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Original Research

Effect of Linseed Oil and Fermented Pomegranate Peel Supplementation on the Growth Performance, Digestive Function, and Muscle Fatty Acid Deposition of Heat-Stress Broiler Chickens

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Abstract

This study aimed to investigate the efficiency of adding dietary linseed oil and fermented pomegranate peel on growth performance, digestibility, antioxidant capacity, and muscle fatty acid deposition in heat-stressed broilers. Three hundred Ross 308 broiler chicks (1 day old) were randomly assigned to four groups (group/five replicates). Dietary treatments included: (CON) corn-soybean meal basal diet; (FPP) basal diet + fermented pomegranate peel 4g/kg; (LIO) basal diet + 3% linseed oil; (FPLO) basal diet + fermented pomegranate peel + linseed oil. Compared with CON, broilers fed FPLO diet showed improved body weight gain and feed conversion ratio, while the experimental diet did not affect feed intake. Broilers fed FPP or FPLO diet had higher dressing percentages and lower relative weight of abdominal fat than other groups, whereas other carcass traits and digestive enzymes activities were not influenced by treated groups. Nutrient digestion enhanced, as the crude protein digestibility increased in chickens fed FPP and FPLO, while the crude fat digestibility increased in chickens fed LIO and FPLO. Cholesterol and triglyceride concentrations decreased, while the HDL concentration increased in the chickens fed FPP and FPLO compared to other groups. The oxidative status was enhanced by the experimental additives, as the SOD and GSH-Px levels increased and the MDA level decreased in the serum in treated groups compared to the control group. Chickens fed FPP and FPLO had an improvement in gut health, as the number of *Lactobacillus* increased and the number of *E. coli* and total coliforms decreased. A significant increase in unsaturated fatty acid and a decrease in the n-6:n-3 ratio was noticed in the muscles of chickens fed LIO and FPLO compared to other groups. This research indicates that diets supplemented with a combination of fermented pomegranate peel and linseed oil experience an improvement in growth performance, blood antioxidant capacities, and fatty acid profile in the muscles of heat-stressed broilers.

KEYWORDS
Broiler chickens, Performance, Antioxidant status, Fatty acid deposition, Linseed oil, Pomegranate peel.

INTRODUCTION

Environmental pressures are the most common problems facing the poultry industry, especially heat stress. However, some strategies have been adopted to counter heat stress effects, such as using environmentally controlled sheds (pad cooling and modern ventilation systems), nevertheless, such strategies have a higher cost than farmers can afford to practice, and this hinders their availability, especially in developing countries. For that reason, alternative strategies are used, such as manipulating the diet by changing the feed compositions or using feed additives. The detrimental effects of heat stress include a reduction in growth and feed intake and a deterioration in performance (Abdel-Moneim *et al*., 2022), antioxidant status, meat quality, metabolism, and physiology (Abdel-Moneim *et al*., 2021; Elbaz *et al*., 2022), in addition to increasing mortality rate (Htin *et al*., 2007). Furthermore, heat stress has been reported to adversely affect digestion by causing intestinal injury, which reduces the digestibility of proteins, carbohydrates, and lipids, via negatively affecting the activities of the enzymes. Besides its adverse effects, excessive production of reactive oxygen species leads to pathophysiological changes at the cellular level, in addition to the disturbed gut, which makes the broilers more susceptible to pathogens, resulting in compromised health (Abdel-Moneim *et al*., 2022; Elbaz *et al*., 2021). Several studies confirmed that the use of certain feed additives had a positive effect in mitigating the harmful effects of heat stress on the bird, such as probiotics, organic acids, oil, and materials containing biologically active compounds, etc. (Chwen *et al*., 2013; Abdel-Moneim *et al*., 2022). In the current study, linseed oil and pomegranate peel were selected as a means of helping the birds resist heat stress.

Pomegranate peel (*Punica granatum* L.) is highly nutritious with numerous bioactive constituents and potent pharmacological properties (Arendse *et al*., 2017), it's the inedible portion of the pomegranate plant that makes up about 50% of the total fruit weight (Fawole and Opara, 2016). Studies showed that pomegranate peel has a high antioxidant effect, in addition, to its antimicrobial, and anti-inflammatory properties (Rajput *et al*., 2011). Pomegranate peel is characterized by its role as an antioxidant (radical scavenging abilities) because it contains large amounts of biologically active compounds, which include: flavanones, flavones, ellagic acid, anthocyanidins, as well as, other polyphenolic constituents (Rajput *et al*., 2011; Fawole and Opara, 2016). It also contains many mineral elements such as phosphorous, calcium, potassium, manganese, and nitrogen (Mirdehghan and Rahemi, 2007), in addition, contains Vitamin E, which is an important antioxidant that regulation several metabolic processes, enhances growth, and immune responses, protects against free radicals (Akuru *et al*., 2021; Habibian *et al*., 2014). From that can be considered pomegranate peel improves the oxidative stability of broiler and meat due to its antioxidant property. Besides the beneficial pomegranate peel properties, there are some limitations to its use in poultry nutrition. it contains a high level of tannin, which is an anti-nutritional factor in poultry nutrition (Serrano *et al*., 2009). However, tannins (anti-nutritional factors) could be reduced through processing such as fermentation (Elbaz *et al*., 2023). Fermentation is the most important biotechnological process used in the recycling of agroindustrial byproducts into useful poultry feeds in developing countries, in addition, it's easy to manage in on-farm conditions. It can lessen the anti-nutritional factors, improve nutrient digestibility and enhance the quality of protein (Kim *et al*., 2007b; Elbaz 2021) thus improving growth performance and immunity, as well as, enhancing gut health by modulating the intestinal microflora in broilers fed by-products fermented with probiotic bacteria (Ahmed *et al*., 2014; Serrano *et al*., 2009).

The addition of vegetable oils in poultry feed is essential, especially since it's the major source of energy, in addition to it has an important role in increasing the efficiency of utilization of the consumed energy, diet palatability, and enhancing the absorption of fat-soluble vitamins (Poorghasemi *et al*., 2013). Moreover, adding oil to the diet reduces the rate of food passage in the gastrointestinal tract, which improves the absorption of nutrients in the diet (Khatun *et al*., 2018, Attia *et al*., 2018), especially during heat stress (Wang *et al*., 2013). The energetic value of oils depends on the length of the carbonic chain, the number of double bonds, the composition of the free fatty acid, and the specific arrangements of (saturated and unsaturated) fatty acids (Kim *et al*., 2007a). Fatty acids, especially omega-3, are proving indispensable to maintaining numerous physiological, reproductive, biological, and beneficial health functions (Khatun *et al*., 2018). Foods that provide ω-3 fatty acids include certain nuts, plants (soybean, olive, corn, palm, sunflower, and rapeseed), and fish oils. Many studies report that nutritional interventions including omega-3 fatty acids have the potential to reduce the risk of oxidative stress and inflammation enhance gut health (Calder, 2012), and improve immune function in broilers (Yu *et al*., 2018; Saleh *et al*., 2009). Numerous studies confirmed that n-3 fatty acids can improve gut health by reducing inflammation in the gastrointestinal tract (El-Katcha *et al*., 2014). The use of linseed oil as a source is good for (ω-3) PUFA, which provides optimum eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) levels, in addition, contain high content (50–55%) of alpha-linolenic acid (18:3 n-3, ALA) (Wang *et al*., 2021). Alpha-linolenic acid (ALA) can be bio-transformed into a long chain ω-3 fatty acids such as EPA and DHA (El-Bahr *et al*., 2021). Omega-3 to humans is important for heart health, as well as brain health and that also supports the immune system and digestion. Poultry meat is one of the most important sources of unsaturated fatty acids after fish meat (Kim *et al*., 2007a). Feeding poultry on a high source of Omega 3 is a good strategy to improve the health of the bird in addition to supporting consumer health.

The present study hypothesized that the use of linseed oil with Fermented pomegranate peel will mitigate the adverse effects of heat stress by enhancing feed utilization, immunity, and oxidative stability, in addition, to improving fatty acid deposition in heat-stressed chicken meat. In the current study, supplementation of broiler diets with linseed oil and fermented pomegranate peel was investigated for their effectiveness on performance, oxidative stress biomarkers, nutrient digestibility, gut health, and fatty acid profiles of broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experimental protocols were reviewed and approved by the Animal Ethics Committee of Desert Research Center, Cairo, Egypt (approval No: 16.2023.2). All protocols were carried out in accordance with the Universal Directive on the Protection of Animals Used for Scientific Purposes.

Experimental Diets, chicks and management

This study was approved and conducted under the guidelines of the Desert Research Center Institutional Animal Care and Use Committee. A total of 300 (one-day-old) Ross 308 unsexed chicks were randomly allocated to floor pens in 4 groups (group/ 5 replicates) that included: group 1, chicks fed on basal a diet (CON); group 2, chicks fed on basal a diet containing fermented pomegranate peel (4 g/kg, FPP); group 3, chicks fed on basal a diet containing linseed oil (3 %, LIO); group 4, chicks fed on basal a diet containing fermented pomegranate peel and linseed oil (FPLO). Each pen was equipped automatic nipple drinker with a bucket feeder. Starter (0 to 21 d) and grower (22 to 35 d) diets were based on corn-soybean meal formulations, diets were formulated to meet the NRC (NRC, 1994) nutrient recommendations according to the Ross 308 guide (Table 1). Applied heat stress in this study, broilers were raised in a controlled environment for 42 d, chickens were subjected to heat at 35°C for 4 hours a day, five days a week during the experiment starting at 10 days old, in addition, relative humidity was maintained at 53 to 65%. All the study period, experimental diets (in pellets form) and water were provided ad libitum. Linseed oil was purchased from TANTA Company for Flax and Oils, Egypt.

Preparation of fermented pomegranate peel (FPP)

A total of 2 strains, including Bacillus subtilis (KCTC 1103) and *Lactobacillus* fermentum (CGMCC No. 0843) were purchased from the Department of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. Pomegranate peel (PP, byproduct) obtained from a juice manufacturing company. The required amount of PP for the experiment was weighed and mixed with distilled water (to raise the humidity) then mixed with the microbe (at a rate of 1 gram per kilogram of PP), to start the fermentation process. Fermentation PP was performed with *Lactobacillus* fermentum and Bacillus subtilis, for 72 h at 40 °C under anaerobes conditions. The fermented PP was dried in the oven at 45°C for 3 days (Elbaz, 2021). The freshly dried fermented PP was ground and kept at room temperature for chemical analysis and preparing the experimental diets.

Performance indicators

Body weight, feed intake, and carcass parameters were measured as performance indicators. Live body weight (LBW) and feed intake (FI) were recorded by replicating at 21, 28, and 42 days of age. From these data, body weight gain (BWG), and feed conversion ratio (FCR) were calculated per group during the experimental period, and Mortality was recorded daily. At 42 d, five broilers from each experimental group were randomly selected for slaughtering to measure the carcass traits. The percentage of dressing, abdominal fat, gizzard, and liver were measured.

Digestive enzymes and nutrient digestibility

At 42 days, during slaughter, twenty samples of intestine contents (5 broiler/ group) were collected and placed in a neutral saline solution for preservation. Start of the analysis, the solution was separated through centrifugation (1792 g for 15 min), and then separated the supernatant part to estimate the digestive enzymes activities, including lipase (Sklan and Halevy, 1985), trypsin

Table 1. Contents and chemical composition of the broiler chick diets.

Ingredients	Starter (1-21d)				Grower (22-42d)			
	CON	FPP	LIO	FPLO	CON	FPP	LIO	FPLO
Corn	52	51.7	52	51.7	58.4	58.1	58.4	58.1
Soybean meal	38	37.9	38	37.9	30.8	30.7	30.8	30.7
Corn Gluten meal	3	3	3	3	4	4	4	4
Soybean oil	3	3	-	-	3	3	-	-
Linseed oil	-	-	3	3	-	-	3	3
FPP	-	0.4	-	0.4	-	0.4	-	0.4
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine Hcl	-	-	-	-	-	-	-	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calcium Carbonate	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.2
Dicalcum phosphate	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8
Vit & min premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated composition								
ME (kcal/kg**)	3000	3000	3000	3000	3100	3100	3100	3100
Crude protein	23	23	23	23	21	21	21	21
Crude fiber	3.82	3.82	3.82	3.82	4.13	4.13	4.13	4.13
Calcium	1.03	1.03	1.03	1.03	0.96	0.96	0.96	0.96
Available phosphorous	0.48	0.48	0.48	0.48	0.45	0.45	0.45	0.45

*Vitamin plus mineral premix provided (per kg of dietary): 2.03 mg vitamin B2, 205 mg vitamin B1, 2.7 mg vitamin K3, 30.6 U vitamin E, 1700 U vitamin D3, 8160 U vit A, 27.2 mg niacin, 10.2 mg calcium pantothenate, 4.1 mg vitamin B6, 2.02 mg vitamin B12, 1.7 mg folic acid, 120 mg ronozyme P500, 0.068 mg biotin, 350 mg choline, 0.34 mg Co, 0.08 mg I, 0.2 mg Se, 70 mg Zn, 70 mg Mn, 50 mg Fe, and 6mg C. ** ME: Metabolizable energy.

Table 2. Effects of fermented pomegranate peel and linseed oil and their interactions on growth performance and carcass traits of heat stressed broiler chickens at 42 days.

Parameter	CON	FPP	LIO	FPLO	SEM	p-value
Growth performance						
Live body weight (g)						
IBW (g)	41.1	40.9	41.2	41.1	0.01	0.07
21d	587^{b}	615^{b}	623^{b}	678^{a}	1.06	0.00
42d	1781^{c}	1869^{b}	1885^{b}	1957^{a}	4.12	< 0.001
Body weight gain (g)						
1-21d	546^{b}	574^{ab}	582^{ab}	637^{a}	0.36	0.04
22-42d	1194^{c}	1254^{b}	1262^{b}	1279^{a}	0.83	< 0.001
1-42d	1740^{c}	1828^{b}	1844^{b}	1916^{a}	1.97	< 0.001
Feed intake (g)						
1-21d	727	731	729	736	2.11	0.40
22-42d	2634	2657	2644	2675	5.06	0.15
1-42d	3361	3388	3373	3412	9.28	0.07
Feed conversion ratio (g:g)						
1-21d	1.332^{a}	1.273^{ab}	1.253^{ab}	1.156^{b}	0.07	0.01
22-42d	2.206^{a}	2.119^{b}	2.095^{b}	2.091^{b}	0.01	0.02
1-42d	1.931^{a}	1.853^{ab}	1.829^{ab}	1.781^{c}	0.02	< 0.001
Carcass traits						
Dressing	69.7^{b}	71.8^{ab}	70.2^{b}	72.6^{a}	1.01	0.00
Gizzard	2.83	2.91	2.85	2.89	0.05	0.10
Liver	1.72	1.68	1.7	1.71	0.12	0.29
Abdominal fat	5.46^{a}	3.35^{b}	4.87^{ab}	3.42^{b}	0.09	0.00

$^{a-c}$: Means in the same row with different superscripts are significantly different at $p < 0.05$. CON: corn-soybean meal basal diet, FPP: basal diet + fermented pomegranate peel 4g/kg, LIO: basal diet + 3% linseed oil, FPLO: basal diet + fermented pomegranate peel + linseed oil

(Sklan *et al*., 1975), and amylase (Pinchasov *et al*., 1990) were estimated. At 42 d, the digestion experiment started. Six chickens from each treatment group were weighed and housed in metabolic cages individually then starved for 12 hours to emptying gut. During the age of 42 to 45 d, excreta was collected, three times a day from the bottom of each cage, weighed dried, and then frozen (-20 0C) to analyze and the digestibility determination for dry matter (DM), crude fat (CF), and crude protein (CP), according to Association of Official Analytical Chemists, (AOAC, 2004), as showed in Table 2.

Serum parameters

Blood samples were drawn into non-heparinized sterile tubes during slaughter to obtain serum, then centrifuged (3400×g for 9 min) and then stored at -20 °C until further biochemical analysis. Serum triacylglycerol, total cholesterol, high-density lipoprotein (HDL), and low–density lipoprotein (LDL) levels were determined colorimetrically (Milton Roy, Ivyland, PA, USA) according to the manufacturer's instructions (Spinreact Co., Girona, Spain). Assays of indicators of antioxidant capacity, including malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and were examined using commercial kits (BioAssay Systems, USA and Cayman Chemical Company, USA).

Microflora enumeration

During slaughter, five samples per group were taken from the cecum directly, about 5 g, and placed in a homogeneous solution and a serial of decimal dilution was prepared. *Lactobacillus*, Total Coliform, and Escherichia coli were enumerated (using conventional microbiological techniques) using nutrient agar, and immunization with the time and temperature required for each microbe (Elbaz *et al*., 2023). Results were estimated as log10 colony-forming units per gram of cecal digesta.

Muscles fatty acids deposition

On day 42, after slaughter, five broilers per group were chosen to measure the left breast muscle fatty acid profile, and it was kept at - 20 °C until analysis. A freeze dryer was used to lyophilize about 30 g of muscle for 60 h. To extract total fatty acid from the homogenized breast muscle tissue, a solvent mixture of chloroform and methanol (2:1, vol/vol) was used. Fatty acid profile in muscles was determined using gas chromatography (GC, 6890 series, Agilent Technologies, Wilmington, DE, USA) was used.

Statistical analysis

The data were analyzed with the Statistical Analysis System (SAS, 2004), using one-way analysis of variance (ANOVA), and the generalized linear model (GLM) procedure. Significance was evaluated at the level of $P<0.05$.

RESULTS

Growth performance and carcass traits

Growth performance and carcass traits evaluated in heat-stress broilers are shown in Table 2. During 0-21 and 22-42 days, BWG and LBW increased in broilers fed LIO, FPP, and FPLO compared with broilers fed CON ($p< 0.05$). However, during 0-42 days, there was significantly increased BWG and LBW in broilers-fed FPLO, LIO, and FPP compared with the control group ($p < 0.05$), while the best BWG in broiler fed on FPLO. FI was not affected ($p< 0.05$) by experimental supplemental in all periods experimental. FCR improvement in chickens fed on LIO, FPP, and FPLO compared with broilers fed on CON, during 0-21 and 22-42 days ($p< 0.05$). FCR significantly decreased in chickens fed FPLO compared with chickens fed LIO, FPP, and CON, during 1-42 days. There was a significantly increased dressing carcass and decreased abdominal fat in chickens fed on FPLO and FPP ($p< 0.05$) compared to other groups, nevertheless, gizzard, and liver were not influenced by experimental dietary.

Digestibility and digestive enzymes activities

Table 3 shows the effect of feed additives on nutrient digestion and digestive enzyme activity. There was a significant improvement in the digestibility of nutrients in chickens fed FPP, LIO, and FPLO compared to the control ($p< 0.05$). Enhanced dry matter and crude protein digestion in chicken fed FPP and FPLO ($p< 0.05$) compared to chicken fed LIO and CON. Enhanced crude fat digestion in chicken fed LIO and FPLO compared to chicken fed FPP and CON. Digestive enzymes were not affected by the experimental treatments ($p< 0.05$).

Serum lipid profile, and antioxidative enzymes

Table 4 shows the effect of feed additives on serum lipid profile and antioxidative enzymes activities. Reduced cholesterol and triglyceride levels in chicken fed FPP and FPLO than that LIO and control ($p< 0.05$), while increased HDL level in chicken fed FPP and FPLO than that LIO and control. However, the LDL level was not affected ($p< 0.05$) among the experimental groups. Exper-

Table 3. Effects of fermented pomegranate peel and linseed oil and their interactions on nutrient digestibility (%) and digestive enzymes activities (U/g) of heat stressed broiler chickens at 42 days.

Parameter	CON	FPP	LIO	FPLO	SEM	p-value
Nutrient digestibility						
Dry matter	69.4^{b}	72.5^{a}	70.3^{b}	73.1^{a}	1.14	0.03
Crude protein	71.2^{b}	76.6^{a}	71.0^{b}	77.2^{a}	2.71	0.00
Crude fat	63.4^{b}	64.1^{b}	68.4^{a}	67.8^{a}	0.50	0.00
Digestive enzymes						
Amylase	149	161	152	167	9.25	0.09
Lipase	67.5	68.8	67.1	69.7	5.06	0.13
Trypsin	83.6	85.4	84.5	86.2	11.05	0.21

$^{a-c}$: Means in the same row with different superscripts are significantly different at $p < 0.05$. CON: corn-soybean meal basal diet, FPP: basal diet + fermented pomegranate peel 4g/kg, LIO: basal diet + 3% linseed oil, FPLO: basal diet + fermented pomegranate peel + linseed oil imental dietary supplementation showed significant improvement in oxidative stress. MDA level decreased, and SOD level increased ($p< 0.05$) in chickens fed on FPP, LIO, and FPLO than in the control group ($p < 0.05$), whilst the level of GSH-Px increased in chickens fed on FPLO than in other groups.

Microbial enumeration

Table 5 shows significant changes in the gut microbial content due to experimental supplementation. The number of *Lactobacillus* increased in chickens fed FPLO and FPP compared to LIO and control groups ($p< 0.05$), while the number of Escherichia coli and Total coliforms decreased.

Muscle fatty acids deposition

The effect of experimental additives on fatty acid deposition in the muscles is shown in Table 6. There was a significant decrease in C14:0, C18:0, and C22:0 in the muscle of rabbits fed LIO and FPLO ($p< 0.05$) compared with FPP and CON. A significant increase in C18:1 ω9, C18:3 ω3, C20:1 ω9, C22:5 ω3, and C22:6 ω3 was observed in the muscle of rabbits fed compared with LIO and FPLO ($p < 0.05$) compared with FPP and CON, while a decrease in C18:2 ω6. Rabbits fed on LIO and FPLO diets led to

Table 4. Effects of fermented pomegranate peel and linseed oil and their interactions on serum lipid profile, and antioxidative enzymes activities of heat stressed broiler chickens at 42 days.

Parameter	CON	FPP	LIO	FPLO	SEM	p-value
Lipid profile						
Triglycerides (mg/dl)	196.1[a]	151.6[b]	187.2[a]	156.5[b]	3.02	0.00
Cholesterol (mg/dl)	229.4[a]	179.3[b]	216.9[a]	183.2[b]	2.16	0.02
HDL (mg/dl)	56.7[c]	76.3[a]	64.5[b]	75.9[a]	1.81	< 0.001
LDL (mg/dl)	121.5	118.4	120.7	119.3	4.13	0.08
Antioxidative enzymes						
MDA (nmol/ml)	1.752[a]	0.971[c]	1.265[b]	0.761[d]	0.29	< 0.001
SOD (U/ml)	123.4[c]	149.0[b]	157.4[ab]	168.2[a]	2.09	< 0.001
GSH-Px (U/ml)	20.7[b]	21.4[b]	20.9[b]	23.1[a]	1.13	0.04

[a-c]: Means in the same row with different superscripts are significantly different at $p < 0.05$. CON: corn-soybean meal basal diet, FPP: basal diet + fermented pomegranate peel 4g/kg, LIO: basal diet + 3% linseed oil, FPLO: basal diet + fermented pomegranate peel + linseed oil, HDL: high-density lipoprotein, LDL: low–density lipoprotein, MDA: malondialdehyde, SOD: superoxide dismutase, GSH-Px: glutathione peroxidase.

Table 5. Effects of fermented pomegranate peel and linseed oil and their interactions on microbial enumeration of heat stressed broiler chickens at 42 days.

Parameter	CON	FPP	LIO	FPLO	SEM	p-value
Lactobacillus	3.105[b]	4.805[a]	3.021[b]	4.553[a]	0.52	0.00
Escherichia coli	6.081[a]	3.190[b]	5.853[a]	3.207[b]	0.01	< 0.001
Total coliforms	3.012[a]	2.577[b]	2.918[a]	2.432[b]	0.28	0.04

[a-c]: Means in the same row with different superscripts are significantly different at $p < 0.05$. CON: corn-soybean meal basal diet, FPP: basal diet + fermented pomegranate peel 4g/kg, LIO: basal diet + 3% linseed oil, FPLO: basal diet + fermented pomegranate peel + linseed oil

Table 6. Effects of fermented pomegranate peel and linseed oil and their interactions on muscles fatty acids deposition of heat stressed broiler chickens at 42 days.

Parameter	CON	FPP	LIO	FPLO	SEM	p-value
C12:0	0.01	0.02	0.01	0.03	0.00	0.31
C14:0	2.81[a]	2.68[a]	2.31[b]	2.27[b]	0.01	0.02
C16:0	27.1	27.6	26.2	26.5	0.13	0.09
C18:0	13.19[a]	13.67[a]	12.18[b]	12.05[b]	0.08	0.03
C20:0	0.03	0.02	0.02	0.03	0.21	0.16
C22:0	0.91[a]	0.57[b]	0.64[b]	0.52[b]	0.09	0.01
C16:1 ω7	2.16	2.01	2.13	2.09	0.18	0.11
C18:1 ω9	27.1[c]	27.6[c]	29.3[b]	30.1[a]	0.04	< 0.001
C18:2 ω6	23.5[a]	23.1[a]	21.6[b]	21.1[b]	0.16	0.04
C18:3 ω3	0.82[b]	0.76[b]	1.56[a]	1.64[a]	0.11	< 0.001
C20:1 ω9	0.33[c]	0.41[b]	0.68[a]	0.73[a]	0.07	< 0.001
C20:4 ω6	0.07	0.08	0.06	0.07	0.27	0.64
C22:5 ω3	1.09[c]	0.84[c]	1.58[a]	1.45[b]	0.03	< 0.001
C22:6 ω3	0.58[b]	0.52[b]	1.41[a]	1.36[a]	0.19	< 0.001
∑ SFA	44.05[a]	44.56[a]	41.36[b]	41.40[b]	0.42	0.00
∑ USFA	55.65[b]	55.32[b]	58.32[a]	58.54[a]	0.90	0.00
∑ PUFA	26.39	25.71	26.89	26.35	0.05	0.09
UFA: SFA	1.265[b]	1.243[b]	1.410[a]	1.414[a]	0.48	0.00
ω6:ω3 ratio	9.46[a]	10.93[a]	4.76[b]	4.75[b]	0.02	< 0.001

[a-c]: Means in the same row with different superscripts are significantly different at $p < 0.05$. CON: corn-soybean meal basal diet, FPP: basal diet + fermented pomegranate peel 4g/kg, LIO: basal diet + 3% linseed oil, FPLO: basal diet + fermented pomegranate peel + linseed oil, SFA: saturated fatty acids, USFA: unsaturated fatty acids, PUFA: polyunsaturated fatty acids.

a decrease in saturated fatty acids (SFA) and an increase in unsaturated fatty acids (USFA) compared to other groups (p< 0.05), while the PUFA was not affected by the experimental treatments (p< 0.05). However, there was a significant decrease in the ω6:ω3 ratio in rabbits fed LIO and FPLO (p< 0.05) compared with FPP and CON.

DISCUSSION

The exploitation of byproducts and vegetable oil as a source of supplement dietary under environmental and economic pressures is a promising field. Consequently, the harmful effects of heat stress can be mitigated through the use of feed additives or some byproducts (Abdel-Moneim *et al*., 2021). Therefore, our hypothesis was based on adding linseed oil and fermented pomegranate peel lead to mitigate the effects of heat stress on broilers.

Our results showed a significant improvement in the performance of heat-stressed broilers fed on a diet that includes a mixture of fermented pomegranate peel with linseed oil, compared to the rest of the groups. Numerous studies reported that adding pomegranate peel or linseed oil to broiler chicken fed improved performance (Akuru *et al*., 2021; Attia *et al*., 2018). Similar to the current findings, enhanced growth performance in broilers fed on 4 g/kg pomegranate peel and the control group (Akuru *et al*., 2021). In similar experiments, researchers proved that the inclusion of linseed oil (2 to 4%) in diets enhanced the performance of broilers (Panda *et al*., 2015). Improved the performance of birds fed FPLO may perhaps be due to the growth-promoting benefits of linseed oil and fermented pomegranate peel which has been linked to its antimicrobial and antioxidant properties, in addition to enhance feed utilization. Pomegranate peel has a positive impact on intestinal bacteria that enable broilers to increased absorb nutrients, leading to an enhancement in their feed utilization and growth performance (Middha *et al*., 2013), as well as, the antioxidant effect of pomegranate peel, which is due to its possession of proanthocyanidins, that enables it to improve gut digestive enzyme and pancreatic functions, and prevent the deleterious impact of free radicals on intestinal enterocytes; thus, enhance nutrient digestion and absorption (Middha *et al*., 2013; Abdollahzadeh *et al*., 2011). This is consistent with the results of previous studies, which stated that adding oil enhanced the growth performance of chickens exposed to heat stress (Chwen *et al*., 2013), this improvement can be explained by the fact that the addition of oil led to enhances absorption of fat-soluble vitamins, palatability of diets, and decreases the rate of food passage within the digestive system (Nobakht *et al*., 2011; Ghazalah *et al*., 2008) thus improving the utilization of nutrients, as well as, an appositive impact on humoral immunity, which is lead to enhance growth performance. In addition, the fermentation of unconventional feed ingredients improves performance by lowering the content of antinutritional factors, modification of the microbial content, and enhancing feed utilization (Elbaz *et al*., 2023), which leads to improved gut health and performance. Our results can be summarized that the combination of linseed oil and fermented pomegranate peel improved the performance of the heat-stressed broiler.

The deterioration of carcass characteristics is one of the most important economic losses the poultry industry suffers from during heat stress, the results of the current study confirm this. There was a significant decrease in carcass traits in the control group compared to other groups. Our results showed a significant improvement in the dressing carcass with a decrease in belly fat in chickens fed on fermented pomegranate peel alone or with linseed oil. The observed increase in dressing carcass in broilers fed FPP and FPLO might be due to the positive impacts of the fermentation process, including increasing digestive enzyme activity, balancing beneficial microbial populations (Shim *et al*., 2010), and nutrient utilization leading to more gain in body weight and carcass. Our results agree with Bidura *et al*. (2007), who found a significant decrease in abdominal fat in chickens receiving probiotics compared to control. Likewise, the carcass content of belly fat decreased in chickens fed on a fermented diet (Elbaz *et al*., 2023). The decreased abdominal fat in the groups fed on fermented pomegranate peel can be explained by the role of beneficial bacteria (probiotics) in the rate of fatty acid synthesis being reduced through a decrease in the activity of acetyl-CoA carboxylase (the rate-limiting enzyme in fatty acid synthesis) or by the conversion of excess energy from the metabolism process, by Santoso *et al*. (1995). However, the reason for the decrease in abdominal fat may be due to the role of oil in regulating the deposition of fat in the carcass, or by the role of dietary oils in inhibiting the absorption of dietary fat and fatty acid biosynthesis and promoting fatty acid β-oxidation (by decreasing the number of abdominal adipose cells) (Yi *et al*., 2016). Our results confirm the effect of supplementing oil and fermented PP on regulating the distribution of fat inside the carcass, which has a significant economic role in addition to enhancing the dressing carcass.

In this study, the results demonstrated that broilers fed with FPP or FLOP had significantly higher digestibility of DM and CP compared to the other group, while broilers fed with LIO or FLOP had significantly higher digestibility of CF compared to the other group. Fermentation of dietary with eligible microbes breakdown the feed components by microbial enzymes production leading to an increase in the nutritional value of fermented feeds (Feng *et al*., 2007; Elbaz *et al*., 2023) and reducing the anti-nutritional factors of feed; as well as, influencing the microbial modulation into the gut and enhancing digestion of nutrients, which improvement of average daily gain (Zhang *et al*., 2006). Hamady *et al*. (2015) reported that the dietary inclusion of a pomegranate peel in the broiler's diet significantly increased nutrient digestion by modulating digestive secretions and enzymatic functions and regulating pre-cacal nutrient digestion, through a decrease in bacteria colony counts. likewise, several studies reported that adding oil to broiler chicken diets reduces the rate of food passage into the digestive tract (Cherian, 2015), which enhances feed utilization by increasing the effect of enzymes and thus promoting digestion and absorption. Fermented pomegranate peel by probiotics might improve its utilization and nutritional quality, as well as reduce the anti-nutritional factors such as tannin, in addition to role oil in enhancing digestion. Consequently, the improved nutrient digestion by a mixture of linseed oil with pomegranate peel in the present study.

The results of the current study showed a significant effect on the blood lipids profile through the experimental additions, as the cholesterol and triglyceride levels decreased, while the HDL level increased. Studies have shown that plant waste and its extracts affected chicken fat profile by regulating the level of serum cholesterol and triglyceride and the expression of enzymes, genes, and signaling pathways related to fat synthesis, transportation, and decomposition in liver, abdominal fat, and muscle (Ding *et al*., 2023). Moreover, direct-feed microbial could be related to inhibited hepatic 3-hydroxy-3-methylglutaryl coenzyme reductase activity or a lowered recycling of bile salts in the gut (Zhang *et al*., 2012). Dietary supplementation of FPLO was able to increase the anti-oxidative enzyme activity of the broiler. Medicinal plants struggle lipid peroxidation in biological systems either through scavenging for free radicals and ROS or enhancing the innate defense mechanisms of the cells, by the activation of antioxidant enzymes (Verma *et al*., 2009). In addition, antioxidant enzymes provide the first line of defense against the deleterious impact of free radicals, which inhibit the lipid peroxidation of hydrogen and peroxide toxicity at the cellular level (Oloruntola *et al*., 2019). An increase in SOD and GSH-Px activity as observed in blood in the birds fed FPLO is an indication that the antioxidant enzyme could work faster to remove free radicals. Previous studies on pomegranate peel showed that it increased from the plasma antioxidant enzymes levels (like CAT levels) in broilers (Akbarian *et al*., 2015; Doostan *et al*., 2017). The reports of Lindblom *et al*. (2019) showed that supplementation of oil enhances the oxidative capacity of broilers in broilers, which contributes to reducing oxidative degradation, leading to strengthening immune functions and stability of poultry. As well, the low level of MDA indicates that the cells are not exposed to any stress, this is due to the increased production of free amino acids, like tyrosine, methionine, and lysine, during the fermentation process, which has a positive impact as an antioxidant (Wang and De Mejia, 2005). The results of this study confirm the beneficial effect of adding linseed oils with fermented pomegranate peel to diets on enhancing blood lipid profile and the oxidative capacity of broilers exposed to heat stress.

Intestinal microbes play a major role in host immune defense mechanisms against pathogens, enhancing feed utilization, and intestinal morphology, which positively influences the productive performance of broilers. The results of the current study showed a significant improvement in the microbial content of the intestine via increasing the number of *Lactobacillus* and decreasing the number of Escherichia coli and Total coliforms. In numerous kinds of research, dietary fermentation has been proven to be beneficial in promoting gut health and supporting immunity by reducing broiler enteric diseases and inflammation (Engberg *et al*., 2009; Elbaz *et al*., 2023). Hence, the fermentation of pomegranate peel had a catalytic role in the health of the intestines of heat-stressed broilers by beneficial modulator effects on the gut microflora in the current study.

It is well-known that meat fatty acid composition is an important indicator to estimate the nutritional value and meat quality (Tang *et al*., 2021). It is generally believed that higher dietary intakes of SFA have a relationship with increasing the risk of coronary heart disease via raising the total cholesterol (Zong *et al*., 2016). In the present study, total SFA significantly decreased and total UFA significantly increased in the muscle, which indicated that the muscles from the broiler fed with fermented pomegranate peel alone or community with linseed oil might be more beneficial to consumer health. Numerous reports confirmed our findings of increased Linoleic, linolenic acids, and total UFA and reduced palmitic acid in broilers fed on probiotics (Tang *et al*., 2021). The results of the current study demonstrated that dietary linseed oil (as a source of n-3 PUFA) supplementation could effectively impact oxidative stress and lipid oxidation in serum that enhancing the antioxidant capacity of muscles in broilers (Rymer *et al*., 2010), furthermore, increasing levels of T-AOC and SOD illustrates the improvement of the antioxidant defense system in broilers (Long *et al*., 2018, Kim *et al*., 2007a). Enhanced antioxidant status in the muscle of broilers supplemented with linseed oil diets was mainly due to the ability of n-3 PUFA to scavenge H_2O_2, free radicals, and lipid peroxides, as well as suppress the production of pro-inflammatory cytokines leading to the mitigation of oxidative stress and inflammatory while modulating lipid metabolism. The beneficial nutritional recommendations in consumer diets of the ω-6/ω-3 ratio to human health should not exceed and should be nearly 1:1 to 3:1 (ideal ratios). These ideal ratios could be enhancing metabolism, bioavailability, and the incorporation of fatty acids into membrane phospholipids (Kim *et al*., 2007a). Interestingly, the ω-6/ω-3 ratio obtained in the muscles of broilers in the current study almost met the recommended guidelines. In summary, linseed oil alone or combined with FPP could improve the composition of muscle fatty acids, thus improving the meat quality, with consumer health support.

CONCLUSION

This study demonstrated that the combination of fermented pomegranate peel with linseed oil in broiler diets positively impacts productive performance and health during heat stress. The detrimental impacts of heat stress on growth, nutrient digestibility, antioxidant status, and gut health (bacterial community structure) were significantly lower in broilers fed on a mixture of fermented pomegranate peel and linseed oil. Furthermore, the synergistic impact of fermented pomegranate peel with linseed oil was observed to exhibit a better improvement in nutrient digestibility, antioxidant status, gut health, and fatty acid deposition in muscles thus improving productive performance.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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