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Effect of betaine supplementation in diets containing oxidized oil on growth performance, blood metabolites, meat quality and oxidative stability of broiler chickens

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Abstract To investigate the effect of betaine supplementation in diets containing oxidized oil on growth performance, blood metabolites and meat quality of broiler chickens, a 2×2 factorial experiment with four treatments and four replicates was performed using 144 one-day-old male Ross 308 chicks. The experimental groups included basal diet with fresh soybean oil (Peroxide value (PV): 3 meq kg⁻¹), basal diet with fresh soybean oil and 0.1% betaine, basal diet with oxidized oil (PV: 200 meq kg⁻¹), and basal diet with oxidized oil and 0.1% betaine. Performance traits were measured at the end of each period. At the end of the trial, two chicks/replicate were randomly selected, weighed, and the blood metabolites and meat quality traits were evaluated. The results showed that the use of oxidized oil led to a significant decrease in feed consumption and weight gain and an increase in feed conversion ratio in the grower, finisher, and the whole breeding period ($P<0.05$), while the addition of betaine supplementation led to an increase in feed consumption, weight gain and a decrease in feed conversion ratio ($P<0.05$). The effect of oxidized oil on blood metabolites was not significant while betaine supplementation lowered blood cholesterol and triglycerides ($P<0.05$). In chickens fed oxidized oil, water holding capacity of breast meat decreased and cooking loss, drip loss, and meat peroxide value increased ($P<0.05$), while betaine supplementation increased the water holding capacity and decreased cooking loss, drip loss, and meat peroxide value ($P<0.05$). Oxidized oil increased the amount of malondialdehyde in breast and thigh meat within 30 days after slaughter ($P<0.05$). The addition of betaine resulted in a reduction in breast and thigh malondialdehyde content ($P<0.05$). Therefore, oxidized oil may reduce growth performance, meat quality and oxidative stability, while betaine supplementation plays an effective role in improving the growth performance, quality and oxidative stability of meat of broiler chickens.

Keywords: betaine, broiler, meat quality, oxidized oil, oxidative stability

Introduction

Appropriate energy levels are necessary for adjusting the diets of broiler chickens to achieve optimal production performance. Energy requirements account for about 70% of the total cost of poultry diets. Fats produce 2.25

times more energy than proteins and carbohydrates. Vegetable oils, energy sources commonly used in poultry diets, are effective in increasing feed palatability and efficiency (Attia et al., 2019). The high cost of edible vegetable oils encourages breeders to use oxidized

restaurant oils. Vegetable oils contain high levels of polyunsaturated fatty acids that undergo oxidation process during storage or heating, which reduces the quality of the oil and diet (Vossen et al., 2011). Also, oxidation leads to changes in taste and smell of oil and consequently reduces the palatability of the diet. Oxidation of unsaturated fatty acids leads to the production of free radicals that compromise poultry health (Kaleem et al., 2015). Free radicals reduce the absorption of nutrients by reacting with proteins, lipids, and vitamins (Tavarez et al., 2011). On the other hand, oil peroxidation reduces the antioxidant capacity in poultry (Tan et al., 2018). Oxidized oils, such as restaurant oils, can reduce the growth and weight of broiler chickens by reducing feed intake (Bayraktar et al., 2011; McGill et al., 2011). Increases in feed conversion ratio and blood malondialdehyde content in broiler chickens consuming oxidized fish oil have already been reported (Tan et al., 2019). In an experiment, feeding broilers with oxidized oil (PV: 70 meq kg⁻¹) negatively impacted on the growth performance, carcass characteristics, gut morphology and meat quality. Also using oxidized oil, reduced digestibility of fat and protein, leading to poor feed conversion ratio. These researchers stated that oxidized oil in broiler diet, can damage fat-soluble vitamins, amino acids, and pigments, affecting growth and potentially causing oxidative stress (Yaseen et al., 2021). Feeding oxidized oils, resulted in reduced growth performance, including reduced average daily feed intake, average daily gain, and gain-to-feed ratio in broilers. Feeding oxidized oil also affected markers of oxidative stress (Lindblom et al., 2019). The use of antioxidant compounds can be a good solution to reduce or stop the oxidation of oxidized oils. Betaine, or 3-methyl-glycine, is a nutrient that is naturally produced from sugar beets or in the form of hydrochloride in industry. Betaine is synthesized from choline in cells mitochondria, but this synthesis does not take place in sufficient quantities in the body. Choline is necessary for the production of acetylcholine and the transmission of nerve messages; however, the amount of choline in corn-soybean basal diet is not enough to meet the need of cells for methyl groups, so the addition of betaine to the diet helps to maintain the stores of choline and methionine to supply methyl groups (Sakomura et al., 2013). The antioxidant properties of betaine have already been proven by researchers. In a study, the addition of 1 g/kg betaine supplement to the diet of broiler chickens under heat stress improved feed intake, weight gains and feed conversion ratio, and also increased breast muscle weight and the activity of glutathione peroxidase enzyme compared to control group. These researchers attributed these results to the antioxidant properties of betaine in combating with heat stress (Shakeri et al., 2018). In an experiment, the use of 0.1 and 0.2% levels of betaine supplementation in the diet of broiler chickens under heat stress improved live weight, and reduced mortality rate caused by heat stress (Nofal et al., 2015). Therefore, due to the presence of

peroxidation products in oxidized oil and the possibility of oxidation progression during its use in the diet and also due to the antioxidant role of betaine, this experiment was designed to investigate the effect of betaine supplementation on growth performance and blood metabolites of broiler chickens fed oxidized oil.

Material and methods

Birds, diets and experimental design

This experiment was conducted as a 2×2 factorial experiment 2 levels oxidized oil (0, 1) and 2 levels of betaine (0, 1), four replications in a completely randomized design using 144 one-day-old male Ross 308 broiler chicks. The experimental diets for the starter, grower and finisher periods were adjusted based on the requirements expressed in the Ross 308 breeding manual using UFFDA software. In Table 1, the feed components and analysis are reported. Betaine supplementation (Eminesan, Tehran, Iran) was added to the feed as powder form and the oxidized oil used was supplied from recycled restaurant oil and completely replaced with fresh soybean oil in the treatments with oxidized oil.

Table 1. Ingredients and chemical composition of the control diet fed to broilers

	Starter (1-10 d)	Grower (11-25 d)	Finisher (26-42 d)
Ingredients (%)			
Corn	50.60	50.40	58.41
Soybean meal	41.77	37.81	33.68
Oil*	3.52	3.73	4.43
Limestone	1.28	1.29	1.14
Dicalcium phosphate	1.62	1.66	1.27
Sodium chloride	0.31	0.31	0.30
DL-Methionine	0.16	0.12	0.10
L-Lysine	0.23	0.17	0.17
Vitamin premix ^[1]	0.25	0.25	0.25
Mineral premix ^[2]	0.25	0.25	0.25
Calculated analysis (DM basis)			
Metabolizable energy (kcal/kg)	3000	3050	3150
Crude protein (%)	23.00	21.50	20.00
Calcium (%)	0.96	0.96	0.81
Available phosphorus (%)	0.48	0.48	0.41
Lysine (%)	1.44	1.29	1.19
Methionine (%)	0.56	0.51	0.45

^[1] Vitamin premix supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 1500 IU; tocopherol, 60 IU; phyloquinone, 2 mg; thiamine, 2.4 mg; riboflavin, 4.8 mg; niacin, 30 mg; pantothenic acid, 16 mg; pyridoxine, 3 mg; folic acid, 1 mg; vitamin B12, 0.03 mg; biotin, 0.15 mg; and choline chloride, 50 mg. ^[2] Mineral premix supplied the following per kilogram of diet: Mn, 80 mg; Fe, 120 mg; Zn, 60 mg; Cu, 100 mg; I, 0.95 mg; and Se, 0.25 mg. * Oxidized restaurant oil completely replaced with fresh soybean oil in the treatments with oxidized oil.

Measurement and sample collection

To measure the peroxide value (PV) of fresh soybean oil and oxidized oil, one gram of oil was mixed with 25 mL of chloroform and acetic acid solvent mixture (2:3 V/V) and homogenized. Then, 1 mL of saturated potassium iodide solution was added to it and stored in the dark for five minutes. Then, 75 mL of distilled water and 0.5 mL of 1% starch solution were added to the mixture. At the end, the mixture was titrated against 0.01 N sodium

thiosulfate until the blue color was disappeared due to iodine release. Thus, based on the amount of sodium thiosulfate consumed, the amount of peroxide number was calculated based on mEq peroxide per kilogram of oil (Jonaidi Jafari et al., 2018).

Peroxide value = [(normality × volume of sodium thiosulfate consumed) / oil weight] × 1000

The peroxide values of fresh soybean oil and oxidized oil were 3 and 200 mEq per kg of oil, respectively.

Feed intake and bird weight per pen were separately measured at the beginning and end of each period. Feed conversion ratio in each period was calculated by dividing the feed consumed by the weight gain. At the end of the experiment, two birds per replicate were randomly chosen, weighed, and blood samples were collected from the brachial vein; the birds were then slaughtered by cutting the jugular vein. Serum biochemical parameters were measured using an auto-analyzer (Cobas Mira Plus, Roche Diagnostics Systems, Inc., Montclair, NJ, USA). Breast meat samples were separated to determine the quality of the meat. Traits related to meat quality were measured one day after slaughter. Also, to determine the peroxide value and malondialdehyde content, breast and thigh meat samples were stored in a freezer at -20°C for 30 days.

To measure pH, five grams of raw meat sample were stirred in 25 milliliters of distilled water to become uniform, and after filtering, the pH of the samples was read with pH meter. To measure the water-holding capacity of the meat, four grams of the sample were wrapped in a sterile gas and centrifuged at 1500 rpm for four minutes, then gently dried and weighed. After that, the sample was dried in an oven at 70 degrees Celsius for 24 hours and weighed again. The water-holding capacity was determined from the difference between the weight after centrifugation and the weight after drying, divided by the initial weight, multiplied by 100 (Castellini et al., 2002).

To measure the drip loss, a piece of breast meat was wrapped in pure linen cloth, transferred to a plastic bag and stored at 4 °C for 24 hours after which, the meat was gently rubbed on a linen cloth and weighed again (Christensen, 2003). The drip loss percentage was calculated as:

Drip loss = [Initial weight (g) - Final weight (g)] / Initial weight (g) × 100

To measure the cooking loss, one cubic centimeter of the breast meat was cut and weighed, and placed in a warm bath at 85°C for 10 minutes. The sample was then gently wiped with a linen cloth and weighed (Bertram et al., 2003). The percentage of cook loss was calculated as:

Cook loss = [Initial weight (g) - Final weight (g)] / Initial weight (g) × 100

In order to measure the fat peroxide value (PV) of the meat (chest and thigh muscles), the oil in meat samples was extracted according to the method described by Zouari et al. (2010). At the first, 50 g of the ground

sample were homogenized in 150 mL of chloroform-methanol solution (2:1 V/V) using a blender (Arshia Company, BL 118-2290, Germany) for one minute and filtered through Whitman filter paper No. 1; then, 50 mL of 0.88% potassium chloride aqueous solution was added to the mixture. After dehydration by potassium chloride, the top phase of the mixture was separated, the aqueous phase (lower phase) was collected, and oil extraction was performed two more times with 100 mL of 0.88% methanol-potassium chloride solution (1:1 V/V). The oil obtained from this step was prepared for PV testing (Zouari et al., 2010).

The amount of malondialdehyde in the breast and thigh muscles was measured based on nanomoles per mg of protein according to the method described by Ahn et al. (1998). For this purpose, at first, 5 g of minced meat were homogenized in 45 mL of normal saline (0.9%) by vortex and centrifuged for 15 minutes at 2800×g (Hettich, EBA 200, Germany). Then, two mL of the supernatant (supernatant phase) were added in an experimental tube containing 4 mL of stock trichloroacetic acid/thiobarbituric acid solution (15% TCA (mass/v) and 0.375% TBA (mass/v/v) in 0.25 M hydrochloric acid) and 100 µL butylated hydroxyanisole (7.2% v/v) and homogenized using vortex. The resultant solution was incubated for 30 minutes in a bain-marie at 95 °C to induce a color reaction. Then, the samples were cooled at room temperature and centrifuged at 2800×g for 15 minutes. The adsorption against the blank (2 ml of 0.9% normal saline and 4 mL TCA-TBA solution) at 532 nm wavelength was recorded spectrophotometrically, and the outputs were obtained as nanomoles per mg protein using the standard curve prepared with reagent concentrations of 1,3,3,3 tetraethoxypropane (Ahn et al., 1998).

Statistical analysis

The data were analyzed using PROC GLM and the means were compared by the Tukey's test at 5% probability level (SAS statistical software, 9.4).

Results

The effect of treatments on the performance traits is shown in Table 2. The use of oxidized oil in finisher period and whole period led to a reduction in feed consumption ($P < 0.05$) and betaine supplementation increased feed intake during the grower, finisher, and whole period ($P < 0.05$). The interaction effect of treatments on feed intake was not significant. Replacement of oxidized oil in lieu of fresh soybean oil resulted in a reduction in broiler weight at all rearing periods ($P < 0.05$), but this effect was not significant during the grower period. Betaine supplementation increased the weight gain in all periods ($P < 0.05$). The interaction effect of the treatments on weight gain at starter, finisher and whole period was significant; chickens that were fed with fresh soybean oil and betaine had the greatest weight gain and chickens fed oxidized oil had the lowest weight gain ($P < 0.05$). Chickens fed

with oxidized oil had a higher feed conversion ratio in the grower period and the whole breeding period ($P<0.05$), but betaine supplementation led to a decrease in feed conversion ratio in grower, finisher, and whole periods ($P<0.05$). The interaction effect of treatments on feed

conversion ratio in finisher and whole periods was significant; chickens fed on fresh soybean oil and betaine had the lowest feed conversion ratio and chickens fed with oxidized oil had the highest feed conversion ratio ($P<0.05$).

Table 2. The effects oil type (fresh or oxidized) and betaine supplementation on growth performance of broilers

Treatments/traits ¹	1-10 d			11-25 d			26-42 d			1-42 d		
	FI (g/b)	WG (g/b)	FCR	FI (g/b)	WG (g/b)	FCR	FI (g/b)	WG (g/b)	FCR	FI (g/b)	WG (g/b)	FCR
Oxidized Oil (OX)												
0	167.71	134.44 ^a	1.25	935.27	542.84	1.73 ^b	2757.62 ^a	1445.12 ^a	1.91	3860.61 ^a	2122.41 ^a	1.82 ^b
1	160.88	126.36 ^b	1.27	958.05	522.38	1.84 ^a	2598.05 ^b	1358.94 ^b	1.92	3717.00 ^b	2007.69 ^b	1.86 ^a
SEM	3.32	2.19	0.01	15.81	9.56	0.018	12.41	10.56	0.010	19.73	14.16	0.008
P-Value	0.17	0.02	0.12	0.32	0.15	0.0008	0.0001	0.0001	0.57	0.0001	0.0001	0.0001
Betaine												
0	153.74 ^b	120.49 ^b	1.27	878.19 ^b	477.83 ^a	1.84 ^a	2628.04 ^b	1337.39 ^b	1.96 ^a	3659.97 ^b	1935.73 ^b	1.89 ^a
1	174.86 ^a	140.31 ^a	1.25	1015.14 ^a	587.40 ^a	1.73 ^b	2727.64 ^a	1466.66 ^a	1.86 ^b	3917.64 ^a	2194.38 ^a	1.78 ^b
SEM	3.32	2.19	0.01	15.81	9.56	0.018	12.41	10.56	0.010	19.73	14.16	0.008
P-Value	0.0007	0.0001	0.09	0.0001	0.0001	0.0009	0.0001	0.0001	0.0001	0.0002	0.0001	0.008
Interaction OX*Betaine												
0*0	152.64	121.02 ^c	1.26	882.77	492.86	1.79	2719.68	1405.79 ^b	1.93 ^b	3755.10	2019.69 ^b	1.85 ^b
0*1	182.77	147.86 ^a	1.23	987.78	592.83	1.66	2795.55	1484.44 ^a	1.88 ^{bc}	3966.11	2225.14 ^a	1.78 ^c
1*0	154.83	119.97 ^c	1.29	873.61	462.81	1.89	2536.39	1268.99 ^c	1.99 ^a	3564.83	1851.77 ^c	1.93 ^a
1*1	166.94	132.75 ^b	1.25	1042.50	581.97	1.79	2659.72	1448.88 ^{ab}	1.83 ^c	3869.17	2163.61 ^a	1.79 ^c
SEM	4.70	3.09	0.01	22.36	13.52	0.025	17.56	14.94	0.015	27.90	20.02	0.012
P-Value	0.07	0.04	0.82	0.17	0.49	0.53	0.20	0.005	0.003	0.12	0.02	0.02

a, b: Within column, means with common superscript (s) do not differ ($P>0.05$). ¹FI=Feed intake, WG=Weigh gain, FCR=Feed conversion ratio. Oxidized oil: 0: diet without OX and 1: diet with OX. Betaine: 0: diet without betaine and 1: diet with betaine.

The effect of oxidized oil on blood metabolites was not significant (Table 3). In chickens fed with betaine, blood cholesterol and triglyceride decreased ($P<0.05$).

The interaction effect of the treatments on blood triglycerides was significant; chickens fed oxidized oil and betaine had lower blood triglycerides.

Table 3. The effects oil type (fresh or oxidized) and betaine supplementation on blood metabolites of broilers at 42 days of age

Treatments/metabolites	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Oxidized Oil (OX)			
0	247.75	102.87	45.75
1	256.25	101.62	44.75
SEM	3.57	1.63	0.44
P-Value	0.11	0.59	0.13
Betaine			
0	252.62	104.87 ^a	46.37 ^a
1	251.37	99.62 ^b	44.12 ^b
SEM	3.57	1.63	0.44
P-Value	0.81	0.04	0.003
Interaction OX*betaine			
0*0	243.50	107.00	47.75 ^a
0*1	252.00	98.75	43.75 ^c
1*0	261.75	102.75	45.00 ^b
1*1	250.75	100.50	44.50 ^c
SEM	5.05	2.31	2.53
P-Value	0.07	0.22	0.01

a,b: Within column, means with common superscript (s) do not differ ($P>0.05$). Oxidized oil: 0: diet without OX and 1: diet with OX. Betaine: 0: diet without betaine and 1: diet with betaine.

The effect of treatments on meat quality characteristics of broiler chickens is shown in Table 4. The effect of oil type (fresh or oxidized) and betaine supplementation on meat pH was not significant. In chickens fed with oxidized oil, water holding capacity decreased and cooking and drip loss increased ($P<0.05$). Also, the use of oxidized oil led to an increase in breast and thigh peroxide value ($P<0.05$). In chickens fed betaine, water holding capacity increased and cooking and drip loss decreased. Also, betaine

supplementation led to a decrease in breast and thigh peroxide value ($P<0.05$). The interaction effect of the treatments on meat water holding capacity was significant; the highest water holding capacity was observed in chickens receiving fresh soybean oil and betaine supplementation and the lowest one was observed in chickens fed with oxidized oil ($P<0.05$). In chickens fed with oxidized oil, the amount of malondialdehyde in breast and thigh meat increased at 30 days after slaughter ($P<0.05$), while betaine

supplementation led to a decrease in malondialdehyde levels in meat ($P < 0.05$). The use of oxidized oil increased the peroxide value of breast and thigh meat

but betaine supplementation caused a decrease in this index ($P < 0.05$).

Table 4. The effects oil type (fresh or oxidized) and betaine supplementation on meat quality, peroxide value (mEq peroxide/kg of oil) and malondialdehyde content (nanomoles/mg protein) of broilers

Effects/traits*	pH	WHC (%)	Cook loss (%)	Drip loss (%)	PV Breast	PV Femur	MDA Breast	MDA Femur
Oxidized Oil (OX)								
0	5.81	63.69 ^a	28.45 ^b	11.24 ^b	123.28 ^b	140.51 ^b	10.12 ^b	10.78 ^b
1	5.82	61.00 ^b	30.98 ^a	12.79 ^a	135.93 ^a	160.80 ^a	10.92 ^a	11.31 ^a
SEM	0.01	0.32	0.42	0.24	1.69	1.99	0.23	0.17
P-Value	0.53	0.0001	0.001	0.0007	0.0002	0.0001	0.03	0.04
Betaine								
0	5.82	61.46 ^b	30.46 ^a	12.68 ^a	133.84 ^a	156.32 ^a	11.09 ^a	11.57 ^a
1	5.81	63.22 ^a	28.97 ^b	11.35 ^b	125.37 ^b	144.99 ^b	9.94 ^b	10.52 ^b
SEM	0.01	0.32	0.42	0.24	1.69	1.99	0.23	0.17
P-Value	0.44	0.002	0.03	0.002	0.004	0.002	0.004	0.001
Interaction OX*betaine								
0*0	5.81	62.31 ^b	29.70	12.13	127.35	149.48 ^b	10.77	10.53
0*1	5.83	65.06 ^a	27.20	10.35	119.21	131.55 ^c	10.41	10.16
1*0	5.81	60.62 ^c	31.22	13.22	140.33	163.17 ^a	11.49	11.82
1*1	5.82	61.37 ^b	30.75	12.35	131.53	158.44 ^{ab}	1.84	10.64
SEM	0.01	0.45	0.59	0.34	2.39	2.82	0.18	0.25
P-Value	0.87	0.04	0.11	0.20	0.89	0.03	0.78	0.35

a, b: Within column, means with common superscript (s) do not differ ($P > 0.05$). * WHC=Water holding capacity, PV=Peroxide value, MDA= malondialdehyde. Oxidized oil: 0: diet without OX and 1: diet with OX. Betaine: 0: diet without betaine and 1: diet with betaine.

Discussion

In this experiment, feeding of oxidized oil negatively impacted on broiler performance as already reported by several investigators (Tavarez et al., 2011; Lindblom et al., 2018; Tan et al., 2018; Yaseen et al., 2021). However; some researchers reported no effect of oxidized soybean oil with different peroxide numbers (3.69, 25.37, 56.83 and 73.21 mEq/kg) on growth rate of broiler chickens. This lack of effect may be due to the low degree of oxidation of these oils as well as the low level of oxidized oil in the diet (Tan et al., 2018). Vegetable oils contain polyunsaturated fatty acids with better digestibility and a greater tendency to oxidation. Overheating and frying vegetable oils at different temperatures cause thermal oxidation reactions leading to physical and chemical changes and the production of various lipid peroxidation products (Zhang et al., 2012). Lipid oxidation produces various oxidation products, including esters, ketones, polymerized oils, and aldehydes, which may produce sour odors and flavors, which are ultimately associated with reduced feed palatability (Qaisrani et al., 2021). Also, byproducts oil oxidation reacts with fat-soluble vitamins and proteins in the diet, thus reducing its nutrient content. Some of these oxidation products are toxic and have harmful effects on the intestinal absorptive cells, thereby slowing down the growth of broiler chickens (Liang et al., 2015). In addition, oxidized oil may cause oxidative stress and reduced growth performance in birds due to the production of reactive oxygen species (McGill et al., 2011).

Addition of betaine to the diet improvement the growth performance parameters in this experiment. In an experiment, addition of betaine supplementation to the diet of broiler chickens under heat stress conditions increased feed intake, increased weight gain and

decreased conversion ratio (Chand et al., 2017). Other researchers also reported improved growth performance of broiler chickens under stress conditions by adding betaine to feed (Liu et al., 2019; Sakomura et al., 2013; Singh et al., 2015; He et al., 2015). It has been reported that the addition of betaine to the diet, significantly increased the weight and decreased the conversion ratio during the entire breeding period (Shakeri et al., 2018; Chen et al., 2018). Betaine is an active plant substance that acts as a donor of methyl group and organic osmolyte. Betaine biosynthesis takes place *in vivo* through the oxidation of choline in the liver, but not in sufficient quantities (Sakomura et al., 2013). An important aspect of adding betaine is maintaining stores of choline and methionine, as these compounds play a role in supplying methyl groups, thereby improving growth performance (Rao et al., 2011; DiGiacomo et al., 2016). Dietary betaine also enhances the fat metabolism and provides more energy for growth (Ahmad et al., 2018) and can be used as an effective antioxidant in the nutrition of broiler chickens (Liu et al., 2019).

According to the results of this experiment, oxidized oil did not have a significant effect on blood parameters, but betaine supplementation reduced blood cholesterol and triglyceride levels. The lack of effect of oxidized oil on blood cholesterol and triglyceride concentrations in broiler chickens was reported by Jafari et al. (2021). In another study, serum triglyceride and cholesterol levels in broiler chickens fed with oxidized oil were significantly reduced compared to healthy oil (Moradi et al., 2021). However, the use of oxidized oil led to a decrease in blood cholesterol and triglycerides in broiler chickens, which was attributed to decreased synthesis of fatty acids (Bayraktar et al., 2011). These inconsistent finding can be due to different levels of application, the degree of oxidation of the oil, or different test and measurement

conditions. In one experiment, increasing betaine levels from 600 to 2400 mg/kg in the geese diet, significantly reduced serum triglycerides and cholesterol (Yang et al., 2021), which is consistent with the results of this study. In another study, it was reported that the use of betaine supplementation under normal temperature and heat stress conditions had no effect on blood glucose levels (Shakeri et al., 2019). In one study on broiler chickens, the addition of 0.1% betaine to the diet of broiler chickens under heat stress led to a decrease in triglycerides and blood cholesterol in broiler chickens (Shaojun et al., 2015). Also, the researchers reported a reduction in triglycerides and blood cholesterol by adding 0.15% betaine to the Japanese quail diet which they attributed to increased lipase activity and lipid degradation (El-Bahr et al., 2021).

In this experiment, in chickens fed with oxidized oil, water holding capacity decreased and cooking and drip loss, peroxide index and malondialdehyde content of meat increased. Zhang et al. (2011) investigated the effect of adding 5% oxidized oil to the diet of broiler chickens and reported that oxidized oil reduced water holding capacity and increased drip loss of breast meat. According to Bayraktar et al. (2011), addition of oxidized sunflower oil with peroxide number of 100 to the diet of broiler chickens under heat stress did not show a significant effect on pH and color characteristics of meat. Feeding oxidized oil to poultry, can have negative effects on the quality, taste and smell of meat by increasing the oxidation products. Increased glycogen oxidation in muscle leads to an increase in lactic acid production and a decrease in pH, which is followed by a decrease in the holding capacity of the meat and an increase in drip and cooking loss, and deteriorates the meat quality (Mir et al., 2017; Zhang et al., 2011).

In the present study, chickens fed oxidized oil, had greater peroxide number and malondialdehyde content in breast and thigh meat, indicating higher lipid oxidation in their meat. This is in line with other studies on the effect of feeding various oxidized oil to chickens (Bayraktar et al., 2011; Liang et al., 2015; Tan et al., 2018; Ghasemi-Sadabadi et al., 2021).

Betaine supplementation in this study increased the water holding capacity and decreased the cooking and drip loss, malondialdehyde and peroxide index of breast and thigh meat. These findings support the findings of Shakeri et al., 2020 and Rajaei et al., 2024. In another study, betaine supplementation had no significant effect on the cooking and pH of breast and thigh meat of broiler chickens under heat stress (Liu et al., 2019). The water retention property of cells by betaine can be the reason for increasing the holding capacity of meat and reducing cooking and drip loss by adding betaine supplementation in this experiment.

Conclusion

Oxidized oil had some negative effects on growth performance, meat quality traits, and oxidative stability, while betaine supplementation at the level of 0.1%

improved the growth performance, meat quality and oxidative stability in broilers. Betaine supplementation may be recommended, especially when oxidized oil is fed to chickens, to mitigate the reduction in growth performance and the progress of oxidation of meat.

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Conflict of interests

There is no conflict of interest.

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