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Impact of dietary supplementation of unsaturated and saturated fatty acids on bone strength, fatty acids profile of thigh muscle and immune responses in broiler chickens under heat stress

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Abstract

Background: There have been some reports that supplementation of fat could alleviate the negative effects of heat stress on performance in broilers. However, information regarding compensation for the adverse effects of heat stress with diets differing in fatty acids source on immune system, bone strength and carcass quality of heat-distressed broilers is limited.

Objectives: The objective of this study was to investigate the effects of diets differing in fat source on performance, immune system, bone strength, and carcass quality of heat-distressed broilers.

Methods: In a completely randomized design with 4 × 2 factorial arrangement of the treatments, 320 24-day-old Ross 308 chickens, with average initial weight of 1220 ± 10 g were divided into eight treatments included sesame oil, tallow, sunflower oil and palm oil in either 22 or 32 degree of centigrade temperature. The broiler performance of each fat source-treated group was not different in this experiment and decreased significantly in heat stress condition.

Results: Heat stress showed a significant increase on fat, energy and ash content of thigh muscle. Tibia absolute length, width, ash and bone breaking strength were affected by fat source and increased when sesame and sunflower oil were used. Data analysis revealed that hot temperature decreased tibia weight, length, width, ash and bone breaking strength. Heat stress led to decrease of immune system parameters.

Conclusion: Results suggest that there is no beneficial effect of broiler performance due to adding different sources of fat in broiler chicken diet under hot condition. Furthermore, the unsaturated fatty acids could improve the profile of fatty acids in thigh and enhance immune responses in broiler chickens.

KEYWORDS
bone status, broiler chickens, fat source, fatty acids profile, immunity, thigh composition

1 | INTRODUCTION

As environmental temperature increases above the thermo-neutral temperature, heat stress (HS) may occur and thus feed consumption, growth rate, feed efficiency, and survival ability all decline (Yu et al., 2008). High ambient temperature is one of the major environment stressors in broiler production in many regions of the world. Birds are sensitive to HS because they possess weak sweat glands, and the genetic breeding for meat production creates raised body heat generation (He et al., 2018). The researchers reported that HS adversely affected health status and performance of broilers, causing economic losses (St-Pierre et al., 2003). Broilers exposed to HS would induce multiple physiology disturbances, such as immune suppression (Wang et al., 2018) and oxidative stress (Ganesan et al., 2017; Zhang et al., 2018). In addition,, it had been mentioned that broilers exposed to HS have a low meat quality by increasing muscle glycolysis metabolism and oxidative status (Zaboli et al., 2019). On the other hand, it should be noted that HS decreases nutrients digestibility and enhanced mineral excretion (Belay & Teeter, 1996). This could lead to decreased bone strength and bone weight and consequently have an impact on broiler production. Also, at high ambient temperature, respiratory alkalosis is a state due to excess loss of CO_2 from the body (Hillerman et al., 1985). Increasing respiratory rate requires increased muscle activity and this resulted in an increased energy requirement which is associated with HS.

In recent years, numerous nutritional strategies have been used to alleviate the detrimental effects of HS on animals, and dietary supplementation with fat/oil extracted increasing attention. Fat supplementation increases the energy intake and reduces the specific dynamic effect of the diet, which helps birds to cope better with HS. HS decreases the consumption of feed, nutrients and metabolisable energy (ME) (N. Daghir, 2008; NRC, 1994; Y. A. Attia et al., 2011). In a similar case, the researcher reported that higher fat supplementation in diets improved heat tolerance in broiler chickens (N. J. Daghir, 2008) and growth performance of broilers exposed to HS (Al-Harthi et al., 2002; Ghazalah et al., 2008; Lou et al., 2003; Raju et al., 2004; Y. A. Attia et al., 2011). Increasing dietary polyunsaturated/saturated fatty acid ratio raised heat production of birds (Sanz et al., 2000) which could exaggerate the HS consequences. Accordingly, it appears that the various sources of fat supplemented in the diet could influence the growth performance traits of broilers under high ambient temperatures differently. In birds, lipogenesis takes place primarily in the liver, whereas adipose tissue serves as the storage site for triacylglycerols (TG, composed of 80%–85% esterified fatty acids). However, both the nature (unsaturation level, ω3 or ω6 series) and the allocation (such as constituents of complexed lipids) of polyunsaturated fatty acid (PUFA) are very important to evaluate their function in lipid metabolism (Cartoni Mancinelli et al., 2022).

Based on the biological function of fat/oil, this study hypothesized that dietary fat/oil supplementation could alleviate the disadvantageous effects of HS. Therefore, the aim of this study was to evaluate the effects of different dietary fat/oil supplementation on immune system, bone strength, carcass quality and performance of broilers under HS.

2 | MATERIALS AND METHOD

2.1 | Animals, diets and housing

All animal care and procedures were approved by the Institutional Animal Care and Use Committee in Iran. Each pen was covered with fresh shaving sand equipped with separate feeders and drinkers. Feed and water were given ad libitum. The experiment was completely randomized design with 4 × 2 factorial arrangement of the treatments. The broilers were allocated to 32-floor pens with eight treatments, during the experimental period. A total of 320 24-day-old Ross 308 male broiler chicks with an average initial BW of 1220 ± 10 g were weighed and placed in two controlled environment rooms for 35 days. The rooms were identical in terms of size, constructing materials, climatization equipment, feeders and nipple-type water device. The chicks in one of the two rooms were exposed to heat exposure, whereas the broilers in another room were kept in a normal thermal environment, which served as the control treatment. The fat sources were each sesame oil, tallow, sunflower oil and palm oil as a source of fat with of basal diet. The ingredient and chemical composition of basal diet with different fat sources is shown in Table 1. The fatty acid compositions of fat sources are also shown in Table 2. Each treatment involved five replicates with eight birds. To induce the HS, the room temperature was set at 32°C throughout the whole experimental period. It has been noted that broiler exposed to continuously high temperatures (32°C) resulted in chronic HS (Sahin et al., 2002). The ambient temperature and humidity values were recorded using an electronic data recorder.

2.2 | Growth performance

Live body weight and feed intake per pen were recorded on 55 days of age to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), and these parameters were corrected for mortality. The chickens were inspected daily, and dead birds were removed following registration of date and body weight (BW). When calculating FCR, the BW of dead birds was also taken into account.

2.3 | Slaughter and sampling procedure

At 35 days of age, two birds per replicate (10 birds per treatment) close to the mean body weight were selected and after bleeding were killed by cervical dislocation, bled, eviscerated, and immediately sampled prior chilling. Carcasses from all broilers were dissected and measured for thigh, spleen and bursa weight.

After carcass measurements, approximately 10 g of skinless thigh meat were collected for determining total lipids, fatty acid composition, total cholesterol, and energy and protein contents. The samples were ground using a food processor (3 × 5 s), vacuum packed, and stored at −80°C until required. Right side of the thigh was used for bone quality determination.

TABLE 1 Ingredients and chemical analyses in basal diet with different fat sources in the grower periods

	Fat source			
Ingredients (%)	Sesame oil	Sunflower oil	Palm oil	Tallow
Ground corn	61.82	61.82	61.82	61.82
Soybean meal	30.24	30.24	30.24	30.24
Sesame oil	4.65	-	1.33	1.33
Sunflower oil	-	4.65	-	-
Palm oil	1.33	1.33	4.65	0.2
Tallow	0.2	0.2	0.2	4.65
Dicalcium phosphate	0.53	0.53	0.53	0.53
Sodium chloride	0.16	0.16	0.16	0.16
Limestone ground	0.25	0.25	0.25	0.25
Calcium carbonate	0.12	0.12	0.12	0.12
DL-methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Vitamin premix*	0.25	0.25	0.25	0.25
Trace mineral premix**	0.25	0.25	0.25	0.25
ME, kcal/kg	3200	3200	3200	3200
Crude protein	20.03	20.03	20.03	20.03
Crude fat	6.35	6.35	6.35	6.35

*Premix vitamin supplement is provided per kilogram of diet: vitamin A (retinylacetate), 9500 U; vitamin E (DL-α-tocopherol acetate), 30 U; vitamin K_3 (menadione sodium bisulphate), 2.65 mg; vitamin D_3 (cholecalciferol), 2500 U; vitamin B_{12} (cyanocobalamin), 0.025 mg; biotin, 0.30 mg; nicotinic acid, 50 mg; folic acid, 1.25 mg; D-pantothenic acid, 12 mg; riboflavin, 6.5 mg; pyridoxine hydrochloride, 6.0 mg; thiamine mononitrate, 3 mg.
**Premix mineral supplement is provided per kilogram of diet: zinc, 80 mg; manganese, 100 mg; copper 8 mg; iron, 80 mg; iodine, 0.35 mg; selenium, 0.15 mg.

TABLE 2 Fatty acid compositions of fat sources (g/100 g fat)

Fatty acid	Sesame oil	Sunflower oil	Palm oil	Tallow
Myristic	-	4.8	3.0	6.0
Palmitic	7.0	5.4	44.0	31.0
Palmitoleic	5.0	4.0	0.1	0.1
Stearic	6.0	5.0	5.0	25.0
Oleic	35.0	30.0	36.0	34.0
Linoleic	35.0	42.1	8.0	2.2
α-Linolenic	1.0	3.0	3.0	0.3
Icozopantanoic	5.1	3.4	-	-
Decozopantanoic	5.0	3.0	-	-
Others	0.1	2.4	0.9	1.5

2.4 | Proximate composition analysis

Thigh fat was extracted by the method of Folch et al. (1957) and determined gravimetrically. Cholesterol content of extracted lipids was determined by high-performance liquid chromatography, and fatty acid composition was measured by gas chromatography (Crespo & Esteve-Garcia, 2001). Gross energy of thigh muscles was measured using an adiabatic bomb calorimeter and total ash (942.05) and CP (990.03) contents of the thigh meat samples were analyzed according to the method described by the AOAC (2000).

2.5 | Bone quality

Tibia was thawed at room temperature in a plastic bag (to prevent dehydration). Fully thawed bones were defleshed manually, and weighed. Length and width (in the medial portion of the bones) were measured using digital calipers (Mitutoyo Absolute Digimatic, Mitotoyo, Kruibeke, Belgium). Subsequently, tibia breaking strength was determined using a shear test (ASAE, 2000). Sheared tibiae pieces were collected, defatted, and ashed at 600°C for 16 h to determine ash percentage.

2.6 | Humoral immune response to sheep red blood cell

For experimental immunization, four birds of 34 days old from each group were injected intravenously (brachial vein) with 0.1 ml of 0.5% sheep red blood cells (SRBC). Blood samples were collected after 7 and 14 days of inoculation to measure the antibody production using a microtiter hemagglutination assay (Wegmann & Smithies, 1966).

2.7 | Cellular immune response

To determine Dinitrochlorbenzene (DNCB) challenge on day 32, 12 birds per treatment were sensitized (Verma et al., 2004) by a single percutaneous application of 1-chloro-2,4-dinitrobenzene (DNCB-Merck). Note that 0.25 ml of DNCB (10 mg/ml of acetone and olive oil 4:1) was applied on a featherless area of the right side, while a similar area on the left side received the solvent without DNCB as a control. Changes in mean skin thickness before and 24 h after challenge were assessed using digital calipers (Mitutoyo, Japan).

PHA-M induced lymphoproliferation Phytohemagglutinin-M (Gibco, USA), and T-cell mitogen was injected (100 mg dissolved in 100 ml of sterile PBS) to the right toe web of nine birds per experimental group at 40 days. The increase in toe web thickness was measured 12 and 24 h after injection (Corrier, 1990).

2.8 | Statistical analysis

Data were analyzed using General Linear Model procedures of SAS software (SAS, 2002). The level of statistical significance was set at $p < 0.05$. When a significant *F*-value for treatment was observed in the analysis of variance, treatment means were compared using Duncan multiple range test.

3 | RESULT AND DISCUSSION

3.1 | Performance parameters

The results of performance parameters are shown in Table 3. The mean BW of broilers at the beginning of the trial (21 days of age) was similar for all treatment groups and increased at different rates during the study ($p < 0.05$). The performance (BW, feed intake and FCR) of each fat source-treated group was not different in this experiment. Main effects of the HS, as well as the fat source × heat stress interaction were significant for performance indices ($p < 0.05$). Data showed that HS resulted in a significant decrease in feed intake and BW and increase in FCR throughout the experimental period ($p < 0.05$) which is similar to results of other studies (Cooper & Washburn, 1998; Smith et al., 2003; N. J. Daghir, 2009; Hasio et al. 2005). Also, performance was reduced when broiler chickens were fed diet containing fat sources in HS compared to normal temperature ($p < 0.05$).

Environmental temperature is the most important variable affecting feed intake and consequently weight gain of broilers. Several authors have shown that use of fat in the diet can coordinate this growth depression (Ghazalah et al., 2008; Mateos & Sell, 1981). This practice reduces the specific dynamic effect of the diet, which helps birds to cope better with HS. However, in this study dietary different sources of fat did not affect the weight gain, feed intake and FCR of chickens in HS. The result is in accordance with the findings of other researchers who suggested that dietary PUFA did not affect heat production and growth performance of chickens under high or low ambient temperature (Sanz et al., 2000; Skylan & Ayal, 1989). Although, it showed that 5% added fat helped in reducing the detrimental effect of HS in broilers raised at 29–36°C (Ghazalah et al., 2008).

3.2 | Thigh muscle

3.2.1 | Muscle composition

The results of muscle composition are shown in Table 4. Source of fat had no effect on fat, protein and ash contents of thighs. However, thighs from birds fed a tallow or palm oil diet showed a slightly higher lipid content ($p < 0.05$) and, accordingly, significant higher energy content ($p < 0.05$) than thighs from birds fed sesame and sunflower oil. This result is in agreement with Crespo and Esteve-Garcia (2001) who suggested type of fat had no effect on weights and percentages of thighs and did not significantly influence fat and protein contents of thighs. They found that thighs from birds fed a sunflower oil diet showed a slightly higher lipid content compared to thighs from birds fed tallow. However, in the present study, we found slightly higher lipid content in thighs of birds fed tallow compared to those fed sunflower diet ($p < 0.05$).

HS showed a significant increase on fat, energy and ash content of thigh muscle. It demonstrated that fat contents of meat were higher in chickens reared under HS and this subject led to the increased gross energy of meat (Husseiny & Cregur, 1980; Vakili & Rashidi, 2011). Furthermore, environmental temperature had no effects on dry matter, crud protein, ether extract and ash of breast and thighs + drumsticks of broiler chickens (Faria Filho et al., 2005).

A fat source × heat stress interaction was detected in that fat content increased in thigh muscle when palm oil and tallow were used in HS. In all diets, thigh energy showed an increase when birds were raised under HS conditions. However, in normal temperature, the birds fed with tallow showed higher energy content in their thigh muscles when compared to other groups. Also, ash content of thigh was decreased when fat sources except tallow were used in normal temperature.

3.3 | Fatty acid composition of thigh muscles

Fatty acid composition of thigh muscles is shown in Table 5.

Birds fed tallow and palm oil presented higher values of saturated fatty acids (SFA), mainly palmitoleic and palmitic acids, and monounsaturated fatty acid (MUFA) than those fed sunflower or sesame oil ($p < 0.05$). Birds fed sunflower and sesame oil presented the higher values of PUFA, Decosapantanoic acid (DHA), Eicosapantanoic acid (EPA), linoleic, α-linolenic, $\omega 3$ and $\omega 6$ fatty acids (FA) in thigh muscles ($p < 0.05$). Holman (1960) suggested that the retention of FA in poultry muscles is not the absolute intake of essential ($\omega 6 + \omega 3$) fatty acids, but the ratio of $\omega 6/\omega 3$ fatty acid is responsible for the quality of their biological response. A consequence of these theories (Simopoulos, 2000) is that an optimized diet should provide small but balanced ratios of $\omega 6$ and $\omega 3$ FA. The recommended ratio of $\omega 6/\omega 3$ fatty acids in the human

TABLE 3 Effects of fat source, temperature and interaction on performance growth

Interaction effect		Feed intake (g)	Weight gain (g)	FCR (g/g)
Fat source	**Temperature (°C)**			
Sesame oil	22	4550[a]	2248[a]	2.00[b]
Sunflower oil	22	4514[a]	2269[a]	1.99[b]
Palm oil	22	4574[a]	2231[a]	2.05[b]
Tallow	22	4584[a]	2245[a]	2.04[b]
Sesame oil	32	3900[b]	1578[b]	2.47[a]
Sunflower oil	32	3982[b]	1586[b]	2.51[a]
Palm oil	32	4051[b]	1654[b]	2.45[a]
Tallow	32	3953[b]	1600[b]	2.47[a]
SEM		54.2	28.4	0.023
Main effect				
Fat source				
Sesame oil		4225	1913	2.20
Sunflower oil		4228	1923	2.21
Palm oil		4312	1942	2.22
Tallow		4268	1922	2.22
SEM		52.1	29	0.02
Temperature (°C)				
22		4555[a]	2248[a]	2.02[b]
32		3971[b]	1604[b]	2.47[a]
SEM		50.1	27.6	0.021
Probability				
Fat source × temperature		0.030	0.120	0.021
Fat source		0.200	0.090	0.060
Temperature		0.012	0.020	0.024

Abbreviation: FCR, feed conversion ratio.

diet ranges between 3:1 and 6:1 (Holman, 1960). Interestingly, this is comparable to the ratios obtained in the thigh (3.4:1) of chickens fed the diet containing sunflower oil. Broilers fed the diets in HS condition presented higher percentage of SFA mainly palmitoleic, stearic and oleic and MUFA and lower percentage of PUFA than that observed in the other diets ($p < 0.05$).

The maximum SFA and MUFA were observed when diets containing tallow and palm oil were used. Also, the highest values of SFA and MUFA in thigh muscles were observed in birds fed with palm oil and tallow in HS condition, respectively. The maximum PUFA in thigh muscle was observed in birds fed diets containing sunflowers oil while reared in normal temperature. The DHA, EPA, linolenic, PUFA and ω3 fatty acids were increased when sesame oil was used. The linoleic acid, PUFA and n-6 FAs were increased with use of sunflower oil as a fat source.

There are >600 published papers on Conjugated Linoleic Acid's (CLA) diverse biological applications, and reported effects on body composition are overwhelmingly strong. However, the vast majority of these reports have come from rodent or porcine models, and CLA's potential as an antiobesity nutrient for humans is still a matter of debate. It is very likely that this conflict persists due to the fact that CLA's mechanism of action is isomer specific and that the level of intake is critical, as is the metabolic status of the participants (Brown & McIntosh, 2003).

3.4 | Bone parameters

The results of tibia growth and bone breaking strength are shown in Table 6.

Tibia absolute length, width, ash and bone breaking strength were affected by fat source and increased when sesame and sunflower oil were used; however, no significant differences in bone weight were found between treatments. Researchers showed that when soybean oil, hydrogenated soybean oil, chicken fat, and menhaden oil were used as fat source in the diet, tibia length, weight, and diameter were similar in all experimental groups, and suggested that dietary lipids had no significant effect on these parameters (Liu et al., 2003).

TABLE 4 Effects of fat source, temperature and interaction on muscle composition

Interaction effect		Fat content (%)	Energy (kcal/kg DM)	Ash content (%)	Protein content (%)	Cholesterol (mg/g)
Fat source	**Temperature (°C)**					
Sesame oil	22	5.1^{b}	5029^{c}	23.3^{b}	50.5	0.168
Sunflower oil	22	7.2^{b}	5086^{c}	23.8^{b}	50.8	0.162
Palm oil	22	7.3^{b}	5260^{b}	25.5^{a}	52.4	0.171
Tallow	22	7.4^{b}	5198^{b}	25.1^{a}	51.6	0.171
Sesame oil	32	9.1^{a}	5222^{b}	26.0^{a}	51.6	0.170
Sunflower oil	32	8.8^{a}	5292^{b}	26.8^{a}	51.8	0.165
Palm oil	32	8.7^{a}	5502^{a}	24.0^{b}	50.4	0.176
Tallow	32	9.1^{a}	5489^{a}	27.2^{a}	51.2	0.172
SEM		0.16	30.1	0.55	0.38	0.007
Main effect						
Fat source						
Sesame oil		8.0	5126^{b}	23.1	51.1	0.170
Sunflower oil		8.1	5189^{b}	23.8	51.3	0.160
Palm oil		8.1	5381^{a}	23.1	51.4	0.180
Tallow		8.2	5344^{a}	26.2	51.4	0.170
SEM		0.09	30.3	0.06	0.38	0.007
Temperature(°C)						
22		7.2^{b}	5143^{b}	22.9^{b}	51.3	0.180
32		8.9^{a}	5376^{a}	25.2^{a}	51.2	0.160
SEM		0.6	30.2	0.65	0.18	0.01
Probability						
Fat source × temperature		0.01	0.02	0.03	0.2	0.2
Fat source		0.05	0.04	0.20	0.07	0.3
Temperature		0.02	0.01	0.04	0.07	0.3

Abbreviation: DM, dry matter.

Several studies have shown an increase in osteoblastic bone formation markers when PGE2 production was decreased or inhibited (Mirsaidi et al., 2017). The actions of ω3 fatty acids on bone formation appear to be linked to altering osteoblast functions. Sesame and sunflower oil contained high amount of PUFA and n-6 fatty acids; therefore, they improved tibia growth and bone breaking strength.

Data analysis revealed that hot temperature decreased tibia weight, length, width, ash and bone breaking strength. High ambient temperature stress causes physiological and metabolic alterations in poultry, including the reduction of bone weight and strength and an increase in the incidence of leg problems in broilers (Ernst et al., 1984; Siegel et al., 1973; Smith & Teeter, 1987). Bruno et al. (2007) observed that high environmental rearing temperature reduced bone length at 42 days of age in broilers. Bruno et al. (2007) found that tibia length was smaller in birds reared at cold temperature, suggesting that the linear growth of long bones seems to be differently affected by rearing temperature (Bruno et al., 2007).

On the other hand, Yalcin et al. (1996) demonstrated that high ambient temperature reduced tibia weight, but not bone length in broiler chickens. Bruno et al. (2007) suggested that no statistical differences on tibia weight were found between birds kept at high or lower temperature.

Significant fat source × temperature interaction was observed in tibia growth and bone breaking strength. The tibia length and bone breaking strength were higher when were used as fat source at normal temperature. Furthermore, use of sesame and sunflower oil can improve growth and bone breaking strength of tibia compared to tallow and palm oil in HS condition.

TABLE 5 Effect of fat source, heat stress and interaction on the fatty acid content of thigh muscles (expressed as g/kg fat or dry matter [DM])

Interaction effect		Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	α-Linolenic	EPA	DHA	SFA	MUFA	PUFA	ω-3	ω-6	ω-6/3
Fat source	**Temperature (°C)**														
Sesame oil	22	25.5^{de}	4.6^{c}	5.3^{d}	37.2^{cd}	15.1^{c}	1.40^{b}	1.40^{b}	1.12^{a}	30.9^{d}	42.1^{d}	19.4^{c}	4.20^{b}	15.3^{c}	3.5^{b}
Sunflower oil	22	19.5^{f}	4.0^{d}	7.7^{ab}	31.0^{f}	32.2^{a}	0.18^{c}	0.26^{c}	0.38^{b}	27.3^{e}	35.0^{f}	34.3^{a}	2.10^{c}	32.3^{a}	15.8^{b}
Palm oil	22	30.7^{b}	7.4^{a}	7.7^{ab}	35.6^{de}	13.7^{cd}	0.52^{f}	0.00^{d}	0.01^{c}	38.4^{b}	43.0^{cd}	13.9^{d}	0.26^{e}	13.7^{cd}	57.3^{a}
Tallow	22	28.8^{c}	6.1^{b}	5.7^{d}	40.1^{b}	10.9^{e}	0.05^{e}	0.80^{d}	0.25^{c}	34.5^{c}	46.3^{b}	12.2^{e}	1.20^{d}	11.1^{e}	9.2^{b}
Sesame oil	32	25.0^{e}	6.0^{a}	5.6^{d}	38.8^{bc}	15.1^{c}	1.50^{a}	1.50^{a}	1.14^{a}	30.6^{d}	45.0^{bc}	19.9^{c}	4.70^{a}	15.3^{c}	3.2^{b}
Sunflower oil	32	20.8^{f}	3.9^{d}	7.2^{bc}	34.5^{e}	28.6^{b}	0.70^{cd}	0.37^{c}	0.50^{b}	28.0^{e}	38.5^{e}	30.8^{b}	2.20^{c}	28.7^{b}	13.0^{b}
Palm oil	32	33.0^{a}	7.5^{a}	8.0^{a}	36.3^{de}	12.6^{d}	0.62^{cf}	0.01^{d}	0.70^{c}	41.0^{a}	43.9^{cd}	12.9^{de}	0.28^{e}	12.6^{d}	48.4^{a}
Tallow	32	26.7^{d}	6.1^{b}	8.6^{c}	43.2^{a}	12.7^{d}	0.66^{d}	0.10^{d}	0.03^{c}	33.6^{c}	49.5^{a}	14.2^{e}	1.40^{d}	12.9^{d}	9.1^{b}
SEM		0.21	0.032	0.02	0.011	0.01	0.028	0.037	0.021	0.015	0.031	0.036	0.025	0.015	0.012
Main effect															
Fat source															
Sesame oil		22.8^{d}	5.3^{d}	5.45a	38^{d}	15.1^{a}	1.45^{a}	1.45^{b}	1.13^{b}	30.75^{d}	43.55^{c}	19.65^{a}	4.45^{b}	15.3^{a}	3.35^{b}
Sunflower oil		20.1^{c}	3.95^{c}	7.45c	32.75^{b}	30.4^{b}	1.25^{a}	·0.25a	0.44^{a}	29.05^{c}	36.75^{b}	33.4^{b}	2.150^{a}	23.65^{b}	14.4^{c}
Palm oil		31.2^{a}	7.85^{a}	7.85a	35.8^{c}	13.15c	0.57^{b}	0.57^{c}	0.23^{c}	39.05^{a}	43.45^{b}	13.4^{c}	0.27^{d}	13.3^{c}	55^{a}
Tallow		27.7^{b}	6.1^{b}	7.45^{b}	42.15^{a}	11.8^{c}	0.35^{c}	0.73^{c}	0.14^{c}	34.05^{b}	47.25^{a}	13.2^{c}	1.30^{c}	12.0^{c}	9.15^{bc}
SEM		4.21	0. 32	2.12	0.011	0.01	0.028	0.037	0.021	0.015	0.031	0.036	0.025	0.015	0.012
Temperature (°C)															
22		26.1	5.5^{b}	6.6	36.0^{b}	17.98	0.53	0.85	0.44	32.77^{b}	43.55^{b}	19.95^{a}	1.92	18.1	21.45
32		26.3	5.9^{a}	6.9	38.2^{a}	17.25	0.87	0.49	0.59	33.3^{a}	44.2^{a}	19.45^{b}	2.150	17.37	18.42
SEM		0.021	0.032	0.02	0.011	0.01	0.028	0.037	0.021	0.015	0.031	0.036	0.025	0.015	0.012
Probability															
Fat source × temperature		0.021	0.032	0.020	0.011	0.010	0.028	0.037	0.021	0.015	0.031	0.036	0.025	0.015	0.012
Fat source		0.045	0.030	0.011	0.018	0.031	0.028	0.026	0.022	16.000	0.014	0.080	0.019	0.015	0.013
Temperature		21.000	0.028	0.078	0.011	0.067	0.120	0.092	0.063	0.022	0.045	0.034	0.21	0.110	0.082

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids.

TABLE 6 Effects of fat source, temperature and interaction on tibia growth and bone breaking strength

Interaction effect		Dry weight (g)	Bone width (mm)	Bone length (mm)	bone breaking strength (N/m^2)	Ash (%)
Fat source	**Temperature (°C)**					
Sesame oil	22	12.1[a]	10.0[a]	105[a]	122[a]	48[a]
Sunflower oil	22	11.1[a]	9.0[a]	103[a]	117[ab]	47[a]
Palm oil	22	11.0[a]	7.2[b]	99[b]	93[c]	43[b]
Tallow	22	11.2[a]	7.8[b]	100[b]	94[c]	44[b]
Sesame oil	32	8.9[b]	7.6[b]	98[b]	115[b]	43[b]
Sunflower oil	32	8.0[b]	7.3[b]	95[c]	81[d]	43[b]
Palm oil	32	8.0[b]	6.4[c]	95[c]	80[d]	40[c]
Tallow	32	8.4[b]	6.9[c]	94[c]	78[d]	43[b]
SEM		0.33	0.16	2.6	3.48	1.66
Main effect						
Fat source						
Sesame oil		10.5	8.8[a]	106[a]	110[a]	47[a]
Sunflower oil		9.5	8.2[a]	102[a]	93[a]	44[a]
Palm oil		9.5	6.8[b]	97[b]	80[b]	40[b]
Tallow		9.8	7.4[b]	98[b]	80[b]	42[b]
SEM		0.33	0.16	1.6	1.48	1.56
Temperature(°C)						
22		11.4[a]	8.5[a]	106[a]	122[a]	45[a]
32		8.31[b]	7.0[b]	95[b]	78[b]	42[b]
SEM		0.33	0.16	1.6	1.48	1.54
Probability						
Fat source × temperature		0.02	0.02	0.01	0.03	0.02
Fat source		0.07	0.02	0.01	0.00002	0.0001
Temperature		0.01	0.04	0.03	0.02	0.02

3.5 | Immune system competence

3.5.1 | Lymphoid organ weights

There was HS, but no fat source differences in the relative weight of spleen and bursa of broilers (Table 7). Furthermore, use of fat source in normal temperature led to higher weight of spleen and bursa. The differences in the weight of lymphoid organs may be due to reduction in feed intake, thereby providing fewer nutrients for the proper development of those organs (Bartlett & Smith, 2003).

3.6 | Blood parameters

Increases heterophil to lymphocyte ratios have been evaluated as indicators of physiological stress in birds. HS altered the proportion of heterophils: lymphocytes in blood (Table 7). This result is in agreement with other studies (Altan et al., 2000, 2003; Borges et al., 2004). Also, the main effect of fat source, as well as the fat source × heat stress interaction significantly changed heterophils: lymphocytes ratio. Heterophil to lymphocyte ratio was increased in birds fed diets with sesame and sunflower oil in normal and HS term.

3.7 | Humoral and cellular immune response

Phytohemmagglutinine (PHA) injection was not affected by fat source, whereas skin response to Dinitrochlorbenzene (DNCB) challenge or antibody titre against SRBC was influenced by different fat sources (Table 7). The greatest skin response to DNCB was in tallow and palm oil group, while maximum SRBC titres were observed in the sesame and sunflower oil group. Enhancement of antibody production by ω3 PUFA had been observed previously in mice and broiler chicks (Parmentier et al., 1997; Sijben et al., 2001).

HS leads to decrease of SRBC, PHA and DNCB. Similarly, it demonstrated that HS reduced antibody production in young chickens (Mashaly et al., 2004; Zulkifi et al., 2000). However, Donker et al. (1990) found that heat exposure did not reduce antibody

TABLE 7 Effects of fat source, temperature and interaction on humoral and cellular immune response

Interaction effect		Spleen %	Bursa %	H/L	DNCB 12 h	PHA-M 12 h	PHA-M 24 h	SRBC titres 12 h	SRBC titres 24 h
Fat source	Temperature (°C)								
Sesame oil	22	0.067^{a}	0.050^{a}	0.25^{c}	0.44^{c}	0.45^{a}	0.81^{a}	2.3^{a}	2.8^{a}
Sunflower oil	22	0.066^{a}	0.047^{a}	0.22^{c}	0.60^{b}	0.40^{a}	0.73^{a}	2.2^{a}	2.8^{a}
Palm oil	22	0.082^{a}	0.051^{a}	0.26^{c}	0.65^{b}	0.41^{a}	0.58^{b}	1.6^{c}	2.4^{b}
Tallow	22	0.086^{a}	0.053^{a}	0.28^{c}	0.90^{a}	0.39^{ab}	0.64^{ab}	1.7^{ab}	2.6^{b}
Sesame oil	32	0.048^{b}	0.042^{b}	0.52^{a}	0.42^{c}	0.24^{b}	0.49^{c}	1.9^{b}	2.1^{c}
Sunflower oil	32	0.055^{b}	0.041^{b}	0.55^{a}	0.59^{b}	0.22^{b}	0.48^{c}	1.8^{b}	1.8^{c}
Palm oil	32	0.064^{b}	0.045^{b}	0.41^{b}	0.63^{b}	0.43^{a}	0.61^{b}	1.2^{c}	1.5^{c}
Tallow	32	0.065^{b}	0.047^{b}	0.44^{b}	0.89^{a}	0.28^{b}	0.42^{c}	1.1^{d}	1.2^{c}
SEM		0.033	0.0013	0.0124	0.043	0.011	0.022	0.23	0.32
Main effect									
Fat source									
Sesame oil		0.057	0.046	0.38^{a}	0.51^{b}	0.34	0.71	2.1^{a}	4.9^{a}
Sunflower oil		0.060	0.044	0.38^{a}	0.54^{b}	0.31	0.60	2.0^{a}	4.6^{a}
Palm oil		0.073	0.048	0.33^{b}	0.76^{a}	0.42	0.60	1.4^{b}	3.2^{b}
Tallow		0.075	0.050	0.36^{b}	0.75^{a}	0.33	0.53	1.4^{b}	3.4^{b}
SEM		0.033	0.0013	0.0124	0.043	0.011	0.022	0.23	0.52
Temperature (°C)									
22		0.075^{a}	0.050^{a}	0.25^{b}	0.76^{a}	0.41^{a}	0.69^{a}	1.9^{a}	5.4^{a}
32		0.058^{b}	0.043^{b}	0.48^{a}	0.52^{b}	0.29^{b}	0.50^{b}	1.5^{b}	2.6^{b}
SEM		0.033	0.0013	0.0124	0.043	0.011	0.022	0.23	0.52
Probability									
Fat source × temperature		0.027	0.016	0.020	0.034	0.041	0.037	0.031	0.023
Fat source		0.051	0.080	0.030	0.020	0.220	0.100	0.030	0.011
Temperature		0.040	0.032	0.030	0.042	0.010	0.012	0.048	0.036

Abbreviation: SRBC, sheep red blood cells.

production to SRBC. In a study, Heller et al. (1979) also found significantly increased antibody titres to SRBC following heat exposure (Heller et al., 1979). Furthermore, Borges et al. (2004) suggested that HS caused the immune response to be depressed, thus increasing the susceptibility of flocks to disease challenges.

It was hypothesized that dietary ω3 PUFA could enhance immune responses and diseases resistance in poultry by reducing eicosanoid production, particularly prostaglandin E2. Diets-induced alterations in immune responses might be determined by assessing changes in the proportions of different lymphocyte subsets (Xia et al., 2003).

The recent investigation to study the liver lipid metabolism, with particular attention to non-esterified fatty acids (NEFA), TG, phospholipids (PL), FADS2 gene expression and Δ6-desaturase activity of three chicken genotypes, Leghorn (Leg), Ross 308 (Ross), and their crossbreed (LxR), by liquid chromatography–mass spectrometry analysis. The concentration of single fatty acids in muscle was quantified by Gas Chromatography with flame ionization detection. The results showed that the Ross has a lipid metabolism related mainly to storage and structural roles, exhibiting higher levels of TG, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) that are largely unsaturated. Meanwhile, Leg showed a relevant amount of ω3 NEFA characterized by a higher phosphatidylserine (PS) unsaturation level, FADS2 gene expression and enzyme activity. The LxR seems to have a moderate trend: ω6 and ω3 NEFA showed intermediate values compared with that of the Ross and Leg, and the TG trend was similar to that of the Ross, while PE and PC were largely unsaturated (mainly six and seven) most of the metabolic energy for storage fatty acids in their tissues (TG), whereas the Leg birds were characterized by different lipid metabolism showing in their liver a higher content of ω3NEFA and higher unsaturation level in PS. More details are needed to better attribute the lipid energy to the different metabolic portion (Cartoni Mancinelli et al., 2022).

4 | CONCLUSION

In the conclusion, unsaturated fat sources could improve profile of fatty acids in thigh and enhance immune responses in broiler chickens. However, we did not observe the beneficial effect of broiler performance due to adding different sources of fat in broiler chicken diet under HS conditions. generally, as the composition of different types of oils is variable, the effects on the production performance, immune responses, meat quality and profile of fatty acids in heat-distressed broilers are diverse and there was also a significant synergistic effect of the concentration and type of oil. Therefore, the choice of oils in the diet feed should be based on studies ascribing the effects and needs of oils in meat production in poultry. Therefore, we recommend adding unsaturated fat sources to broiler feed diets in HS.

AUTHOR CONTRIBUTIONS

Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft, and writing—review and editing: Reza Vakili. Formal analysis and validation: Yahya Ebrahimnezhad.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ETHICS STATEMENT

The authors hereby declare all ethical standards have been respected in preparation of the submitted article.

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