liter of drinking water, respectively. T4 and T5, including the addition of 8 or 16 gm of seeds / kg of diet, respectively.**

*** The means with different letters within one column indicate that there are significant differences between treatment (P≤0.05).**

**N.S. : no significant differences.**

The reason for the improvement in the levels of AST enzyme, total protein and creatinine may be due to the role of silymarin in protecting liver cells from free radicals due to its antioxidant properties (Abenavoli et al., 2011). Silymarin also has the ability to promote liver cell protein synthesis (Muhammad et al., 2012) The researcher Nik et al. (2015) also indicated the role of milk thistle in increasing the manufacture of globulin as a result of maintaining the main and secondary immune organs and thus increasing the level of total protein.

Milk thistle, especially whole seeds, also contains quercetin, which has many vital activities within the body, including the ability to protect against oxidative stress, by curbing free radicals. The reason for the decrease in the level of creatinine in the blood serum and the increase in total protein percentage with a decrease in AST enzyme, which is one of the indicators of liver health (Romero et al., 2009 and Morales-Cano et al., 2014) is explained.

**2- Measurements of the liver**

**3-2-1- glutathione**

Figure (1) shows the results of the statistical analysis of the effect of adding milk thistle seeds to the diet or milk thistle seed extract to the drinking water of broiler chickens raised under heat stress on glutathione level in the liver of birds, where T3 excelled with a highly significant level (P≤0.01) the control treatment and the rest of the addition treatments, as T2 was significantly superior to the control and T4 and T5 group, while T5 was significantly superior to control and T4 groups. It can be concluded from these results that the addition of seed extract to drinking water at a level of 250 and 500 mg / liter has increased the level of glutathione in the liver of birds more than adding seeds in its raw form to the feed. The reason for this increase may be due to the higher content of silymarin in the extract compared with raw seeds, which led to a decrease in its oxidation in the liver (Muhammad et al., 2012). Federico et al. (2017) indicated that silymarin is a powerful antioxidant that suppresses free radicals and can affect enzyme systems associated with glutathione.

**Table 5. Effect of adding milk thistle seeds to diet or its extract to drinking water on levels of liver function enzymes, glucose, total protein, uric acid and creatinine in serum of broilers subjected to heat stress (mean ± standard error)**

| treatment | ALT IU/L | AST IU/L | glucose mg/dl | total protein g/dl | uric acid mg/dl | Creatinine Mg/dl |
|---|---|---|---|---|---|---|
| T1 | 4.05±0.54 | 182.5±34.5 $^{a}$ | 246.73±6.15 | 3.15±0.10 $^{b}$ | 4.4±0.58 | 0.49± 0.05 $^{a}$ |
| T2 | 4.01± 0.41 | 138.5±8.5 $^{ab}$ | 245.26±8.22 | 3.33±0.15 $^{ab}$ | 4.96±0.42 | 0.32± 0.03 $^{c}$ |
| T3 | 4.15± 0.16 | 116.5± 10.5 $^{ab}$ | 255.4±10.21 | 3.4±0.16 $^{ab}$ | 4.75±0.29 | 0.47±0.03 $^{ab}$ |
| T4 | 4.18± 0.22 | 95.0±20 $^{b}$ | 266.4±7.61 | 3.58± 0.09 $^{a}$ | 5.13±0.4 | 0.44± 0.04 $^{b}$ |
| T5 | 3.4±0.18 | 135.5±13.5 $^{ab}$ | 256.83±9.72 | 3.29± 0.1 $^{ab}$ | 4.77±0.5 | 0.36± 0.01 $^{bc}$ |
| LSD | NS | * | NS | * | NS | * |

**T1: control treatment, T2 and T3, including the addition of 250 or 500 mg of seed extract / liter of drinking water, respectively. T4 and T5, including the addition of 8 or 16 gm of seeds / kg of feed provided, respectively.**

*** The means that bear different letters within one column indicate that there are significant differences between the treatment (P≤0.05).**

**N.S. : no significant differences**

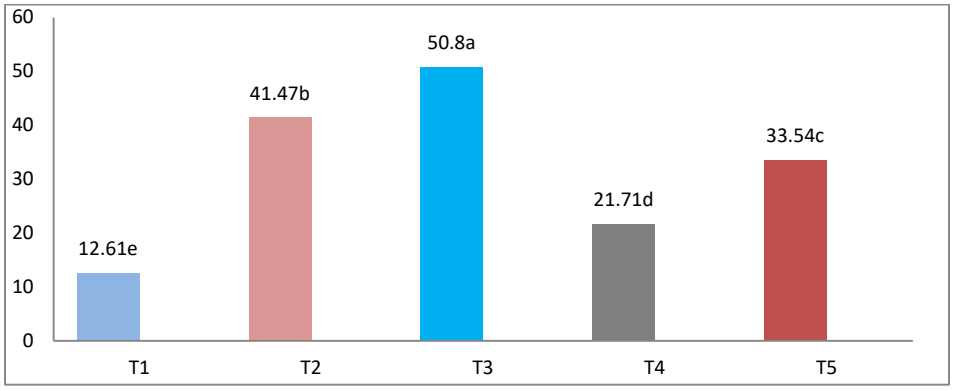


**Figure 1.Effect of adding milk thistle seeds to the diet or its extract to drinking water on the level of glutathione in the liver of broilers subjected to heat stress**

**T1: control, T2 and T3 include the addition of 250 or 500 mg of seed extract / liter of drinking water, respectively. T4 and T5 include the addition of 8 or 16 gm seeds / kg diet, respectively.**

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