Performance, immune response, and oxidative status in broiler chicken fed oxidized oil and *Otostgia persica* leaf extract

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**ABSTRACT**

The aim of this study was to determine the effect of vitamin E (α-Toc) and *Otostgia persica* leaf extract (OPLE) on the performance, immune response, and oxidative status in broiler chicken fed oxidised oil. A total of 350 one-day-old male broiler chickens were randomly attributed to seven dietary treatments. Broilers were fed diets containing fresh oil or oxidised oil without supplement, and diets containing oxidised oil supplemented with 200 mg/kg α-Toc, 100 or 200 mg/kg OPLE, 200 mg/kg α-Toc + 100 mg/kg OPLE, and 200 mg/kg α-Toc + 200 OPLE mg/kg for six weeks. The plasma oxidative status was evaluated by measuring the superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in plasma. Lipid peroxidation in thigh meat was measured by thiobarbituric acid-reactive substances (TBARs). Inclusion of antioxidants in the diet containing oxidised oils or fresh oil was increased body weight, feed intake, and weight gain ($p < .05$). The dietary antioxidants had no effect on plasma GPx in birds fed oxidised oil. Dietary supplementation with OPLE and α-Toc increased SOD activity and reduced TBARs in the thigh meat of broilers fed with a diet containing oxidised oil ($p < .05$). Birds fed with the dietary oxidised oil revealed lower titres of antibody against the sheep red blood cell ($p < .05$), and immune response was significantly improved by the supplementary OPLE. Serum cholesterol, triacylglycerols, and HDL were not affected by the inclusion of antioxidants and the type of oil. In conclusion, *Otostgia persica* leaf extract combined with α-Toc could reduce the negative effects of oxidised oil in the diets of broiler.

**HIGHLIGHTS**

- Performance, plasma oxidative status, and lipid oxidation in broilers fed with oxidised oil were decreased.
- The immune response was significantly improved by the supplementary *Otostgia persica* leaf extract in broilers.
- *Otostgia persica* leaf extract reduced TBARs in thigh meat broilers fed with diet oxidised oils.
- *Otostgia persica* leaf extract along with vitamin E has the ability to reduce the negative effects of oxidised oil in broilers.

**Introduction**

The quality of oil as a source of energy in poultry diets influences growth performance and the health status of the animal. The use of oxidised oil in the diet of broiler chicken causes growth depression in broiler by reducing feed intake (Kishawy et al. 2016; Azimi et al. 2020). Utilising oxidised oil in broiler feed may result in decreased palatability of ration due to rancid odour; also it has health risk on poultry due to the formation of free radicals (Mazur-Küssnirek et al. 2019; Saleh et al. 2018). Vegetable oils, which are high in polyunsaturated fatty acids, are prone to oxidation (Vossen et al. 2011). In addition to reducing the nutritional value of fats, proteins, and fat-soluble vitamins, peroxidation reduces the antioxidant capacity of animals (Tan et al. 2018). Consumption of oxidised oils also reduces tocopherol levels of tissues and blood (Eder and Kirchgessner 1998; Juskiewic et al. 2000) and increases the amount of malondialdehyde (MDA), which indicates an elevation in lipid peroxidation (Bayraktar et al. 2011).

Normally, the body has the ability to remove free radicals. Oxidative stress is an imbalance between free radicals and antioxidants in the body (Mazur-Küssnirek...
et al. 2019). As a result, the body’s antioxidant defense system cannot eliminate unpleasant free radicals. As reported previously, there has been a great deal of attention towards natural or synthetic antioxidant supplements in poultry diets to prevent the adverse effects of free radicals (Tavárez et al. 2011; Kishawey et al. 2016; Saleh et al. 2018).

Several antioxidants of plant origin have been exploited in recent years for their actual or supposed beneficial effect against oxidative stress, such as vitamin E, anthocyanins, flavonoids, and polyphenols (Kishawy et al. 2016; Mazur-Kusnierek et al. 2019; Shen et al. 2019). The safety limits of natural antioxidants are mostly not known, but they are hardly safer than synthetic antioxidants, and demand for products of natural origin continues to increase. Phenolic compounds of plant origin derived from pomegranate and bamboo, which have antioxidant properties, may reduce the negative effects of oxidative stress (Saleh et al. 2017; Hosseini-Vashan and Raei-Moghadam 2019; Shen et al. 2019).

*Otostegia persica* (*O. persica*), is a flowering plant that belongs to *Lamiaceae* and has been used traditionally as herbal medicine in Asian and Arabic folklores as a remedy for various ailments since ancient times. The aerial parts of the plant, in particular, have been demonstrated to possess antibacterial, antifungal, antioxidant, and antiflammatory properties (Shrififara et al. 2003). Some of the important constituents of *O. persica* are flavonoids and phenolic compounds including quercetin, morin, kaempferol, Trans-Cinnamic acid, and Caffeic acid. Some of these compounds may be responsible for the plant’s antioxidant activity. The antioxidant properties of ethanolic extract of *O. persica* have been reported by DPPH free radical scavenging (Sharififara et al. 2007).

Phenolic compounds, the breed, and the age of the birds can affect the immune response of birds to dietary polyphenol supplementation (Nelson et al. 1995; Renaudeau et al. 2012). Some compounds can enhance the proliferation of beneficial bacteria, while reducing the abundance of pathogenic ones through their bactericidal and bacteriostatic activities, thus indirectly improving host immunity and health (Gessner et al. 2017). Vashan and Raei-Moghadam (2019) reported that pomegranate pulp could improve the humoral antibody response and the relative weight of the bursa of Fabricius. Phenolic compounds can be positively regulating the production of cytokines, HSPs, and transcription factors (Lipiński et al. 2017). The aim of this study was to determine the effect of *O. persica* leaf extract and α-Toc and on performance, immune response, and oxidative status in broiler chicken fed oxidised oil.

**Materials and methods**

**Preparation of hydroalcoholic extract of *Otostegia persica* leaf**

Leaf *O. persica* was prepared from the one-year-old plants dried locally (Saravan, Sistan and Baluchestan, Iran). The samples were identified by the Herbarium of the Plant Production Department in the Higher Education Complex of Saravan. The leaves of *O. persica* were finely powdered in a mill. Dried powder (2.5 g) was extracted with 40 ml of ethanol solvent at room temperature for 48 h. The liquid-soluble materials were separated from the solids using vacuum filtration and were concentrated by a rotary evaporator under vacuum at 30 °C (Labrato 4000, Heidelberg, Germany). The concentrated extracts were stored at −20 °C for further use (Saleh et al. 2018). Total polyphenol contents (TPP) were determined by the Folin–Ciocalteu method reported by Saleh et al. 2018. Total flavonoid contents in the extracts were determined by aluminium chloride colorimetric assay (Moghadam and Shaaban, 2018). The total phenol and flavonoids were measured using a spectrophotometric device.

**Birds, feeding, and management**

The Animal Ethics Committee of Saravan Higher Educational Complex approved all the animal protocols used in the current experiment. Three hundred and fifty, one-day-old male chicks of a commercial meat-type (Ross 308) were obtained from a local hatchery and were randomly allotted into 7 groups with 5 replicates containing 10 birds each. The birds were fed *ad libitum* from one to 42 d. Continuous lighting was provided throughout the experiment. The starting temperature was 33 °C then decreased gradually to 2 °C each week until reached 21 °C at the 6th week. At the end of the experimental period, average body weight, body weight gain, feed intake, and feed conversion ratio were measured for each pen. The basal diets were formulated according to the requirements of the guidelines of Ross broiler (2014). Ingredients and nutrient composition of diets are shown in Table 1 and are designed for the starter phase (1–10 d), growth phase (11–23 d), and finisher (24–42). Experimental diets were as follows: 1) The control group with fresh oil (FO), 2) The control group with oxidised oil and without supplement (OO), 3) Diet OO + 200 α-Toc mg/kg, 4) Diet OO+ 100 OPLE
mg/kg, 5) Diet OO + 200 OPLE mg/kg, 6) Diet OO + 200 α-Toc + 100 OPLE mg/kg, and 7) OO + 200 α-Toc + 200 OPLE mg/kg. Consistently, in all diets, 2% of oxidised sunflower oil was added to the experimental diet (Table 1). To preparing oxidised oil, sunflower oil was heated at a temperature of 200°C for 3 d and was aerated for 8 h daily. The processed oils were stored at −30°C before adding to diets (Tan et al. 2018). No antioxidant was added before or during the manufacturing of the experimental diets. Peroxide value in fresh oil was <1 mEq/kg and in the oxidised oil 7.5 mEq/kg.

| Ingredients                   | Starter diet (1–10 d) | Grower diet (11–23 d) | Finisher diet (24–42 d) |
|-------------------------------|-----------------------|------------------------|-------------------------|
| Maize                         | 563.00                | 589.30                 | 624.90                  |
| Soybean meal, 48% CP          | 352.50                | 329.00                 | 289.40                  |
| Maize gluten meal             | 11.80                 | 3.00                   | 0.00                    |
| Sunflower oil                 | 8.00                  | 20.00                  | 32.00                   |
| Oxidised sunflower oil        | 20.00                 | 20.00                  | 20.00                   |
| Oyster                        | 14.00                 | 12.00                  | 10.00                   |
| Dicalcium phosphate           | 17.80                 | 16.00                  | 16.00                   |
| Salt                          | 3.00                  | 3.00                   | 2.50                    |
| DL-Methionine                 | 2.40                  | 1.10                   | 0.10                    |
| L-Lysine                      | 1.10                  | 0.80                   | 0.10                    |
| DL-Threonine                  | 1.40                  | 0.80                   | 0.00                    |
| Vitamin mixtureb              | 2.50                  | 2.50                   | 2.50                    |
| Mineral mixturec              | 2.50                  | 2.50                   | 2.50                    |
| Calculated nutrients and energy |                       |                        |                         |
| ME, MJ/kg                     | 3.00                  | 3.10                   | 3.21                    |
| Crude protein, %              | 230.20                | 215.20                 | 195.10                  |
| L-Lysine, %                   | 14.40                 | 12.90                  | 11.60                   |
| DL-Methionin, %               | 7.00                  | 6.40                   | 5.90                    |
| TSAA, %                       | 10.70                 | 9.80                   | 9.10                    |
| Crude fibre, %                | 2.63                  | 2.56                   | 2.50                    |
| Total calcium, %              | 1.02                  | 1.01                   | 0.81                    |
| Available phosphorus, %       | 0.15                  | 0.14                   | 0.23                    |

aConsistently, in all diets, 2% of oxidised sunflower oil was added to the experimental diet (Peroxide value in fresh oil was <1 mEq/kg and in the oxidised oil 7.5 mEq/kg).

bVitamin premix provided per kilogram of diet: retinyl acetate, 11,000 IU; cholecalciferol, 11 mg; menadione sodium bisulphate, 2 mg; riboflavin, 5.7 mg; pyridoxine hydrochloride, 2 mg; cyanocobalamin mg; 0.024 mg nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250.

cMineral premix provided per kilogram of diet: Mn, 100 mg; Zn, 65 mg; Cu, 3 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg.

Collection of samples and measurements

At 42 d, two blood samples obtained by cardiac puncture for separation of serum, the sera obtained were used for biochemical analysis. Other blood samples were collected at 42d on EDTA and then used for SOD and GPx activity. The activity of SOD and GPx were determined with the use of the Randox kit according to the manufacturer’s recommendations (Randox Laboratories Ltd., Crumlin, County Antrim, UK), respectively (Arthur and Boyne 1985). The assays were conducted on a UV-visible spectrophotometer (model PharmaSpec 1700, Shimadzu, Japan). Wavelength was used for SOD and GPx, 505 and 340 nm, respectively. Total serum cholesterol concentration was measured according to Finley et al. (1978), Serum TAG concentration was determined calorimetrically according to Wahlefeld and Bergmeyer (1974), Serum HDL was determined calorimetrically according to Naito and Kaplan (1984). The serum concentrations of cholesterol, TAG, and HDL were measured by the Gesan chem autoanalyzer instrument (Gesan Chem 200 autoanalyzer, Italy) with ParsAzmon kits (Pars Azmon Ltd Co; Tehran Iran).

One bird from each replicate was weighted before slaughter and after sacrificing the chicks by cervical dislocation after sufficient exsanguinations at 42 d. The percentage content of breast muscles in the carcass and abdominal fat and liver in live body weight was determined. The lymphoid organs (thymus, spleen, and bursa of fabricius) were carefully dissected and weighed. Their relative weights were determined as percentages of the live weight. The sheep red blood cells (SRBCs) were used as an antigen to evaluate the specific antibody responses and were injected into the vein of broilers at 28 and 35 d. The sera were collected 7 d after injection and the antibody titres against SRBCs (Nelson et al. 1995). To determine the cell-mediated immune response the Cutaneous Basophil Hypersensitivity (CBH) response was used. At 37 d of age, 0.1 mg of phytohemagglutinin-P (PHA-P) dissolved in 0.1 ml of saline was injected into the toe web of the right foot between the second and third digits of two birds from each replicate.

From the slaughtered sample, the raw thigh meat was minced twice (4 mm plate) using a grinder and stored at −20°C for further analysis of meat quality, which was performed on 30 d of freezer storage (−20°C). Five grams of each meat sample storage was placed in 15 ml distilled water and homogenised at 1130 g for 1 min. Sample homogenate (5 ml) was transferred to a test tube and lipid oxidation was determined as the 2-thiobarbituric acid-reactive substances (TBARS) value and was expressed as micrograms of malondialdehyde (MDA) per gram of muscle using the procedure described by Ahn et al. (1999).

Calculations and statistical analysis

All the recorded data were analysed using PROC GLM of SAS (SAS Institute 2009) followed by a completely
randomised design. Each treatment consisted of an equal number of replicates. Significant differences between the treatment means were compared using the Duncan multiple range test at the probability level of 5% ($p \leq .05$).

**Results**

**Performances**

The effects of supplementary oil type and vitamin E ($\alpha$-Toc) and *Otostgia persica* leaf extract (OPLE) on broiler performance are shown in Table 2. Oxidised dietary oil without supplementation affected performance during the six-week experimental periods. The applied antioxidants improved the growth performance of broiler chickens fed diets containing oxidised sunflower oil ($p < .05$). In birds fed oxidised oil, groups consuming 200 mg/kg of $\alpha$-Toc combined with 100 OPLE showed better performance than other groups. To some extent, in this experiment, the negative effects of oxidised oil on weight, daily weight gain, daily feed intake, and feed conversion ratio were reduced by feeding some antioxidants used. Statistically, the performance of birds fed oxidised oil supplemented with $\alpha$-Toc and 100 mg/kg OPLE was similar to that of birds consumed fresh oil without antioxidants.

**Slaughter performance**

As shown in Table 3, carcass dressing percentage tended to increase in broilers fed diets without rancid oil and diets containing rancid oil supplemented with $\alpha$-Toc and OPLE (Table 3). However, diets containing oxidised oil had no significant effect on the percentage content of breast muscles and liver in the carcass ($p = .089$ and $p = .128$, respectively). Birds fed with the oxidised oil exhibited significantly higher abdominal fat relative weight compared to those fed with fresh oil and supplemented diets ($p < .05$). No difference was observed for relative weights of a lymphoid organ (Bursa, spleen, and thymus) among groups ($p > .05$).

**Humoral immunity and cell-mediated immunity**

The effect of feeding diets containing rancid oil and supplementation with $\alpha$-Toc and OPLE on humoral immunity and Cell-mediated immunity are shown in Table 4. Birds receiving oxidised oil showed significantly lower titres for IgG, IgM, and total antibody titres against the SRBC in both primary and secondary challenges than those receiving fresh oil.
(p < .05). The highest amount of IgG, IgM, and total antibody titres against the SRBC in both primary and secondary challenge were observed in birds fed diets containing fresh oil and diets with oxidised oil and supplementation with a mixture of OPLE and α-Toc. The cell-mediated immune response determined by the inoculation of PHA-P was not significantly affected by the type of oil and supplement in experimental diets after 12 and 24 h. The increase in the toe web thickness between birds fed OPLE-supplemented diets was not statistically significant and was only numerically greater compared to diets without extracts (p > .05).

### Plasma lipid and lipid peroxidation

As shown in Table 5, the dietary containing oxidised oil significantly increased the MDA content in thigh meat and inversely reduced the activity of GPx and SOD antioxidant enzymes in the blood, although a reduced trend only was observed for GPx activity and was not statistically significant (p = .072). The addition of an antioxidant to diets increased GPx and SOD activity in the blood of bird chickens compared with birds fed diets without antioxidants and contains low-quality oil. Supplementation with antioxidants significantly decreased TBARs values in the thigh meat of broilers. The lowest MAD concentration was observed

### Table 3. Effect of oil quality and antioxidant *Otostgia persica* leaf extract (OPLE) and vitamin E (α-Toc) on slaughter performance of broilers.

|                      | Carcase dressing percentage, % | Breast muscle content of carcasse, % | Abdominal fat content of BW, % | Liver content of BW, % | Bursa content of BW, % | Spleen content of BW, % | Thymus content of BW, % |
|----------------------|--------------------------------|-------------------------------------|--------------------------------|------------------------|------------------------|------------------------|------------------------|
| Fresh oil            | 70.250a                         | 27.120                              | 0.350a                         | 2.432                  | 0.137                  | 0.163                  | 0.615                  |
| Oil oxidised (OO)    | 68.890b                         | 26.450                              | 0.425a                         | 2.224                  | 0.122                  | 0.148                  | 0.536                  |
| OO + 200 α-Toc       | 69.255ab                        | 26.780                              | 0.375ab                        | 2.320                  | 0.126                  | 0.152                  | 0.572                  |
| OO + 100 OPLE        | 69.440b                         | 26.840                              | 0.344b                         | 2.319                  | 0.129                  | 0.160                  | 0.574                  |
| OO + 200 OPLE        | 69.590b                         | 26.890                              | 0.343b                         | 2.333                  | 0.129                  | 0.159                  | 0.574                  |
| OO + 200 α-Toc +100 OPLE | 70.110ab                      | 26.990                              | 0.349b                         | 2.329                  | 0.134                  | 0.161                  | 0.579                  |
| OO + 200 α-Toc +200 OPLE | 70.090ab                     | 26.970                              | 0.346b                         | 2.336                  | 0.134                  | 0.159                  | 0.577                  |
| SEM                  | 1.160                           | 0.320                               | 0.012                          | 0.113                  | 0.010                  | 0.010                  | 0.022                  |
| p Value              | .039                            | .089                                | .23                            | .128                   | .253                   | .145                   | .019                   |

Mean values with different superscripts are significantly different (p < .05); OO: oxidised oil without antioxidant; SEM: standard error of the means.

### Table 4. Effect of oil quality and antioxidant *Otostgia persica* leaf extract (OPLE) and vitamin E (α-Toc) on humeral immunity and cell-mediated immunity.

|                      | Primary antibody response, log2 | Secondary antibody response, log2 | Swelling response, mm |
|----------------------|--------------------------------|----------------------------------|-----------------------|
|                      | IgG                            | IgM                              | Total                |
| Fresh oil            | 2.300a                         | 4.500a                           | 6.800a               |
| Oil oxidised (OO)    | 2.000b                         | 3.600d                           | 5.600b               |
| OO + 200 α-toc       | 2.100b                         | 3.900b                           | 6.000b               |
| OO + 100 OPLE        | 2.000b                         | 4.200b                           | 6.200b               |
| OO + 200 OPLE        | 2.000b                         | 4.250b                           | 6.250b               |
| OO + 200 α-Toc +100 OPLE | 2.000b                       | 4.300ab                          | 6.300ab              |
| OO + 200 α-Toc +200 OPLE | 2.100b                       | 4.500a                           | 6.600a               |
| SEM                  | 0.191                          | 0.311                             | 0.389                |
| p Value              | .041                           | .021                              | .024                 |

Mean values with different superscripts are significantly different (p < .05); OO: oxidised oil without antioxidant; SEM: standard error of the means.

### Table 5. Effect of oil quality and antioxidant *Otostgia persica* leaf extract (OPLE) and vitamin E (α-Toc) on the plasma lipid and lipid peroxidation.

|                      | GPx activity, U/ml| SOD activity, U/ml | MDA content, mg/kg meat | Triglyceride, mg/dl | Cholesterol, mg/dl | HDL, mg/dl |
|----------------------|-------------------|-------------------|------------------------|---------------------|-------------------|----------|
| Fresh oil            | 129.160           | 145.160           | 0.359d                 | 101                 | 119               | 83       |
| Oil oxidised (OO)    | 125.920           | 135.960           | 0.426                  | 113                 | 129               | 73       |
| OO + 200 α-toc       | 127.140           | 137.220           | 0.388b                 | 109                 | 128               | 74       |
| OO + 100 OPLE        | 128.340           | 139.290           | 0.411ab                | 109                 | 127               | 74       |
| OO + 200 OPLE        | 128.140           | 139.880           | 0.397b                 | 106                 | 125               | 76       |
| OO + 200 α-Toc +100 OPLE | 128.010         | 143.080           | 0.367                  | 107                 | 124               | 75       |
| OO + 200 α-Toc +200 OPLE | 128.790         | 142.930           | 0.367                 | 106                 | 122               | 77       |
| SEM                  | 11.266            | 13.025            | 0.024                  | 2.198               | 3.215             | 1.253    |
| p Value              | .072              | .025              | .010                   | .235                | .311              | .167     |

Mean values with different superscripts are significantly different (p < .05); OO: oxidised oil without antioxidant; SEM: standard error of the means; GPx: Glutathione peroxidase; SOD: Duperoxide dismutase; MDA: Malondialdehyde; HDL: High-density lipoprotein.
in birds fed a diet containing 100 α-Toc combinations with 100 α-Toc and 200 OLP compared to diet oxidised oil without antioxidant (p < .05). No significant difference was observed in cholesterol, triglyceride and HDL contents among groups (p > .05). The lowest levels of cholesterol and triglycerides and the highest HDL were observed in the bird-fed fresh oil.

Discussion

Performance

In poultry diets, vegetable oils are commonly used in the diet to increase energy. However, due to their high polyunsaturated fatty acids, these oils are very prone to oxidation. The result of the negative effects of oxidation on the performance of broiler chickens has been inconsistently reported. This may be related to differences in the type of oil and degree of oil oxidation (Rebecca et al. 2014; Tan et al. 2019). Similar results were reported by Mazur-Künsirek et al. (2011). Birds whose diets were supplemented with vitamin E combined with polyphenols had higher final body weights and body weight gains. Nevertheless, Açıkgoz et al. (2011) reported that the applied antioxidants did not improve the growth performance of broiler chickens fed diets containing oxidised sunflower oil.

Some reports suggest that antioxidant compounds have the potential to reduce the harmful effects of oxidation. In the present study, oxidative stress was stimulated by the addition of oxidised sunflower oil. The oxidised oil used in the diet severely reduced the birds’ performance. Depression in the performance of broilers fed oxidised oils may be due to free radicals react with proteins, lipids and vitamins and, nutrients (Saleh et al. 2018). According to our laboratory analysis, the main components that leave Otostgia persica extract (OPLE) were flavonoids and polyphenols (flavonoid concentration is 67 mg per gram of extract, and the polyphenol concentration is 47.32 mg per gram of extract). Leaf extract Otostgia persica and α-Toc supplemented groups had improved growth performance which may be due to their antioxidants effect that prevents the toxic effect of free radicals. Saleh et al. (2017) reported that the antioxidant pomegranate peel extract administered individually or in combination with α-toc, was more effective than a vitamin E dose of 200 mg kg⁻¹. In this regard, Smet et al. (2008) showed that dietary natural antioxidant extracts were less effective than the treatment with synthetic antioxidants combined with α-Toc for protecting against oxidation. The results in the study showed that the addition of a mixture of α-Toc and OPLE of antioxidants improved the growth performance of broilers fed oxidised sunflower oil.

Slaughter performance

Percentage of muscle and fat are the main traits of poultry carcass yield, while excessive fat deposition is a problem in the current poultry industry. In the present study, OPLE contributed to an increase in the carcass dressing percentage of broilers fed oxidised sunflower oil, which could state that phenolic compounds and flavonoids protective effects against the harmful consequences of peroxidation products. Shen et al. (2019) reported that diet supplemented bamboo leaf contains flavonoids could improve carcass yield in broilers. Results of dressing percentage are quite comparable to those reported earlier. Mazur-Künsirek et al. (2019) did not observe differences in the percentage content of liver and breast muscle of broilers whose diets contained rancid oil and were supplemented with vitamin E or polyphenols. Shen et al. (2019) showed that abdominal fat deposition was linearly decreased with bamboo leaf extract inclusion. These findings are similar to the present results. Zdunczyk et al. (2002) did not report differences in the carcass abdominal fat content of turkeys fed oxidised oil. According to Li et al. (2009), hawthorn leaf flavonoids have been reported to reduce fat deposition in broilers. Also stated that flavonoids have been shown to regulate deposition and fat metabolism in animals. Phyogenic flavonoids probably have a similar structure and function (Shen et al. 2019). According to the findings, it is suggested that phenolic compounds in OPLE may contribute to reducing abdominal fat.

The relative weight of lymphoid organs is often used to predict the immune status of an animal. The bursae of Fabricius and thymus were in good condition with a satisfactory size, indicating a functioning immune system and absence of a health challenge (Das et al. 2020). Although the study has reported positive effects on immune organ development with supplementation natural additives (Li et al. 2009), in this research, despite the improvement in the relative weight of the lymphatic organs, no statistical difference was observed between chickens fed different antioxidants. However, the increased antibody titres after SRBC in chickens is probably reflected in the relative weight gain these organs.

Humeral immunity and cell-mediated immunity

Diet manipulation is one of the reported mechanisms to improve the immune response in domestic animals.
Some compounds can enhance the proliferation of beneficial bacteria, while reducing the abundance of pathogenic ones through their bactericidal and bacteriostatic activities, thus indirectly improving host immunity and health (Gessner et al. 2017). The flavonoids and phenolic compounds are known for their antioxidant, anti-inflammatory, antiviral, and antifungal properties (Górniak et al. 2019). Since leaf O. persica is rich in flavonoid and phenolic compounds such as X quercetin, morin, kaempferol, Trans-Cinnamic acid, and Caffeic acid, in the present study, probably feeding with OPLE significantly increased the primary and secondary immune response of chickens.

Mirakzehi et al. (2020) reported that feeding the broilers with diets containing the oxidised oil decreased the production of antibodies during the primary and secondary challenge against an SRBC. As regard, high antioxidant capacity can scavenging free radicals generated during stress, factors such as bioavailability, type of compound, and a number of biological compounds are important to increase the immune response of broilers (Eynj et al. 2014; Hosseini-Vashan and Raei-Moghadam 2019).

In the current study, the broilers did not have a rapid immunological response compared with other studies that have also evaluated CBH response to phytohemagglutinin in birds. Some researchers report that chicken’s CBH response improved with the Withania somnifera supplement, which contains flavonoids (Malik et al. 2007; Mirakzehi et al. 2020). The interdigital reaction to phytohemagglutinin involves the stimulation of T cells; as the thymus is the organ responsible for the maturation of these cells (Moore and Siopes 2002). The absence of a significant difference in relative thymus weight presented in this study is consistent with the response to phytohaemagglutinin.

**Plasma lipid and lipid peroxidation**

Variation in the activity of antioxidant enzymes is observed according to the conditions of different experiments in response to rancid oil treatment. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) are antioxidant enzymes that do not only play a fundamental but indispensable role in the antioxidant protective capacity of biological systems against free radical attack. Endogenous protection against oxidative stress is achieved by enzymes that catalytically remove free radicals and other reactive species (Saleh et al. 2018). Antioxidant defense systems in the body include the consumption of antioxidants in the diet (natural or artificial) and antioxidant enzymes present in the biological system. Increasing the intake of diets rich in antioxidants may maintain high levels of these enzymes SOD, CAT and, GPX in the cell (Saleh et al. 2018). On the other hand, feeding oxidised oils that contain high levels of free radicals may increase the number of free radicals in the animal biological system after consumption (Mazur-Küsnierek et al. 2019). Our results showed that an antioxidant in the feed increase GPX in the broiler fed with the diet of oxidised oil compared to the diet without supplementation, although this difference was not statistically significant. Dietary supplementation with vitamin E with 100 or 200 OPLE influenced SOD activity in the blood of broiler chickens fed low-quality sunflower oil. Dietary antioxidants can neutralise oxidative stress. However, non-nutrient antioxidants in plant foods can increase the power of the antioxidant system and the protective effect of oxidative stress. Some feed components have been identified to possess antioxidant properties; they have some specific activities and usually work synergistically to improve the antioxidant capability of the body (Zang et al. 2000).

Measurement of the levels of ROS production, metabolites of lipid peroxidation such as MDA may give information about the antioxidant status. The findings show broilers fed oxidised oil with antioxidants had less accumulation of MAD in thigh meat. Broilers fed with diets containing low-quality rapeseed oil, supplemented with vitamin E and polyphenols or polyphenols alone, were lower TBARS values in the meat of birds fed with sunflower oil. Dietary antioxidants can neutralise oxidative stress. However, non-nutrient antioxidants in plant foods can increase the power of the antioxidant system and the protective effect of oxidative stress. Some feed components have been identified to possess antioxidant properties; they have some specific activities and usually work synergistically to improve the antioxidant capability of the body (Zang et al. 2000).

Broilers-fed diets containing oxidised sunflower oil were characterised by lower cholesterol and triglyceride activity and higher HDL in the blood. Supplementing diets with antioxidants did not cause any difference with chickens fed with fresh oil. While some reports had no significant effect on rats (Juskiewicz et al. 2000), other researchers have
reported that oxidised dietary oil lowers cholesterol and triglyceride levels, and adding vitamin E in the diet does not affect (Eder and Kirchgessner 1998)). Increased cholesterol excretion, impaired hepatic cholesterol absorption, and increased plasma thyroxine levels may lower cholesterol (Bayraktar et al. 2011). Iritani et al. (1980) suggested that peroxidation products in oxidised oils may accumulate in liver microsomes and mitochondria of broilers impairing cholesterol metabolism.

**Conclusions**

Performance, humoral immunity, plasma oxidative status and lipid oxidation in broilers fed with oil oxide were affected by feeding natural antioxidant extracts. According to the results, it can be suggested that 100 or 200 mg/kg Otostgia persica leaf extract along with vitamin E have the ability to reduce the negative effects of a diet containing oxidised oil in broilers.

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