Effect of adding different levels of mix-oil solution to the drinking water on the blood biochemical traits and oxidative enzymes of broiler (Ross 308) exposed to heat stress

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Abstract

The study was performed at Al-Anwar poultry farm located in Almuradia distract - Babylon Province for 35 day period from 10 / 7 / 2022 until 14 / 8 / 2022 to evaluate the effect of supplementing different levels of mix-oil to the drinking water of broiler on the blood biochemical traits and oxidative enzymes at day 35 under the conditions of heat stress. 300 unsexed one day old broiler chicks (Ross 308) were used in the study. Mix-oil was applied and added to the drinking water of the study from the first day as follows: control treatment without addition; second treatment added 0.25 ml mix-oil/L drinking water; third treatment added 0.50 ml mix-oil/L drinking water; fourth treatment added 0.75 ml mix-oil/L drinking water; and finally fifth treatment added 1 ml of mix-oil/L drinking water. Heat stress temperatures that the birds were exposed to range from 28-35 C°. The results of the study showed that adding mix-oil solution had a significant effect on most of the study characteristics including blood biochemical traits ($p < 0.01$); whereas the highest value of glucose was reordered in T4, total protein was significantly affected by the addition of mix-oil solution as well, specifically in T2, while the highest and the lowest values for the albumin and globulin was recorded for T5 respectively. Also, the addition of mix-oil solution, specifically in T3 and T5 reduced cholesterol and triglyceride respectively compared to the control which is good for the animals, while the HDL trait was reduced in the treated treatments; however, it helped reduce the LDL concentration compared to the control treatment. The addition of the mix-oil solution had a significant ($P\leq 0.01$) effect on all of the oxidative enzymes except that catalase was not significantly affected by the mix-oil solution.

Introduction

Climate change is one of the biggest problems that glob is facing nowadays, which is considered a big challenge for many countries, especially in the Middle East and because of the hot weather these countries have, which as a result reflects on the people, animals, plants, and the environments as a whole (Livingston et al., 2022; Zittis et al., 2022). Thus, it has been listed as one of the sustainable development goals the world is trying to address (Hariram et al., 2023). From the animal standpoint, specifically, broilers because it is the subject of this study and the effect of heat stress on these types of animals. Heat stress occurs when the temperature and humidity become higher than the body temperature of the birds (Apalowo et al.,

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2024). Poultry is recognized as homoeothermic animals independent from the outside temperature, which maintains their temperature at a certain level. When temperature increase higher than that level, the birds are stressed (Bülent Bayraktar et al., 2020). In addition, poultry doesn't have sweat glands which increases heat stress because it cannot control its temperature which depends on the outside temperature. Heat stress negatively affects the birds' physiological traits, specifically blood traits. There are lots of studies certified that heat stress reduced globulin and total protein concentrations and increased cholesterol and glucose in the blood plasma (Yang et al., 1992; Al-Daradji and Al-Hasani, 2000; Vecerek et al., 2002; Bedanova et al., 2003). One of the most important actions to reduce or control heat stress for the birds is providing them with cold water. So they used to drink water to balance their temperature. Many feed additives have been used to prevent stress and obtain good quality and quantity of products. One of these additives that have been used for decades is antibiotics which are now banned by many countries especially in the European Union as a result of the resistant bacteria which in the end affect animals, humans, and the environment negatively (Dibner and Richard, 2005; Tekce et al., 2020). Therefore, one of the alternative feed additives that have been used safely for animals was essential fatty acid which was originally a derivative from medicinal plants. This type of additives have antioxidant, antimicrobial, anti-inflammatory, antiviral, antitumoural, antifungal, antiparasitic effects as stated by (Bishop, 1995; Karpouhtsis et al., 1998; Ultee et al., 2002; Botsoglou et al., 2004; Fu et al., 2007; Monzote et al., 2007; Daroui-Mokaddem et al., 2010; Al-Jafari et al., 2011; Silva et al., 2011; Guimaraes et al., 2013; Kpoviessi et al., 2014; Dezsi et al., 2015). Essential oil has been supplemented to broilers exposed to heat stress which has positively impacted as stated by (Parvar et al., 2013; Gopi et al., 2014; Akbarian et al., 2015; Petrolli et al., 2019). Therefore, this study aims to evaluate the effect of adding different levels of mix-oil solution to the broiler drinking water (Ross 308) under heat stress conditions on the blood biochemical and oxidative enzymes at the age of 35 days.

Materials and Methods

-1Experimental diets and bird management

This experiment was conducted at Al-Anwar Poultry Company located in Almuradia district – Babylon province for 35-day, from 10/7/2022 until 14/8/2022. Three hundred unsexed one day old broiler chicks (Ross 308) were used for the study. Broiler chicks were randomly allocated to five treatments with three replicates and twenty chicks for each replicate. The dimensions for each pen (replicate) used during the study were 1.5 × 1 m. Treatments started using a mix-oil solution from the first day of the study in the following order. Control treatment without addition (T1); 0.25, 0.50, 0.75, and 1 ml of mix-oil solution were added per liter of water for T2, T3, T4, and T5 respectively .

The mix oil solution used in this experiment was a product produced commercially and imported by Sama Al-anwar poultry company, which produced by the Italian company of Animal Wellness. This product has consisted of a highly concentrated essential oil mixture. Table 1 shows the chemical composition of the mixed oil solution that was analyzed by the Iraqi Ministry of Science and Technology (Abdul Reda, H. A. S., 2022.(

Table 1: chemical composition of mix-oil solution

Name of the fatty acids	%
Butyric acid	3.8
Linoleic acid	15.9
Palmitic acid	16.3
a-Lenolinic acid	14.8
Mystiric acid	2.9
Stearic acid	3.6
Eicosapentaenoic	8.6
Docosahexaenoic	7.9
Oleic acid	20.6
Lauric acid	1.3
Arachidonic acid	3.9

Birds were fed on the starter and finisher diets based on Table 2 below.

Table 2: Feed Ingredients and nutrient composition of the experimental diet

Diet ingredients	Starter (%)	Finisher (%)
Corn	30.10	40.00
Wheat	28.16	24.00
SBM (48% CP)	31.74	24.80
Protein concentrate	5.00	5.00
Veg oil	2.90	4.40
Limestone	0.90	0.60
Dicalcium phosphate (DCP)	0.70	0.90
Vitamins and Minerals Premix	0.20	0.20
Nacl	0.30	0.10
Calculated values, %		
ME (kcal/kg)	3021	3195
CP	23.04	20.06
Lysine	1.27	1.07
Methionine	0.41	0.38
Cysteine	0.35	0.30
Methionine + cysteine	0.82	0.78
Available phosphorus	0.41	0.43
Energy: Protein	131.14	159.77

Protein concentrate type W Special 5 – Brocon: Made in China Each kg contains 40% crude protein, 5.3% fat , 1% fiber , 6% calcium , 3% available phosphorus , 25.3% lysine , 90.3% methionine+cysteine , 2.2% sodium , 2100 kcal / kg metabolized energy , 20000 IU vitamin A , 40000 IU vitamin B3 20 mg, 150 mg vitamin B2 + B1 mg , 15 mg vitamin K3, 30 mg vitamin E, 500 mg vitamin D3, 300 mg vitamin B6, 300 mg vitamin B12, 10 mg folic acid , 100 microgram biotin, 1 mg iron , 100 mg Copper, 2.1 mg Manganese, 800 mg Zinc , 15 mg Iodine, 2 mg Se, 6 mg Cobalt , 900 mg Antioxidant (BHT.(

-3Hall breeding temperature

The temperature was recorded 4 times during the day in the experimental room as shown in the table below:

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Table 3: Average temperature used in the study for a period of 1-5 weeks.

Age/week	Time/temperature C°			
	at6 am	at noon(12pm)	at 6pm	at 12am
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

-4blood biochemical measurements

Samples of the blood were collected at the end of the experiment on 35 days of age, which was obtained after slaughtering the birds by using tubes without anticoagulant and placed in the centrifuge at a speed of 3000 rpm /15 minutes to separate the serum. This process was done using College of Veterinary Medicine laboratory- University of Karbala to measure standard blood samples, total protein levels, and albumin. A French company Orphee kit was used to calculate the Albumin which was called the ready-made analysis kit, based on the method of Biuret (Wotton, 1964). In contrast, globulin concentration was measured according to what was pointed out by (Bishop and Hall, 2000) based on this formula.

Globulin level (gm./100ml blood serum) = Total protein level - Albumin level

Glucose and cholesterol levels (mg./100ml) in the blood serum were measured using the same kit used above and according to the method mentioned by (Henry et al., 1982; Franey and Elias, 1968.(

Triglycerides and lipoproteins were measured using a ready-made estimation kit, which read at a wavelength of 546 nm. In contrast, HDL was calculated using a French company kit called BIOLO in the blood serum and the spectrophotometer was used to read the samples (Burstein et al., 1970) while LDL calculated based on the formula of Friedewald (Assmann, 1993) as follow:

LDL = cholesterol – (HDL-VLDL(

-5ALT and AST measurements

The activity of Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) was measured using a kit prepared by the French company Orphee using the method reported by Reitman and Frankel (1957.(

-6Measurements of the oxidative enzymes

Catalase (CAT), Glutathione Peroxidase (GSH-PX), and malondialdehyde (MDA) Enzyme activity were measured using the French company Orpheen kit and based on the methods reported by (Sedlak and Lindsay, 1968; Hadwan and Abed, 2016; Buege and Aust, 1978) for all three of them respectively.

Statistical analysis

Complete Randomized Design (CRD) with [SAS, 2012] was used for analyzing the data. Significant differences between means were compared using Duncan, 1955 multinomial test and the mathematical model:

Yij = μ + Ti + eij

Results and discussion

Blood biochemical parameters

Mix-oil solution has affected all the blood biochemical traits significantly (table 4). Glucose was significantly ($P \leq 0.01$) increased

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in the fourth treatment (0.75 ml) compared to the control; however, T2, T3, and T5 had substantially lower Glucose values in contrast with the control. Total protein showed a significant increase in T2 compared to the control, while other treatments had approximately similar values to the control except for the lowest one (T5). Whereas, T5 significantly had the highest and the lowest values of albumin and globulin respectively. Other treatments had approximately similar values to the control .

Table 4:- Evaluating adding different levels of mix-oil to the broiler (Ross 308) drinking water exposed to heat stress and its effect on the blood biochemical traits (glucose, protein, albumin, and Globulin concentrations) at 35 days of age

Treatments	Mean ± SE Glucose (mg/100ml)	Total protein	Albumin	Globulin
		(g/100ml)		
T1	288.33 ± 1.66^{b}	3.56 ± 0.35^{bc}	1.59 ± 0.04^{b}	1.97 ± 0.38^{ab}
T2	199.66 ± 4.33^{d}	4.43 ± 0.27^{a}	1.60 ± 0.02^{b}	$2.83\ 0.30^{a}$
T3	213.33 ± 1.76^{c}	2.81 ± 0.05^{cd}	1.56 ± 0.05^{b}	1.25 ± 0.04^{bc}
T4	297.33 ± 2.66^{a}	3.83 ± 0.31^{ab}	1.32 ± 0.03^{c}	2.51 ± 0.32^{a}
T5	219.66 ± 0.33^{c}	2.53 ± 0.05^{d}	1.85 ± 0.04^{a}	0.68 ± 0.06^{c}
P-value	**	**	**	**

**Different letters within the column indicate significant differences at the level of ($p < 01.0$) Treatments: T1 was the control treatment (without addition); T2 was used mix-oil solution at the level of 25.0 ml/l; T3 used mix-oil solution at the level of 50.0 ml/l; T4 has used mix-oil solution at the level of 75.0 ml/l; T5 has used mix-oil solution at the level of 1 ml/l.

Many studies have found that increasing temperature in the poultry house leads to heat stress for the birds which in the end increases the concentration of blood glucose (Barrow et al., 1999). As a result, blood glucose increases to meet the energy needed by the birds during heat stress (Park et al., 1997). The results of this study showed a decrease in the blood glucose in treatments T3 and T5 and the reason may be because of the mixture of oil and fatty acids that these treatments have which may increase insulin secretion. Also, insulin secretion may increase the metabolism of glucose and then the energy that would be used in the synthesis of protein and affect the results of total protein, albumin, and globulin (Reitman and Frankel, 1957). These results were obtained by Abdul Reda, H. A. S. (2022) when he used similar levels of mix-oil in his thesis, but with powder and found that T3 and T5 were significantly lower than the control in the glucose and globulin traits which are similar to this result; however, the total protein and albumin were different. This study disagreed with the results reported by Nafel et al. (2024) when they used a mix-oil solution for 14 day- old- broiler on most of the parameters mentioned above .

As shown in Table 5 the addition of a mix-oil solution to drinking water helped reduce cholesterol, triglyceride, and LDL significantly ($P \leq 0.01$) in contrast to the control in most treatments. Whereas, T3 (0.050 ml) significantly showed the lowest values among them, which is a good sign because as long as these values are low, the birds have good health. However, HDL concentration was

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linearly decreased as the level of the mix-oil solution increased
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Table 5:- Evaluating adding different levels of mix-oil to the broiler (Ross 308) drinking water exposed to heat stress and its effect on cholesterol, triglyceride, HDL, and LDL concentrations at 35 days of age

Treatments	Mean ± SE			
	Cholesterol (mg/100ml)	Triglyceride (mg/100ml)	HDL (mg/100ml)	LDL (mg/100ml)
T1	148.20 ± 3.40^{a}	115.42 ± 0.86^{a}	124.55 ± 1.89^{a}	101.55 ± 1.65^{a}
T2	128.74 ± 3.72^{bc}	115.10 ± 2.44^{a}	110.05 ± 0.63^{b}	90.42 ± 0.64^{b}
T3	122.45 ± 1.53^{c}	81.67 ± 2.78^{c}	109.49 ± 0.62^{b}	50.79 ± 3.78^{c}
T4	133.01 ± 1.04^{b}	102.55 ± 1.01^{b}	114.05 ± 1.55^{b}	95.07 ± 2.29^{ab}
T5	121.08 ± 2.70^{c}	102.17 ± 1.53^{b}	104.47 ± 1.71^{c}	38.85 ± 0.91^{d}
P-value	**	**	**	**

HDL=High-density lipoproteins, LDL=Low-density lipoproteins
**Different letters within the column indicate significant differences at the level 0f ($p < 01.0$)
Treatments: T1 was the control treatment (without addition); T2 has used mix-oil solution at the level of 25.0 ml/l; T3 has used mix-oil solution at the level of 50.0 ml/l; T4 has used mix-oil solution at the level of 75.0 ml/l; T5 was used mix-oil solution at the level of 1 ml/l

This study agreed with the results found by Nafel et al. (2024) who reported that adding mix-oil to the water significantly reduced cholesterol, triglyceride, and LDL. Also, the reduction in LDL and HDL in the mix-oil solution treatments may be due to reduced lipoprotein particle secretion in the blood (Tongnuanchan. and Benjakul, 2014). This results disagreed with the results reported by Abdul Reda, H. A. S. (2022) when he used mix-oil powder, the levels of cholesterol, triglyceride, HDL, and LDL increased while in the current study, these values were reduced. Nafel et al. (2024) reported that adding the solution of mix-oil to the drinking water of 14-day-old broiler chicks had reduced the percentages of cholesterol, triglyceride, HDL, and LDL which is similar to our results.
Oxidative enzymes

Table 6:- Evaluating adding different levels of mix-oil to the broiler (Ross 308) drinking water exposed to heat stress on the AST, ALT, GSH - PX, CAT , and MDA concentrations at 35 days of age

Treatments	Mean ± SE				
	AST (IU/L)	ALT (IU/L)	GSH-PX (IU/L)	CAT (IU/L)	MDA (IU/L)
T1	156.51 ± 0.63[b]	20.04 ±0.88[b]	20.18 ± 1.26[c]	85.15 ± 2.61	32.87 ± 1.42[a]
T2	155.81 ± 2.49[b]	24.15 ± 0.29[a]	31.73 ± 1.27[b]	78.07 ± 2.04	19.24 ± 1.64[b]
T3	165.77 ± 1.54[a]	20.02 ± 0.24[b]	20.89 ±0.91[c]	86.77 ± 3.95	16.52 ± 0.32[bc]
T4	157.68 ± 1.00[b]	20.79 ± 1.24[b]	38.14 ± 1.73[a]	81.50 ± 0.78	19.03 ± 0.64[b]
T5	137.84 ± 0.56[c]	22.09 ± 0.42[ab]	36.94 ± 0.68[a]	78.75 ± 2.89	15.23 ± 0.34[bc]
P-value	**	**	**	NS	**

AST = Aspartate Amino Transferees, ALT = Alanine Amino Transferees, GSH - PX= Glutathione Peroxidase, CAT = Catalase, and MDA = Malondialdehyde.

**Different letters within the column indicate significant difference at the level of (p < 01.0) Treatments: T1 was the control treatment (without addition); T2 was used in a mix-oil solution at the level of 25.0 ml/l; T3 used mix-oil solution at the level of 50.0 ml/l; T4 has used mix-oil solution at the level of 75.0 ml/l; T5 was used mix-oil solution at the level of 1 ml/l

Table 6 showed that most of the oxidative enzymes were significantly (P≤0.01) affected by the mix-oil solution compared to the control. T3 was substantially higher than other treatments including the control in the Aspartate Amino Transferase (AST). Alanine Amino Transferase (ALT) was significantly increased in T2 compared to the control and treatments T3 and T4, but not significantly different from T5. Glutathione Peroxidase (GSH-PX) significantly increased in T4 and T5 in contrast to other treatments, whereas no significant differences were found between T3 and T1 (the control) which were significantly lower than T2. No significant difference was found between mix-oil solution treatments; however, all of them were significantly lower than the control treatment in the measurements of Malondialdehyde (MDA). Finally, for the Catalase (CAT) enzyme, no significant difference was found between treatments including control. Approximately similar results were found with Abdul Reda, H. A. S. (2022) in the parameters mentioned above. Nafel et al., 2024 mentioned that adding the solution of mix-oil to the water of 14-day-old broiler chicks reduced the values of ALT and CAT and increased the values of AST, GSH-PX, and MDA which is a little different than our results.

Conclusion

The study concluded that adding the solution of mix-oil to the water of the broiler chicks had significantly improved both biochemical traits and the oxidative enzymes. 0.25 ml of mix-oil solution showed the best results with low blood glucose and high total protein, including (albumin and globulin). Reduced cholesterol, triglyceride, HDL, and LDL were significantly obtained when the level of the mix-oil solution was 0.50 ml/L of water. Oxidative enzymes were significantly affected

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by the level of 0.50 ml of mix-oil solution. Therefore, adding the solution of mix-oil to the drinking water of broilers would improve their ability to resist exposure to heat stress compared to the control

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