Impact of adding vitamin C and crude alcoholic extract of *Petroselinum crispum* leaves and their mixture to drinking water in some physiological traits of broiler exposed to heat stress

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Abstract
The study involved the use of 180 chicks, with an average age of 1 day and a body weight of 40 g, regardless of sex, distributed among four treatments, since one treatment contained 45 bird, each in 3 replicates (15 chicks/ Replicat), for 5 weeks, from the third week to the end of the experiment, chicken temperature (28-36 -30 ± 2 °C) and humidity (40-60-50 ± 2%) time (700-1200- 1900) at the end of the fifth week. Chicks were randomly assigned as follows: - The first treatment (T1) was a control treatment with no supplementation of drinking water, the second treatment (T2) was supplemented with 300 mg/L vitamin C in the water , the third treatment (T3) addition to 300 mg/l water from *Petroselinum crispum* alcoholic extract, fourth treatment (T4) added to 300 mg/l water from *Petroselinum crispum* alcoholic extract and 300 mg/l vitamin C to water to each, study The results showed a significant increase in the proportion of lymphocytes for all additional treatments and a significant decrease in the percentage of heterophil and the ratio of heterophil to lymphocytes (H/L) in favor of the add-on treatment compared to the control treatment, the results also showed that the percentage of total protein in serum was significantly increased in favor of adjuvant therapy, e favored T3 and T4 in glucose, cholesterol, ALT and AST enzyme concentrations and significantly favored T3 and T4 The results also showed that some T3 and T4 parameters significantly increased antioxidant enzymes represented by catalase and glutathione peroxidase in serum. We concluded from the study that using *Petroselinum crispum* alcohol Extracts and Vitamin C and their blends helped to improve the physiological properties in most of the studies, stating that T3 and T4 gave the best results.

Keywords: Alcoholic extract, Broiler, Heat stress, *Petroselinum crispum*, Vitamin C.

Introduction

Increased temperature is a major environmental constraint that can lead to decreased yields of farm animals in general and poultry in particular, as heat stress can cause the body temperature of poultry to rise above 47 °C, resulting in decreased mortality in a natural way Reduced heart failure [6], heat stress leading to deterioration of physiological and production traits, weakened immune system and high mortality in birds [40], increased ambient temperature Temperature is associated with increased oxidative damage that occurs in chickens [7,26], moreover, oxidative damage due to high ambient temperature is the main source of free radicals formed through electron leakage in the respiratory chain during molecular oxygen reduction at the end of oxidative phosphorylation [26]. Much recent research has focused on medicinal plants and their role as antioxidants in the prevention of oxidative stress, which occurs when the body's defense mechanisms fail to scavenge free radicals Occurs to maintain important cellular functions [30]. The leaves of *Petroselinum crispum*, which is renowned for its medicinal properties, are bursting with essential oils that far surpass the concentration found in its roots. The oils contain an abundance of nourishing vitamins, mineral salts, phosphorus, iron, and calcium [36]. Recent experiments have confirmed that *Petroselinum crispum*, considered a natural antioxidant, can improve the efficiency of the immune system due to its vitamin C content and high levels of flavonoids, especially apigenin, which inhibits the breakdown of vitamin C in cancer cells [29]. Experiments

have shown that oil extracted from the plant *Petroselinum crispum* is a natural alternative as an antioxidant and free radical inhibitor [49]. However, the most prosperous discovery has been that the volatile oil myristicin, which is one of the plant's most significant compounds that includes apiole, vitamin C, iron salts, calcium, and iodine, is the active component [33]. Due to its vitamins, minerals, dyes, essential oils, and bioactive compounds [12], it has been used to treat many ailments, which is why it is considered one of the widely used herbal remedies in alternative and in complementary medicine, it is used to treat various ailments such as chest ailments, coughs, asthma and colds, and to treat other ailments such as malaria, as it contains celery and is used as an antiseptic against bacteria and viruses. Due to the plant's iron-rich folic acid, *Petroselinum crispum* is not only important for treating the aforementioned conditions but also for additional ones including anemia [47]. In addition to its potent action in dissolving fat, it helps the liver to secrete bile and is a diuretic, and its juice relieves kidney and urinary tract pain [8]. In view of the above, this study aimed to explore the possibility of using *Petroselinum crispum* alcohol extract and vitamin C and their mixtures to reduce the effect of high ambient temperature on poultry and its effectiveness on poultry. Heat stress and amelioration of some of the consequent collateral damage and prevention of its effects remains a matter of research, experimentation and understanding of the extent to which this extract contributes as an antioxidant and a compound affecting the physiological performance of broilers. Therefore, the aims of this study were: - To demonstrate the alcoholic extraction of *Petroselinum crispum* and the effect of this extract and the active compounds it contains on heat stress. And determine the effect of the extract and its active ingredients on the physiological performance of broilers, and compare and show that the effect of adding *Petroselinum crispum* alcohol extract and vitamin C and their mixture is the best.

MATERIALS AND METHODS

The experiment was carried out in a closed hall with the dimensions 55 x 12 m (length x width) according to the floor training system, on a 5 cm thick mattress, divided into pens, the area of each pen corresponds to 3 m^2 , each case represents a replicas of the experiment. In this experiment, 180 1-day-old unsexed Ross strain broilers, with body weight 40 g/bird, It was distributed into 4 treatments, each with 3 Replication, and each treatment was 45 chicks, 15 chicks per replicate. The birds were exposed to temperatures range of (28-36 -30 ± 2 °C)and humidity (40-60-50 ± 2%)for the times 700-1200-1900 record the temperature and humidity at different times of the day and extract the weekly average value of the experiment. The temperature and humidity are measured with a digital thermometer (HTC-2) made in China, and the hall temperature and reading humidity are distributed in the hall at the same time. The height of the thermometer is at the level of the back of the chicken, the birds were fed on a starter diet from the age of one day until the age of 21 of the birds, after that, it was replaced with growth diet until the end of the experiment at the age of 5 weeks (35 days), the fodder was provided *ad libitum* freely, according to the chemical analysis of the diet according to NRC [28]the diets used and their chemical composition are shown in Table 1.

Four Treatments were involved in the experiment, which was carefully planned:

(T1) was a control treatment without addition .

(T2) add vitamin C at a level of 300 mg/liter.

(T3) add *Petroselinum crispum* alcohol extract at a level of 300 mg/liter.

(T4) add amixture of vitamin C and *Petroselinum crispum* alcohol extract at a level of 300 mg/liter respectively.

Table 1 This report displays the percentages of dietary components that were investigated and their chemical composition.

Feed materials	Primer diet % 1-21 days	Growth diet % 22-35 days
Yellow corn	30	40
Wheat	28.25	24
Soybean meal (48% protein)	31.75	24.8
Protein concentrate *	5	5
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium Phosphate (DCP).	0.7	0.9
Salt	0.3	0.1
Mixture of vitamins and minerals	0.2	0.2
the total	100	100
Calculated chemical composition **		
Crud protein (%)	23.04	20.06
Metabolic energy (kcal/kg feed)	3027.10	3194.92
Lysine (%)	1.29	1.11
Methionine + Cysteine (%)	0.86	0.78
Calcium	0.91	0.82
Available phosphorous (%)	0.49	0.51
Energy ratio: protein C/P %	131.83	159.62

*** Use the Jordanian-produced Holde Mix concentrated protein, which has the following nutrients per kilogram: 40% crude protein, 3.5% fat, 1% crude fiber, 6% calcium, 2100 kcal of energy, 3% phosphorus, 2.20% salt, and 3.25% lysine. Methionine 3.5% Methionine with cysteine, 3.90% Vitamin B6 300 mg with vitamin D3 40,000 IU. Vitamin B1 15mg, Vitamin B12 300mg, Vitamin K3 30mg, Biotin 100mg, Copper 100mg, Manganese 1200mg, Iodine 15mg, Selenium 2mg, Folic Acid 10mg, Vitamin E 50mg, Niacin 200mg, Iron 1000mg, Cobalt 6mg, Zinc 800 mg, Vitamin A 20,000 IU.**

**** The chemical composition of feed ingredients in the diets was calculated according to the NRC (1994) recommendations.**

Prepare the alcoholic extract

Petroselinum crispum were obtained from a local market, dried in the shade at room temperature in a well-ventilated environment, and then ground into powder with an electric grinder for the preparation of the alcoholic extract, which was prepared by mixing 50 g curly *Petroselinum crispum* powder is placed in a glass flask with a capacity of 1000 ml, and 250 ml of ethanol (ethanol) 70% is added. Then place the beaker on a magnetic stirrer and mix at room temperature for 24 hours, then filter the mixture with a gauze layer, then put the prepared extract into a flask of a rotary evaporator at 45°C, remove ethanol and remove water until a dry powder is obtained, The amount of extract produced is 2g.

Vitamin C

Vitamin C was added to drinking water during the experiment at a rate of 300 mg per liter of vitamin C ascorbic acid (white fine

powder), known to come from Iraq, from animals and with a purity of 25%.

Qualitative chemical detection

The presence of some active compounds of the extracts has been detected as follows:-

a- **Detection of flavonoids:** Add many drops of concentrated sulfuric acid to the alcohol extract of *Petroselinum crispum*, and the red color is a positive test [18].

b- **Detection of alkaloids**: Meyer reagent method is used for the detection of alkaloids. Mercury chloride HgCl2 and potassium iodide KI are added to appropriate amount of distilled water, and a few drops of Meyer reagent are added to the extract. White precipitates indicate the presence of alkaloids [41].

c- Detection of saponins: The detection of saponins adopts the method provided by [18],using a mercury chloride detector composed of mercuric chloride HgCl and distilled water, adding mercuric chloride reagent dropwise in the extract, and a white precipitate appears to indicate that it contains saponin.

d- **Detection of turbines:** Using an aldehyde reagent consisting of glacial acetic acid and sulfuric acid, a few drops of the reagent were added to the extract, and a brown precipitate indicated the presence of terpenes [18].

e- **Detection of tannins:** The ferric chloride reagent is used to detect the presence of tannic acid. This reagent consists of ferric chloride and water. A few drops of ferric chloride reagent are added to the extract. The appearance of blue-green color indicates the presence of tannic acid [18].

vitamin C, ascorbic acid, obtained from the local market in Iraq with a purity of 25%.

Table 2: Qualitative chemical detection of the active compounds in the alcoholic extract of *Petroselinum crispum* leaves

Compound	Detector	Guide	The result
Flavonoids	**Concentrated sulfuric acid H_2SO_4**	**The appearance of a red color**	**+**
Alkaloids	**Meyer detector**	**White precipitate**	**+**
Saponins	**Mercuric chloride**	**White precipitate**	**+**
Turbines	**Anas aldehyde**	**Brown precipitate**	**+**
Tannins	**Ferric chloride**	**Bluish green**	**+**

(+) means active compounds are present in the extract.

Estimated lymphocytes (L) and heterophiles (H), and the ratio of heterophiles to lymphocytes (H/L).

Blood smears were made by placing a drop of blood on a glass slide taken directly from the wing vein of a bird, 6 birds per treatment, 2 birds per treatment, and then blood was spread on another slide Put a drop of blood on the first slide at a 45 degree angle, do not press hard and let it dry for 15 minutes, then fix the slide with 90% methanol for 2 minutes Blood dry staining according to procedure [39] were stained with Wright-Giemsa for 10 minutes. Then washed with distilled water and dried, and counted under the microscope according to the method of [10], for apurpose is to counting the lymphocytes and heterophiles cells, the ratio of heterophiles cells to lymphocytes according to the following formula. Divide the heterophiles ratio by the lymphocyte ratio to calculate H/L:-

$$\text{H/L ratio} = \frac{\text{Percentage of Heterophiles per slide}}{\text{Percentage of Lymphocytes per slide}}$$

Biochemical serum test

Use a medical syringe to collect blood from the wing vein of chickens, 6 roosters are treated once, and 2 chickens are repeated every time, the blood collection volume is 5ml at the age of 5 weeks (35 days), and the blood sample were placed in tubes that did not contain an anticoagulant of 8ml Test tubes, and then use a centrifuge (centrifuge) to separate the serum at a speed of 3000rpm for 15 minutes, and detect the chemical properties of the serum, that is, the content of total protein in the serum. Serum

total protein concentrations (g/dl) were calculated using the method of [42]. As for the estimation of blood glucose concentration (mg/dl), it was measured according to the method of [43]. Total cholesterol concentrations (mg/dl) were estimated using the method of [48]. The concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were estimated according to the method of [34] .

Determination of some oxidative stress indicators in serum

Serum glutathione (GSH) levels were determined using the method of [15]. Superoxide dismutase (SOD) levels were estimated using the method of [24]. Serum catalase (CAT) levels were estimated using the method of [16] . Glutathione peroxidase (GPX) levels in serum were determined using the method of [35] .

Statistical analysis:

Using a completely randomized design (CRD), the trial data were pored over to investigate the impact of the treatments being studied on different traits. The means that had noteworthy disparities between them were compared through [14] multiple range test, while the SAS program[37] was utilized to carry out the statistical assessment by following the mathematical model:

Yij = μ + Ti + eij

So: - **Yij**: Observation j for transaction i.

μ: The population mean of the feature.

Ti: The effect of treatment i (since the study includes the effects of the 4 treatments above).

eij: random error normally distributed with zero mean and σ^2e variance.

Results and Discussion

Table 3 displays the impact of vitamin C supplementation and *Petroselinum crispum* alcohol extract, in broiler exposed to heat stress circumstances. T2, T3, and T4 significantly outperformed T1 in comparison to these groups ($P \leq 0.01$) Studying the number of L lymphocytes at 5 weeks of age, it was found that the heterophilic H cell results showed a significant reduction ($P \leq 0.01$) for all additional treatments compared to T1, with T4 being preferred. However, the results of the heterophiles count and lymphocyte H/L showed a significant reduction ($P \leq 0.01$) for all additional treatments compared to T1.

Table 3 Effects of vitamin C and *Petroselinum crispum* alcohol extract on lymphocytes, heterophiles and H/L ratio in broiler exposed to heat stress (mean ± standard error)

Studied traits	Treatments				Significant level
	T1	T2	T3	T4	
Lymphocytes	48.00±0.58^{b}	54.00±0.88^{a}	53.67±0.58^{a}	55.67±2.33^{a}	**
Heterophil cells	27.00±0.58^{a}	23.67±0.88^{b}	23.67±0.33^{b}	21.67±0.33^{c}	**
H/L	55.93±2.25^{a}	43.77±0.35^{b}	43.28±1.27^{b}	38.90±1.85^{b}	**

Significant differences between treatments are denoted by different letters in the same column, and a ** denotes a significant level difference ($P \leq 0.01$). (4) treatments: T1 is a control without addition, T2 add vitamin C at a level of 300 mg/liter, T3 add *Petroselinum crispum* alcohol extract at a level of 300 mg/liter, and T4 add amixture of vitamin C and *Petroselinum crispum* alcohol extract at a level of 300 mg/liter respectively.

This ratio is significantly reduced (H/L) during heat stress with the addition of vitamin C in T2 and T4, which can be explained by the exposure of poultry to heat stress, which reduces the availability of the adrenal glands and increases the hormone corticosterone , the addition of vitamin C helps to increase the activity of the thyroid gland, and this increase leads to a decrease in the release of the stress hormone corticosterone, which is caused by a decrease in the ratio (H/L), a decrease in blood glucose concentration and Protein was increased, which is consistent with [5] in his report that in broiler breeder the weight and ratio (H/L) of adrenal glands decreased, plasma glucose concentration and plasma protein fraction decreased in vitamin C supplemented treatments. After applying the

Petroselinum crispum alcohol extract leaves, the treatment of T3 and T4 lymphocytes significantly increased, while the number of heterophiles cell and the H/L ratio significantly decreased. This might be because the extract contains flavonoids shown in a table(2) [2]. They are categorized as antioxidant compounds, and by possessing hydroxyl groups, they function to protect the plasma membrane surrounding lymphocytes from harm by increasing the activity of antioxidants by reducing the oxidation of fatty substances and chemicals. The body's antioxidant defense mechanisms provide hydroxyl and peroxy radicals an atom of hydrogen, stabilizing them and preventing their detrimental effects[11,23] By preventing free radicals from oxidizing these membranes, in addition to these membranes and their optional permeability [22], providing phospholipids and their antibacterial, anti-inflammatory, and oxidative actions increases the responsiveness of immunological systems[13]. Alternately, the vitamin E included in the extract may be the cause of the greater number of lymphocytes in the experimental treatments, particularly T3 and T4 therapies[9,29]. Thus, it increases the resistance of birds to disease[3,4].

Table 4 displays the findings of various serum characteristics for birds aged 5 weeks, expressed as levels of total protein, glucose, cholesterol, AST, and ALT enzymes. The results for total protein were significantly better than T1 and T2 ($P \leq 0.01$). T3 and T4 levels were significantly lower than T1 levels, but glucose and cholesterol levels were significantly higher ($P \leq 0.01$). Although all further treatments significantly reduced AST enzyme levels compared to T1 ($P \leq 0.01$), preference was given to T3 and T4, which were then superior to T2. Results from the ALT enzyme level test revealed that T4 had a significantly lower level than T1, T2, and T3 ($P \leq 0.01$).

Table 4 Effects of adding vitamin C and *Petroselinum crispum* alcohol extract on some characteristics of blood serum of 5-week-old in broiler exposed to heat stress (mean ± standard error)

Studied traits	Treatments				Significant level
	T1	T2	T3	T4	
Total protein(g/dl)	4.37±0.12^{b}	4.47±0.04^{b}	4.92±0.14^{a}	4.97±1.85^{a}	**
Glucose(mg/dl)	231.67±5.81^{a}	222.00±5.69^{ab}	212.33±3.38^{b}	211.33±3.76^{b}	**
Chlesterol(mg/dl)	179.33±2.73^{a}	171.33±5.93^{ab}	160.00±6.08^{b}	155.67±5.21^{b}	**
AST(U/L)	88.52±5.23^{a}	65.18±5.90^{b}	65.18±1.79^{c}	38.86±1.68^{c}	**
ALT(U/L)	3.67±0.17^{a}	3.57±0.11^{a}	3.34±0.06^{a}	2.54±0.13^{b}	**

Significant differences between treatments are denoted by different letters in the same column, and a ** denotes a significant level difference ($P \leq 0.01$). (4) treatments: T1 is a control without addition, T2 add vitamin C at a level of 300 mg/liter, T3 add *Petroselinum crispum* alcohol extract at a level of 300 mg/liter, and T4 add amixture of vitamin C and *Petroselinum crispum* alcohol extract at a level of 300 mg/liter respectively.

Exposure to high temperatures causes stress in birds, resulting in elevated serum levels of the hormone corticosterone, which forms glucose from non-carbohydrate sources by breaking down proteins during gluconeogenesis to help maintain adequate levels of glycemic energy supply[32]. The increased percentage of protein in serum during adjuvant therapy, especially during T3 and T4 treatment, may be due to the high flavonoid content in the *Petroselinum crispum* alcoholic extract[46], because of the Flavonoids reduce prostaglandin production by inhibiting cyclooxygenases responsible for converting fatty acid arachidonic into prostaglandins that cause hyperthermia[44]. Regarding the results of the T3 and T4 treatments, the significant drop in serum glucose levels can be attributed to the presence of phenolics[27], antioxidants[21], magnesium,

and phosphorus[36], which increase glucokinase levels in hepatocytes and lower blood glucose levels by regulating the islets of Langerhans in the pancreas Moreover, phenolic chemicals activate undifferentiated cells in the pancreatic islets of Langerhans , transform them into newly differentiated -cells through the process of glycolysis[23].increases insulin production, which is followed by a drop in blood sugar levels.

The reason for the significant reduction in serum cholesterol levels when using the alcoholic extract of *Petroselinum crispum*, especially for both treatments T3 and T4, can be attributed to its active substances such as flavonoids and alkaloids[2] , they are considered as antioxidants that prevent the oxidation of fat membranes[46] . Since these compounds limit the action of metal ions that stimulate the oxidation process, having multiple hydroxyl groups can donate hydrogen atoms to free radicals, leading to their stabilization and stopping their role in fat oxidation [45]. In addition to increasing the production of the Paraoxonase1 enzyme, Paraoxonase1 plays an important role by binding to HDL, preventing its oxidation[38].

In most organisms, transaminases (ALT, AST) transfer amino groups from amino acids to ketoacids and vice versa. As a result, they play a crucial part in life processes[19].Since it contains a considerable quantity of flavonoids, which have a protective impact on the cell membrane through their inhibitory action, the addition of the alcoholic extract of *Petroselinum crispum* alcoholic extract dramatically lowered both enzymes in the blood, notably in both treatments T3 and T4. Free radicals are affected by oxidation because they contain hydroxyl groups that contribute hydrogen atoms, which stabilize and render them inactive at the start of the fatty acid oxidation chain on the cell membrane[25].

Table 5 shows how supplemental vitamin C and *Petroselinum crispum* alcoholic extract affect the levels of catalase , and glutathione in broiler exposed to heat stress. The table below shows representative effects of partial oxidation index levels on superoxide dismutase and glutathione peroxidase at 5 weeks of age. T3 and T4 in catalase and GPX were significantly ($P \leq 0.01$)better than T1, and the experimental coefficients of glutathione and Superoxide Dismutase content did not differ significantly from each other.

Table 5 Effects of vitamin C and *Petroselinum crispum* alcohol extract on oxidative partial indexes of blood serum of 5-week-old in broiler exposed to heat stress (mean ± standard error)

Studied traits	Treatments				Significant level
	T1	T2	T3	T4	
Glutathione (µmol/L)	57.08±2.35	60.73±3.17	66.99±3.80	67.53±4.67	NS
Catalase (KATAL/ML)	0.07±0.02^{b}	0.11±0.01^{ab}	0.15±0.03^{a}	0.18±0.02^{a}	**
Superoxide Dismutase(U/L)	632.44±4.75	730.39±3.43	784.36±7.55	803.91±1.27	NS
Glutathione peroxidase(U/L)	317.17±5.53^{b}	444.40±1.63^{b}	595.44±4.82^{a}	605.95±3.36^{a}	**

Significant differences between treatments are denoted by different letters in the same column, and a ** denotes a significant level difference ($P \leq 0.01$). (4) treatments: T1 is a control without addition, T2 add vitamin C at a level of 300 mg/liter, T3 add *Petroselinum crispum* alcohol extract at a level of 300 mg/liter, and T4 add amixture of vitamin C and *Petroselinum crispum* alcohol extract at a level of 300 mg/liter respectively.

The use of crude alcoholic extracts from *Petroselinum crispum* may have caused the serum's high levels of oxidative stress enzymes as a result of the flavonoid compounds they contain[27]. These flavonoids, which are classified as antioxidants, protect the cells that

produce antioxidant enzymes, resulting in an increased concentration of these enzymes in serum[13]. The stability of flavonoids makes them unable to attack adipocyte membranes, thus preventing harmful effects [17]. Flavonoids are also responsible for the synthesis of Y-glutamylcysteine, which elevates glutathione levels and boosts its formation rate, and hence increases glutathione peroxidase concentration[50] .Vit E contained in the raw alcoholic extract of *Petroselinum crispum* is responsible for the increased oxidation index in serum because it is a natural antioxidant that works with glutathione Peptide peroxidase, together with elemental selenium to prevent the oxidation of unsaturated fatty acids in body cells[1]. The comparison of vit E with the control reveals that it keeps the antioxidants at the standard level, thereby reducing fat oxidation [3]. Vit E also combats the formation of hydrogen peroxide[31], which helps prevent damage to cell membranes[31]. It serves as the first line of defense against heat stress-induced hydrogen peroxide, thus reducing the depletion of antioxidant enzymes and elevating their levels in serum, as seen in T3 and T4 treatments.

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