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Effect of Adding Different Concentrations of Mix-Oil Solution to Drinking Water of Broiler Chickens Ross 308 and Breeders at Elevated Temperatures on Blood Biochemical Characteristics and Oxidative Enzymes at the Age of 14 Days

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ABSTRACT: This study was conducted at Al-Anwar Poultry Station located in Babil Governorate for a period of 35 days, from 10/7/2022 to 14/8/2022, to demonstrate the effect of the mix-oil solution added to the drinking water of broiler chickens on some blood biochemical characteristics and oxidation enzymes at the age of 14. day and under conditions of heat stress, where 300 Ross308 broiler chicks were used, one day old, unsexed, and the average weight of the chicks was 40 gm. The mix-oil was added to the drinking water from the first day, as follows: T1: control treatment without addition, T2: adding 0.25 ml of mix-oil / liter of water, T3: adding 0.50 ml of mix-oil / liter of water, T4: adding 0.75 ml of mix-oil / liter of water, T5: Add 1 ml of mix-oil / liter of water. The experimental birds were exposed to periodic temperatures (28-35-28).

The most important results we obtained are summarized as follows:

• A highly significant ($p \leq 0.01$) increase in the blood serum glucose concentration of the T5-treated birds compared to the control treatment. A significant increase was also observed ($p \leq 0.01$) in the concentration of total protein, albumin and globulin in the T4 treatment compared to the control treatment, while serum cholesterol was observed. The results of our experiment witnessed a "significant" rise and within the normal limits in the concentration of cholesterol for T4-treated birds on T5, T3, and T2 treatments, but it was "significantly similar" in its concentration with the T1 control treatment.

• The results showed that there was a highly significant increase ($p \leq 0.01$) in the rate of triglycerides for the T4 treatment compared to "with all treatments, while high-density lipoproteins (HDL) and low-density lipoproteins (LDL) witnessed a significant decrease ($p \leq 0.01$) in all addition treatments compared to" control treatment.

• There was a "significant" decrease ($P \leq 0.01$) for all addition treatments in the concentration of ALT enzyme compared to the "control treatment", while the T3 treatment had a significant ($P \leq 0.01$) superiority in the concentration of AST and GSH-PX enzyme compared with the control treatment, while the concentration of the enzyme CAT value decreased in all addition treatments compared to the control treatment, and the rate of MDA increased in the blood serum of T2 treatment birds compared to the control, which recorded the lowest measured rates, but it did not differ significantly with the T5 treatment in its concentration.

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I. INTRODUCTION

The rise in environmental temperatures is one of the problems affecting the production of domestic birds, especially in areas with a hot climate and during the summer months, which leads to a decrease in the productive and physiological performance of the birds. The statistics conducted indicated a rise in temperatures every decade at a rate of 0.2 m, and this rate of rise is likely to increase

(Al-Darraji & Al-Hasani, 2000: Al-Khafaji et al., 2019 & 2022). Many studies have confirmed that heat stress negatively affects the blood characteristics of birds, as a decrease in the value of PCV (packed cell volume) and Hb (hemoglobin) and a decrease in the concentration of blood plasma globulin and total protein and an increase in the concentration of glucose and cholesterol when exposing broilers to high temperatures (Al-Darraji, 1995: Al-Jebory, 2023 a) (Allen et al., 2013) (Assmann, 1993) (Bedáňová et al., 2003). In order to reduce the stress caused by high temperatures, various types of antibiotics have been widely used in the poultry industry (Bishop & Hall, 2000: Al-Jebory, 2023 b,c), but many countries have banned the use of these antibiotics as growth stimulants in the nutritional program (Buege & Aust, 1978), which encouraged the development and finding of alternatives, including Essential oils and fatty acids (Burstein, Scholnick, & Morfin, 1970: Al-Jebory, 2023). Essential oils are only aromatic oily liquids that are extracted through steam distillation or cold pressure from plant parts, such as flowers, buds, seeds, leaves, fruits, roots, etc., and the real essence of using these oils is that they are an "effective" ingredient for medical use (Franey & Amador, 1968: Al-Jebory, 2021). Essential oils are characterized by their strong aroma and varied composition and are often used in alternative medicine as a treatment (Freeman, 1987: Al-jebory, 2021). The value of essential oils and their digestibility may be affected by a major factor that plays an important role in the effect of these oils on the performance of broiler chickens and the enhancement of physiological and immunological characteristics when used in diets. This factor is the length of the chain of fatty acids constituting these oils and the percentage of saturated and unsaturated fatty acids in them. A mixture of essential oils (Hadwan & Abed, 2016). Fatty acids can be used as an alternative to growth stimulants with antibiotics which can improve the performance of broiler chickens, as well as fat deposition, improve some physiological characteristics, and reduce metabolic disorders and deaths in broiler chickens (Christie, 2016). Oxidation enzymes, this study aimed at the effect of adding Mix-oil solution at different concentrations to the drinking water of broiler chickens on some blood biochemical characteristics and oxidation enzymes at the age of 14 days and under conditions of heat stress.

II. MATERIALS AND METHODS

This experiment was conducted in the fields of Al-Anwar Company in Babil Governorate for a period of 35 days, from 10/7/2022 to 14/8/2022, where 300 unsexed one-day-old chicks were used, which were randomly divided into 5 treatments with 3 replications for each treatment. Each replicate contained 20 chicks, and the replicates were distributed within pens of dimensions 1.5 x 1 m. The Mix-oil solution was added to the water drinking since the first day of the experiment and the treatments were as follows:

T1: control treatment without addition
T2: adding 0.25 ml of mix-oil / liter of water
T3: adding 0.50 ml of mix-oil / liter of water,
T4: adding 0.75 ml of mix-oil / liter of water,
T5: add 1 ml of mix-oil / liter water.

A. The materials used in the experiment

Mix-oil solution was used, which is a commercial product consisting of a mixture of highly concentrated essential oils, from the Italian company Animal Wellness Products. Imported by Sama Al-Anwar Company for Veterinary and Agricultural Services.

1. Food treatment

The chicks were fed on a starter diet (protein content 23.04% and energy quantity 3021.45 kilo calories/kg of feed) from the age of one day until the third week of the birds' life, after that it was replaced with a growth diet (protein ratio 20.06 and energy quantity 3194.92 kilocalories/ kg of feed) until the end of the fifth week , and the feed with its additives of nano selenium and astaxanthin mixed in the concentrations shown above and water were provided freely . The feed used is as shown in Table 1.

Table (1) shows the percentages of the components of the diet used in

Feed material	Feed startup	Feed growth
Yellow corn	30	40
Wheat	28.25	24
(48% protein) soybean	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
A mixture of vitamins and minerals	0.2	0.2
Nacl	0.3	0.1

Total	100	100
Crude Protein (%)	23.04	20.06
Calculated representative energy (kcal / kg feed)	3021.45	3194.92
Lysine %	1.27	1.07
Methionine%	0.41	0.38
cysteine %	0.35	0.30
Methionine cysteine %	0.82	0.78
Phosphorous %	0.41	0.43
c/p Energy Ratio: Protein%	131.14	159.77

Chemical analysis of the suspension was calculated according to (Henry, Sobel, & Kim, 1982)

2. Preventive program

Use the preventive health program mentioned in Table (2) as follows:

Table 2: Preventive Program

age / day	**Vaccine or vitamins used**
1	**Oily vaccine (Newcastle + IB + Camboro)**
2-5	**Vitamins AD3E + C + B-Complex + Antibiotics**
14	**Newcastle Vaccine 30 IB + Clone ophthalmic instillation**

3. Breeding room temperature

The temperature inside the hall was recorded daily at 600, 1200, 1800 and 2400 by 4 thermometers distributed inside the hall, as shown in Table (3)

Table 3: Average weekly cyclic temperatures for the period 1-5

age / week	the hour			
	average temperature at 600 ° C 6 am	Average temperature at 1200 ° C 12 noon	Average temperature at 1800 ° C 6 pm	Average temperature at 2400 ° C 12 at night
1	33.60	35.14	35.90	33.24
2	29.84	35.28	35.45	29.46
3	28.71	35.57	36.17	28.10
4	29.52	36.67	36.33	28.24
5	27.65	36.80	36.54	29.60

B. Characteristics studied

1. Biochemical characteristics of blood

At the age of 14 days, blood samples were taken from the birds immediately after the slaughter process and collected in tubes that did not contain anticoagulant, and then the serum was separated from the blood using a centrifuge at a speed of 3000 rpm for 15 minutes in the laboratory of the College of Medicine. The veterinarian / University of Karbala, blood standards were measured, and the level of total protein and albumin in the blood serum was estimated using the ready-made analysis kit (kit) from the French company Orphee, which was based on the Biuret method, based on (Jones, Hancock, Harmon, & Walker, 1992). As for globulin, its level in the serum was estimated according to what was indicated by (Kaplan & Larson, 1985) and according to the following law:

Globulin concentration (gm / 100 ml of blood serum) = total protein level - albumin level

Glucose concentration (mg / 100 ml) in blood serum was also measured using a measuring kit (kit) from the French company Orphee and according to the method (Lee et al., 2014), and cholesterol concentration (Cholesterol) mg / 100 ml blood was measured using a kit prepared from French Orphee and based on the method referred to by (Elwinger et al., 2016) and the examination was conducted based on the steps indicated by the processing company in the attached guide.

As for measuring triglycerides and lipoproteins, a ready-made estimation kit was used, which is based on the sedimentation of LDL, VLDL, and Chylomicron by Phosphotungestic acid and magnesium ions Mg + 2, and the survival of HDL in the upper filtrate,

which can be estimated using the cholesterol estimation kit, and the reading was done at a wavelength of 546 nm. The high-density HDL proteins were calculated in the blood serum by using a kit from the French company (BIOLO), and the examination was carried out based on the steps indicated by the equipped company in the attached manual. The samples were read using a spectrophotometer (Londok & Rompis, 2019). And low-density proteins (LDL) were calculated according to the Friedewald formula (Long et al., 2018) ias:

(LDL = cholesterol - (HDL + VLDL).

- **Measurement of the concentrations of aminotransferase (ALT) and (AST) enzymes**.

To estimate the activity of the enzyme ALT () Alanine Amino Transferase, using a kit prepared from the French company Orphee and according to the method of (Malheiros et al., 2003), and the test was conducted based on measuring the activity of the enzyme by colorimetric methods by measuring pyruvic acid from alanine, as pyruvic acid is reacted with a compound DNPH to form a complex with a red color was measured at a wavelength of 546 nm and was estimated according to the international unit / liter. As for the estimation of the activity of the Aspartate Amino Transferase (AST) enzyme, a kit (kit) prepared from the French company 0rphee was used for this measurement according to the method of (Malheiros et al., 2003). In turn, it reacts with 2,4-dinitrophenyl hydrazine (DNPH) to form a red complex, measured at a wavelength of 546 nm.

- **Estimation of the status of oxidative enzymes**

These measurements include Glutathon Peroxidase (GSH-PX), Catalase (CAT) and Monaldehyde (MDA).

- **Determination of Glutathon Peroxidase (GSH-PX) Activity**

Glutathione was measured using a method (Millet & Maertens, 2011)that is based on the use of a precipitation solution containing metaphosphoric acid (Na2EDTA) and the addition of sodium chloride (NaCl) and placing the solution in a centrifuge at 4500 rpm for 10 minutes. The value of glutathione was estimated as the difference in the absorbance values for samples in the presence or absence of DTNB and at a wavelength of 340 nm.

- **Determination of Catalase (CAT) Enzyme Activity**

A kit (kit) equipped from the French company Orphee was used and based on (National Research Council & Subcommittee on Poultry Nutrition, 1994) and the method that supports spectrophotometry to estimate the activity of catalase, and that method depends on measuring the amount of hydrogen peroxide broken down by the catalase enzyme and using Redox dye and obtaining a change in color intensity at a wavelength of 570 nm or Fluorescence at a wavelength of 530/545 nm, which indicates the activity of the catalase enzyme in the sample.

- **Estimation of malondialdehyde (MDA) level**

Its concentration was measured using a measuring kit (kit) from the French company Orphee based on (Al-Saeedi, Ajafar, & Al-Jeobry, 2024), where this method determines the amount of lipid peroxides by measuring aldehyde, which is one of the products of lipid peroxide decomposition, and it is done by the reaction of one molecule of Malondialdehyde and two molecules of thiobarbituric acid to form a compound MDA-TBA is red in color and can be measured at a wavelength of 535 nm.

III. RESULTS AND DISCUSSION

• The effect of adding different concentrations of the mix-oil solution to the drinking water of Ross 308 broiler chickens subjected to heat stress on blood biochemical characteristics at the age of 14 days.

1. Serum glucose, total protein, albumin, globulin and cholesterol concentration.

The results of Table (4) indicate the effect of adding different concentrations of the mix-oil solution to the drinking water of broiler chickens exposed to heat stress on glucose concentration, total protein rate, albumin and globulin, as well as serum cholesterol at the age of 14 days. Where a highly significant increase ($p \leq 0.01$) was observed in the glucose concentration of the T5 treatment over all studied treatments. Followed by treatment T3, which was superior to treatment T4, T2, but it did not show any significant difference with treatment treatment T1. As for the percentage of total protein, it was superior, at a highly significant level ($P \leq 0.01$), treatment T4 over all treatments of the experiment, and a "significant" decrease was observed for the treatments. T5, T3, T2 in the total protein rate compared to the control treatment, while the T5 treatment was superior to T3, T2, and the T2 treatment had the lowest rates.

It was noted in the statistical analysis table a highly significant superiority ($P \leq 0.01$) of treatment T4 over all studied treatments in serum albumin concentration, followed by treatment T3 which outperformed treatments T5, T2, T1 and we did not notice any significant difference between the last treatments mentioned in the same concentration. As for serum globulin, its concentration increased significantly "and at a level of ($P \leq 0.01$) in the birds of T4 treatment, followed by T5 treatment, which did not show any significant difference with the control treatment on the one hand, and outperformed the T2 and T3 treatments on the other hand. It was also noted that the T2 treatment was superior. on T3 in the serum globulin concentration. Also, "a highly significant increase

(P≤0.01) was seen in the cholesterol concentration of the T4 treatment over all the addition treatments and did not differ significantly" with the control treatment T1, also T5 and T3 were superior to the T2 treatment. Which gave the lowest rates in the same concentration.

Numerous studies have shown that high temperatures in the breeding hall and exposure of birds to heat stress increases the concentration of blood sugar (Barrow, Oyen, & Dung, 1999). Relatively glucose to meet the body's energy needs during exposure to stress (Park et al., 1997). But the significant decrease in the concentration of glucose for T4 and T2 treatment birds in our study may be due to the fact that the mix-oil solution contains a mixture of oils and fatty acids that increase pancreatic activity and insulin secretion, and thus increase glucose metabolism and oxidation, and the energy gained from this process is used in protein synthesis. (Reitman & Frankel, 1957) This is the reason for the high rate of total protein in the T4 treatment shown in Table No. (4), or it may be the reason for the high rate of total protein, albumin, and globulin in some addition treatments. It may be due to the improvement of liver function and the increase in fatty acid levels due to the essential oils that make up the mix-oil solution. Thereby increasing the creation of protein and globulin in the blood serum (Preston, 2011). An increase in the room temperature may lead to an increase in the concentration of serum cholesterol, and the reason may be the increase in the rate of corticosterone secretion in response to heat stress (Sedlak & Lindsay, 1968), When adding a mix-oil solution to drinking water caused a significant decrease in the concentration of cholesterol in the addition treatments compared to the control treatment. 3) which is responsible for the synthesis of serum cholesterol.

Table 4: The effect of adding different concentrations of the mix-oil solution to the drinking water of broiler chickens subjected to heat stress on glucose concentration, total protein rate, albumin and globulin, as well as serum cholesterol at the age of 14 days

Transactions	Averages ± standard error				
	Glucose (mg / 100 ml)	Total protein (g / 100 ml)	Albumin (g / 100 ml)	Globulin (g / 100 ml)	Cholesterol (mg / 100 ml)
T1	1.85±211.33 B	0.00±2.63 b	0.06±1.47 c	0.06±1.17 b	0.04 ±110.02 a
T2	4.09±198.66 C	0.01±2.24 e	0.01±1.47 c	0.01±0.77 c	2.27±80.88 c
T3	2.33±215.66 B	0.02±2.34 d	0.01±1.68 b	0.00±0.66 d	0.87±90.76 b
T4	2.00±196.00 C	0.01±4.01 a	0.02±1.87 a	0.03 ±2.14 a	1.62±107.87 a
T5	1.00±238.00 A	0.01±2.58 c	0.01±1.37 c	0.003±1.20 b	1.18 ±93.53 b
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**** Different letters within one column indicate a significant difference at (p≤0.01) level.**

2. The concentration of triglycerides and high density lipoprotein (HDL) and the concentration of low density lipoprotein (LDL) at the age of 14 days.

The results of Table (5) indicate the effect of adding different concentrations of the mix-oil solution to the drinking water of broiler chickens exposed to heat stress on the concentration of triglycerides and high-density lipoproteins (HDL) and low-density lipoproteins (HDL) and LDL in serum at the age of 14 days. (p≤0.01) for the T4 treatment in triglyceride concentration when compared with all The studied treatments, and we also notice a "significant decrease for the treatments T5, T3, T2 compared to" the control treatment, while the treatment T3 was superior to the treatment T5, T2 and the treatment T2 gave the lowest rates measured in the concentration of triglycerides. As for high-density lipoproteins (HDL), the results of our study witnessed a "significant" decrease at (p≤0.01) for all addition treatments when compared with the control treatment, followed by T5 treatment, which outperformed T4, T3, and T2 treatments as well. T2 on the two treatments T4 and T3, which did not show any significant difference in the concentration of high-density lipoproteins (HDL).

Also, the results of the statistical analysis showed that there was a significant decrease (P≤0.01) for all addition treatments compared to the control treatment, followed by treatment T3, which was superior to T5, T4, T2, and treatment T2 was superior to treatment T5, T4, while treatment T5 was recorded. The lowest levels of low-density lipoproteins (LDL).

High environmental temperatures may expose birds to heat stress, which leads to an increase in the secretion of hormones (epinephrine and norepinephrine), and thus causes high blood fats as a result of the decomposition of fatty tissues storing it and the occurrence of hyperlipidemia, as mentioned by the researcher (Shokrollahi, Yavari, & Kordestani, 2014). As for the high triglycerides in the T4-treated birds in our study, which are shown in Table (5), the reason may be due to the linoleic fatty acid present in the mix-oil solution, which increases the lipid formation activity by increasing the decomposition of adipose tissue (St-Pierre, Cobanov, & Schnitkey, 2003). As for the decrease in high-density and low-density lipoproteins in the addition treatments compared to the control treatment, the reason may be the decrease in the secretion of lipoprotein particles in the blood (Tongnuanchan & Benjakul, 2014).

Table 5: The effect of adding different concentrations of the mix-oil solution to the drinking water of broiler chickens subjected to heat stress on the concentration of triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) at the age of 14 days.

Transactions	Averages ± standard error		
	Triglycerides	High-density lipoproteins (HDL)	Low-density lipoproteins (LDL)
T1	2.86 ± 96.21 b	0.92 ±88.60 a	0.87 ± 93.39 a
T2	1.68 ± 58.26 e	0.04 ± 69.30 c	0.50 ± 71.98 c
T3	0.56 ± 88.28 c	0.86 ± 58.80 d	0.85 ± 83.90 b
T4	1.14 ± 103.76 a	0.68 ± 57.24 d	0.35 ±67.31 d
T5	0.87 ± 67.35 d	1.08 ±76.33 b	0.04 ± 24.03 e
	**	**	**

**** Different letters within one column indicate a significant difference at (p≤0.01) level.**

• The effect of adding different concentrations of the mix-oil solution to the drinking water of Ross 308 broiler broilers subjected to heat stress on the concentration of aminotransferase and oxidation enzymes at the age of 14 days.

The results of Table (6) indicate the effect of adding different concentrations of the mix-oil solution to the drinking water of broiler chickens exposed to heat stress. in the concentration of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Glutathon Peroxidase (GSH-PX), Catalase (CAT) and Malondialdehyde (MDA) in blood serum at the age of 14 days. It was observed that a highly significant decrease (P≤0.01) occurred in all addition treatments compared to the control treatment in the concentration of ALT enzyme, followed by treatment T5, which was superior to treatment T4, T3, T2, while treatment T2 gave results that were superior to them at a significant level (P≤0.01).) on treatment T4, T3, and also, it was observed that treatment T4 was the lowest recorded in the concentration of this enzyme. As for the AST enzyme, the statistical analysis table showed a significant increase (P≤0.01) for treatment T3 over all treatments of the experiment, followed by treatment T4, which outperformed T1, T5, and T2. As for treatment T2, it gave the lowest measured rates in the concentration of AST enzyme compared to all treatments and even Less than treatment T5, T1.

As for the oxidative enzymes, we notice a highly significant increase (P≤0.01) in the concentration of the glutathione peroxidase enzyme in treatment T3 compared to all treatments of the experiment, followed by treatment T4, which was superior to treatment T5, T2, T1, and a significant decrease (P≤0.01) for the two treatments T5. T2 compared to the control treatment. As for the CAT enzyme, we notice a "significant" decrease (P≤0.01) for all addition treatments compared to the "control treatment". Also, treatment T3 did not show any significant difference with treatment T4, but they outperformed treatment T5 and T2. Treatment T5 suffered a "significant" decrease in the concentration of Catalase enzyme compared with all studied treatments. As for the malondehyde compound, a "significant" (P≤0.01) increase was seen for the T2 treatment over all the studied treatments, followed by the T3 treatment, which was similar to the T4 treatment in MDA concentration, but it was superior to T1 and T5, and we note that there is no significant difference between the last-mentioned treatments in same focus.

When reviewing the results of our study in Table (6), we notice an increase in the concentration of the AST enzyme in the T3 treatment, and the reason may be due to the exposure of the birds to heat stress. The liver, including the AST enzyme, leads to an

increase in its activity in the blood (United States Environmental Protection Agency, 2001) (Vecerek, Strakova, Suchy, & Voslarova, 2002). There is also a relationship between thyroid hormones and the activity of the AST enzyme, as it was shown through an experiment that he carried out (Wootton, 1964). A decrease in thyroid activity accompanied by an increase in AST enzyme activity, and then a decrease in protein synthesis, and this was observed in the T3 treatment, which was accompanied by a decrease in the percentage of protein in the blood serum of its birds, an increase in the concentration of AST enzyme. Also, we note that the significant effect of the mixture of fatty acids Or the MIX-OIL solution on indicators of oxidation may be due to the antioxidant property of the solution, as it enables it to reduce fat oxidation in the cells and tissues of the broiler body, especially tissues that contain a high percentage of unsaturated fatty acids, which results in peroxides, fats, oxycitrol and malondehyde (Yang, Wu, Yu, & Lin, 1992).

Table 6: The effect of adding different concentrations of the mix-oil solution to the drinking water of broilers subjected to heat stress on the concentration of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Glutathon Peroxidase (GSH-PX), Catalase (CAT) and Malondialdehyde (MDA) in serum at 14 days of age.

Transactions	Averages ± standard error				
	ALT (U/L)	AST (U/L)	GSH-PX (U/L)	CAT (U/L)	MDA (U/L)
T1	41.42± 0.43 a	129.00± 0.57 d	16.46± 0.63 c	90.82± 0.70 a	4.61± 0.18 C
T2	31.30± 0.35 c	100.84± 0.27 e	13.83± 0.14 d	69.66± 0.21 c	25.65± 0.27 A
T3	28.66± 0.73 d	166.78± 0.30 a	44.92± 0.30 a	85.44± 0.33 b	17.78± 0.11 B
T4	20.99± 0.30 e	143.82± 0.13 b	20.59± 0.25 b	85.88± 1.14 b	20.37± 3.68 B
T5	40.03± 0.07 b	139.92± 0.07 c	11.97± 0.13 e	61.54± 0.35 d	6.86± 0.08 C
	**	**	**	**	**

**** Different letters within one column indicate a significant difference at (p≤0.01) level.**

REFERENCE

1. Al-Darraji, H. J. (1995). A study of some physiological characteristics and thermal resistance of Fauber broiler chickens and their comparison with some commercial broiler crosses (Master's thesis). College of Agriculture, University of Baghdad.
2. Al-Darraji, H. J., & Al-Hasani, D. H. (2000). The effect of heat stress on blood characteristics of some broiler breeds. Iraqi Journal of Agricultural Sciences, 31, 319–336.
3. Al-Jebory, H.H. and S.A.H. Naji. 2021. Effect of Pelleted Fermented Feed in Production Performance of Laying Hens. Fourth International Conference for Agricultural and Sustainability Sciences I.O.P. Conf. Series: Earth and Environmental Science 910 (2021) 012007 IOP Publishing doi:10.1088/1755-1315/910/1/012007.
4. Al-Jebory, H.H., M. K. I. Al-Saeedi., I. L. Al-Jaryan., and F.R.Al-Khfaji.,2023 a. Impact of Neem (Azadirachta Indica) leaves powder on growth performance of broiler (Ross 308) exposed to H.S. Research Journal of Agriculture and Biological Sciences, 15(2): 1-5. DOI: 10.22587/rjabs.2023.15.2.1
5. Al-Jebory, H.H., M. K. I. Al-Saeedi., I. L. Al-Jaryan., and F.R.Al-Khfaji. 2023 b Impact of Neem (Azadirachta Indica) leaves powder on growth performance of broiler (Ross 308) exposed to heat stress. Research Journal of Agriculture and Biological Sciences, 15(2): 1-5. DOI: 10.22587/rjabs.2023.15.2.1.
6. Al-Jebory, H.H., M.A. Elsagheer, H.Q. Baqer, M.K.I. Al-Saeedi, I.L. Al-jeryan, and F. Al-Khfaji. 2023 c. Histological Study of Jejunum in Broiler Chicks Fed in the Embryonic Period with Silver Nanoparticles and Exposed to Heat Stress. Syrian Journal of Agricultural Research – SJAR. 10(5): 138-149.

7. Al-Jebory, H.H., M.K.I. Al-Saeedi, S.A. Sakr, F.R. Al-Khafaji, N.A.L. Ali, B.A.M. Lehmood, H. Taheri. A.A. A. Qotbi, and S. Ghazi. 2023 d. Improving Chicken Growth Performance with Nano Silver Added to Drinking Water. International Journal of Scientific Research in Biological Sciences. 10 (6): 01-04.
8. Aljebory, H.H.D S.A.H. Naji. 2021. Effect of Pelleted Fermented Feed-in Egg Quality of Laying Hens. Diyala Agricultural Sciences Journal 13 (1): 41-57. https://dx.doi.org/10.52951/dasj.21130105.
9. Al-Khafaji, F. R., and AL-Jebory, H. H. 2019. Effect Of Injection In Hatching Eggs With Different Concentrations Of Nanosilver At 17.5 Days Age In Some Hatching Traits And Blood Parameters For Broiler Chickens (Ross 308). Plant Arch., 19(2):1234–1238.
10. Al-Khafaji, F.R.A., H.H.D. Al-Gburi, and N.M.A. Al-Gburi. 2022. Effect of injecting hatching eggs with different concentrations of nanosilver at the age of 17.5 days from the age of the embryos on the qualitative traits for the carcass and some lymphatic organs for the broiler chickens (Ross 308). NeuroQuantology. 20(11): 2768 -2774. doi: 10.14704/NQ.2022.20.11.NQ66281.
11. Allen, H. K., Levine, U. Y., Looft, T., Bandrick, M., & Casey, T. A. (2013). Treatment, promotion, commotion: Antibiotic alternatives in food-producing animals. Trends in Microbiology, 21(3), 114–119.
12. Al-Saeedi, M. K. I., Ajafar, M., & Al-Jeobry, H. H. (2024). Immunity and glycogen metabolism of laying hens fed diets supplemented with manganese sulfate during the forced molting. Journal of Animal Health Production, 12(3), 413–419.
13. Assmann, S. M. (1993). Signal transduction in guard cells. Annual Review of Plant Physiology and Plant Molecular Biology, 44, 345–375.
14. Barrow, S., Oyen, L. P. A., & Dung, N. X. (1999). Plant Resources of South-East Asia No. 19. Essential-Oil Plants. Kew Bulletin, 54(2), 502.
15. Bedáňová, I., Voslářová, E., Večerek, V., Strakova, E., & Suchý, P. (2003). The hematological profile of broilers under acute and chronic heat stress at 30±1°C level. Folia Veterinaria, 47, 188–192.
16. Bishop, A. L., & Hall, A. (2000). Rho GTPases and their effector proteins. Biochemical Journal, 348(2), 241–255.
17. Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In Methods in Enzymology (Vol. 52, pp. 302–310). Academic Press.
18. Burstein, M. S. H. R., Scholnick, H. R., & Morfin, R. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Journal of Lipid Research, 11(6), 583–595.
19. Christie, L. G. (2016). Use of essential oils on the development of academic and social skills in an autistic child (Doctoral dissertation, Master of Education Thesis, University of Canterbury). https://doi.org/10.13140/RG.2.2.10783.18080
20. Elwinger, K., Fisher, C., Jeroch, H., Sauveur, B., Tiller, H., & Whitehead, C. C. (2016). A brief history of poultry nutrition over the last hundred years. World's Poultry Science Journal, 72(4), 701–720.
21. Franey, R. J., & Amador, E. (1968). Serum cholesterol measurement based on ethanol extraction and ferric chloride-sulfuric acid. Clinica Chimica Acta, 21(2), 255–263.
22. Freeman, B. M. (1987). The stress syndrome. World's Poultry Science Journal, 43(1), 15–19.
23. Hadwan, M. H., & Abed, H. N. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief, 6, 194–199.
24. Henry, R. J., Sobel, C., & Kim, J. (1982). Determination of uric acid. In N. W. Tietz (Ed.), Fundamentals of Clinical Chemistry. W.B. Saunders Company.
25. Jones, D. B., Hancock, J. D., Harmon, D. L., & Walker, C. E. (1992). Effects of exogenous emulsifiers and fat sources on nutrient digestibility, serum lipids, and growth performance in weanling pigs. Journal of Animal Science, 70(11), 3473–3482.
26. Kaplan, M. M., & Larson, P. R. (1985). The Medical Clinics of North America (thyroid disease) (Vol. 69). W.B. Saunders Company.
27. Lee, J. H., Cho, S., Paik, H. D., Choi, C. W., Nam, K. T., Hwang, S. G., & Kim, S. K. (2014). Investigation on antibacterial and antioxidant activities, phenolic and flavonoid contents of some Thai edible plants as an alternative for antibiotics. Asian-Australasian Journal of Animal Sciences, 27(10), 1461.
28. Londok, J. J. M. R., & Rompis, J. E. G. (2019, November). Supplementation of lauric acid and feed fiber to optimize the performance of the broiler. In IOP Conference Series: Earth and Environmental Science (Vol. 387, No. 1, p. 012082). IOP Publishing.
29. Long, S., Xu, Y., Wang, C., Li, C., Liu, D., & Piao, X. (2018). Effects of dietary supplementation with a combination of plant oils on performance, meat quality, and fatty acid deposition of broilers. Asian-Australasian Journal of Animal Sciences, 31(11), 1773.

30. Malheiros, R. D., Moraes, V. M., Collin, A., Decuypere, E., & Buyse, J. (2003). Free diet selection by broilers is influenced by dietary macronutrient ratio and corticosterone supplementation. Poultry Science, 82(1), 123–131.
31. Millet, S., & Maertens, L. (2011). The European ban on antibiotic growth promoters in animal feed: From challenges to opportunities. The Veterinary Journal, 187(2), 143–144.
32. National Research Council, & Subcommittee on Poultry Nutrition. (1994). Nutrient requirements of poultry: 1994. National Academies Press.
33. Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E., & Pariza, M. W. (1997). Effect of conjugated linoleic acid on body composition in mice. Lipids, 32(8), 853–858.
34. Preston, R. A. (2011). Acid-base, fluids, and electrolytes are made ridiculously simple.
35. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28(1), 56–63.
36. Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry, 25, 192–205.
37. Shokrollahi, B., Yavari, Z., & Kordestani, A. H. (2014). Effects of dietary medium-chain fatty acids on performance, carcass characteristics, and some serum parameters of broiler chickens. British Poultry Science, 55(5), 662–667.
38. St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by U.S. livestock industries. Journal of Dairy Science, 86, E52–E77.
39. Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science, 79(7), R1231–R1249.
40. United States Environmental Protection Agency. (2001). Quality assurance guidance document-model quality assurance project plan for the PM ambient air (Vol. 2, p. 12).
41. Vecerek, V., Strakova, E., Suchy, P., & Voslarova, E. (2002). Influence of high environmental temperature on production and hematological and biochemical indexes in broiler chickens. Czech Journal of Animal Science, 47(5), 176–182.
42. Wootton, I. D. P. (1964). Micro-analysis in medical biochemistry.
43. Yang, Q. M., Wu, Q. W., Yu, Z. H., & Lin, H. (1992). A study of the influence of environmental temperature on some biochemical indices in serum of broilers.