

Effects of artichoke (*Cynara scolymus* L.) extract on antioxidant status in chicken thigh meat

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Key words:

Artichoke extract, broiler chicken, glutathione peroxidase, lipid stability

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Received: 3 November 2015

Accepted: 18 January 2016

Abstract:

BACKGROUND: Artichoke extract (AE), containing natural antioxidant compounds, can be considered as a good source of antioxidant potential. **OBJECTIVES:** The aim of this study was to evaluate antioxidant abilities of AE on broiler meat quality. **METHODS:** 200 Ross chicken broilers were divided into five equal groups and received 100, 200, 300, and 500 mg/liter of AE in drinking water and pure water in the control group, respectively. Antiradical activity and phenolic content of AE were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and gallic acid measurement before adding extract into drinking water. The broilers received AE extract from 21-35 day of growing phase and the samples from thigh muscles were taken for biochemical analysis in the 42 day of the growing phase. **RESULTS:** Antiradical activity of AE was 35% and phenolic content was 3.3 g/100g of dry extract. Regarding antioxidant enzymes, such as glutathione peroxidase (GPx) and catalase (CAT), the AE with dosage of 200 mg/l indicated maximum antioxidant ability compared to the other groups ($p < 0.05$). Supplementation of AE 200 mg/l also demonstrated the lowest GPx and CAT activities, compared to the control and AE 300 mg/l groups ($p < 0.05$). Regarding performance weight gain, average daily weight gain, percentage of weight gain in 21 to 35 as well as final weight were similar in control and AE-received groups and AE indicated similar effect for all the treatments. **CONCLUSIONS:** This study showed that administration of 200 mg/l AE in drinking water during growing phase decreased GPx and CAT activities in chicken meat presumably due to down-regulation of gene expression for antioxidant enzymes.

Introduction

It is well-known that lipid peroxidation is one of the main factors limiting the quality and acceptability of meat (Morrissey et al., 1998). Usually, lipid peroxidation occurs when antioxidant defenses (non-enzymatic and enzymatic antioxidants) are overcome by peroxida-

tion mechanisms and lipid peroxidation refers to oxidation of lipids in the presence of oxygen (Gutteridge, 1995). During the oxidation of lipids, unsaturated fatty acids are oxidized in presence of reactive free radicals, such as superoxide anion, hydroxyl radical, and hydrogen peroxide. Unsaturated fatty acids exposed to oxygen produce compounds, such as

malondialdehyde (MDA), therefore, MDA has been widely used as an indicator of lipid peroxidation in meat (Alirezai et al., 2012). The antioxidant system contains non-enzymatic and enzymatic antioxidants (Sies, 1997). Glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are enzymatic antioxidants. SOD converts superoxide anion into hydrogen peroxide (H₂O₂). Then, CAT removes hydrogen peroxide by converting it into water and oxygen. GPx also removes hydrogen peroxide by oxidizing the glutathione into its oxidized form (Gutteridge, 1995), and also converts H₂O₂ into water. Natural components including phenolic acids, flavonoids, polyphenols, retinoids, tocopherols, and ascorbic acid act as non-enzymatic antioxidant (Chu et al., 2000).

A number of byproducts as sources of natural antioxidant compounds have been studied, such as Oregano, Sage, and Rosemary on meat quality of broilers (Bustoglou et al., 2002; Lopez-Bote et al., 1998). Artichoke (*Cynarascolymus* L.), as a rich source of non-enzymatic antioxidants, is a potential good source of antioxidant activity since it contains poly phenolic compounds, with mono- and dicaffeoylquinic acids as the major chemical components (Table 1). The most well-known caffeoylquinic acid derivative identified in artichoke is cynarin. The other phenolics are the flavones apigenin and luteolin, and the anthocyanidins cyanidin, peonidin, and delphinidin have also been found in artichoke (Lattanzio et al., 2009).

The aim of the present study was to evaluate antioxidant effect of AE in drinking water on meat quality of broilers. This effect was evaluated by antioxidant enzyme activities, such as GPx and CAT and lipid peroxidation marker as shown by MDA content.

Materials and Methods

Birds and treatments: A total of 200 one-day-old broiler chickens (Ross) obtained from

a commercial hatchery (Dorbal Company, Bo-roojerd, Iran) were used in the present study. The chickens received 5% sugar and lemon juice mixture for 15 hours as soon as they arrived to the hall. The chickens were fed and reared according to Ross guidelines with similar diets throughout the experiment. All chickens were fed pre-starter (0.0-10 days), starter (11-21 days), grower (22-35 days), and finisher (36-42 days) diets. The ingredients and nutrient contents of the diets are shown in Table 2. The chickens had freely accessed to drinking water and exposed to 23 hours of light and 1 hour of darkness, daily. Vaccinations were performed against Newcastle and Bronchitis on the 4th and 18th days of the experiment. From day 21, chickens were randomly allocated into five treatments. Each treatment included 40 birds (male and female equally). AE obtained from Barij Essance Company (Kashan, Isfahan, Iran) (99% purity) was added to the chickens' drinking water from day 21 to day 35 of the experiment (Nateghi et al., 2013).

Drinking water was either with 100 (AE100), 200 (AE200), 300 (AE300), and 500 (AE500) mg per liter of AE or without AE (control group). At the end of the experiment (42 d), 40 chickens (8 chickens per each treatment) were randomly selected and slaughtered. Meat samples were taken from thigh muscles and were stored at -80°C for one month. Animal care, slaughter, and sampling methods used in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Lorestan University.

Antiradical activity and phenol content determination: Radical scavenging activity of AE was determined using DPPH method according to Burits and Bucar (2000). In brief, different concentrations of ascorbic acid (0.0, 2, 4, 6, 8 and 10 µmol) were taken in different test tubes. The volume was adjusted to 100 µl by adding methanol. For drawing curve calibration, five ml of a 0.1 mM methanolic solution of DPPH was added to the standard

tubes and shaken vigorously. The tubes were incubated at 27°C for 30 min. Similarly for AE samples, 50 µl and five ml of a 0.1 mM methanolic solution of DPPH were mixed and shaken vigorously. The control was prepared as above without addition of AE extract. The absorbance of the standards and AE samples (three samples) were measured at 517 nm by a spectrophotometer (52000 UV Model; WPA; Cambridge, UK). Radical scavenging activity was expressed as the percentage of inhibition and was calculated using the following formula:

$$\% \text{ Radical scavenging activity} = (\text{Control Absorbance} - \text{Sample Absorbance} / \text{Control Absorbance}) \times 100.$$

The concentration of phenolic compounds in AE extract was determined according to the Singh et al. (2002). The AE (1 g) was dissolved in 100 ml distilled water (w/v). Then 20 µl of the dissolved AE were mixed with 1.58 ml water and 100 µl of Folin-Ciocalteu reagent as well as 300 µl of sodium carbonate solution (7%). The mixture were held for 30 min at the dark condition in room temperature. The absorbance was measured at 765 nm by a spectrophotometer. The estimation of phenolic compounds in AE was determined in triplicate and the results were averaged. Results were expressed as gallic acid equivalents. The free radical scavenging potential of Artichoke extract (AE) was 35% and the phenolic content of AE was 3.3 ± 0.2 g per 100 g of dry extract.

Muscle preparation for protein measurement, MDA detection and enzyme assay: Thigh samples were thawed and manually homogenized via liquid nitrogen in cold phosphate buffer (pH 7.4, 0.1 M) containing 5mM EDTA, the debris removed by centrifugation at 5000g for 10 min (Centrifuge 5415 R; Rotofix 32 A, Germany) (Alirezai et al., 2012). Supernatants were recovered and used for measurement of protein, antioxidant enzyme activities and MDA concentration.

Protein measurement: Protein content of

the tissue homogenates was determined using the colorimetric method of Lowry with bovine serum albumin as a standard (Lowry et al., 1951).

Measurement of glutathione peroxidase (GPx) activity: The activity of Glutathione peroxidase (GPx) was evaluated with Randox®GPx detection kit according to the manufacturer's instructions as described previously (Alirezai et al., 2012). GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance was measured spectrophotometrically against blank at 340 nm (52000 UV Model; WPA; Cambridge, UK). One unit (U) of GPx was defined as 1 µmol of oxidized NADPH per min per milligram of tissue protein. The GPx activity was expressed as unit per milligram of tissue protein (unit/mg protein).

Measurement of catalase (CAT) activity: Tissue catalase activity was assayed using the method described by Cliaborne (1986) and reported by Alirezai et al. (2012). In brief, 900 µl of potassium phosphate (5 mM), 50 µl H₂O₂ (19 mM), and 50µl of homogenized tissue were added into a tube. The reaction was initiated by adding H₂O₂ and absorbance change was measured at 240 nm for 30 seconds (52000 UV Model; WPA; Cambridge, UK). The molar extinction coefficient for H₂O₂ is 43.6/Mcm. The CAT activity unit (U) was defined as 1 µmol of H₂O₂ consumption per min per milligram of tissue protein (U/mg protein).

Measurement of MDA content: The amount of lipid peroxidation was indicated by the content of thiobarbituric acid reactive substances (TBARS) in the homogenates. Tissue MDA was determined by following production of thiobarbituric acid reactive substances as described previously (Subbarao et al., 1990). In short, 40 µl of supernatant was added to 40

μl of 0.9% NaCl and 40 μl of deionized H₂O, resulting in a total reaction volume of 120 μl . The reaction was incubated at 37°C for 20 min and stopped by the addition of 600 μl of cold 0.8 mol/l hydrochloric acid, containing 12.5% trichloroacetic acid. Following the addition of 780 μl of 1% TBA, the reaction was boiled for 20 min and cooled at 4°C for 1 hour. In order to measure the amount of TBARS produced by the homogenate, the cooled reaction was spun at 1500 g in a microcentrifuge for 20 min and the absorbance of the supernatant was spectrophotometrically read at 532 nm, using an extinction coefficient of $1.56 \times 10^5 / \text{mol cm}$ (52000 UV Model; WPA; Cambridge, UK). The blanks for all of the TBARS assays contained an additional 40 μl of 0.9% NaCl, instead of homogenate as just described. MDA results were expressed as nmol per milligram of tissue protein (nmol/mg protein).

Statistical analysis: This experiment was conducted in a complete randomized block design. The data obtained from the experiment were statistically analyzed using GLM procedure by SAS software (SAS, 2003). Significant differences among the control and treatment groups were compared using Duncan's multiple range tests with a 5% probability (Duncan, 1955). REG procedure was used to make a correlation between AE and content of TBARS ($r = -0.14$, $p = 0.37$).

Results

Effect of AE on GPx and CAT activities in broiler thigh muscles was shown in Figures 1 and 2, respectively. The results showed that AE significantly decreased the GPx activity in AE100, AE200, and AE500 mg/l, compared to control group ($p < 0.05$). In respect to antioxidant enzymes, such as GPx and CAT, the AE with dosage 200 mg/l indicated maximum antioxidant ability compared to the other groups. AE 200 mg/l also demonstrated the lowest GPx and CAT activities when compared to the con-

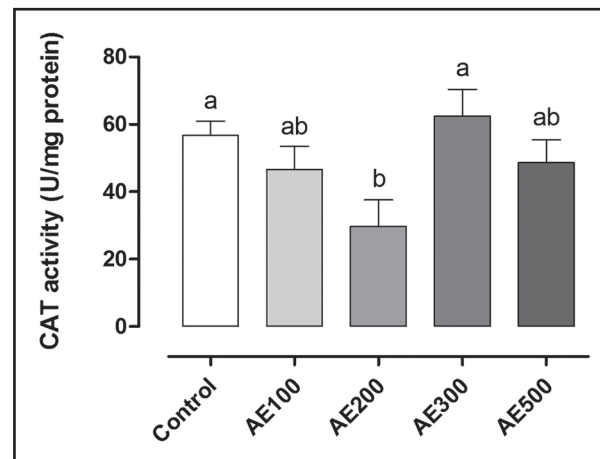


Figure 1. Means \pm SD of glutathione peroxidase (GPx) activity in broiler thigh meat in control, AE 100 (100 mg/l), AE 200 (200 mg/l), AE 300 (300 mg/l) and AE 500 (500 mg/l) of Artichoke extract in liter drinking water. a,b means with different superscripts differ significantly ($p < 0.05$). AE decreased significantly GPx activity in dosages 100, 200 and 500 mg/l in comparison with control group ($p < 0.05$).

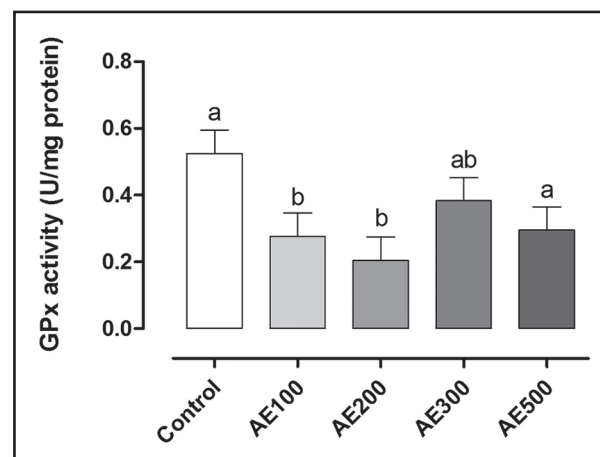


Figure 2. Means \pm SD of catalase (CAT) activity in broiler thigh meat in control, AE 100 (100 mg/l), AE 200 (200 mg/l), AE 300 (300 mg/l) and AE 500 (500 mg/l) of Artichoke extract in liter drinking water. a,b means with different superscripts differ significantly ($p < 0.05$). AE 200mg/l demonstrated the lowest CAT activity. AE decreased significantly CAT activity in AE200 when compared with control, AE300 and AE500 groups ($p < 0.05$).

control and AE 300 mg/l groups ($p < 0.05$).

Supplementation of 100-500 mg/l of AE decreased MDA concentration as a lipid peroxidation marker (Fig. 3). Although the AE-treated groups indicated lower lipid peroxidation marker than the control group, the difference tendency was decreased ($p = 0.08$).

Water Consumption was similar in control and AE-treated groups ($p > 0.05$), the extract

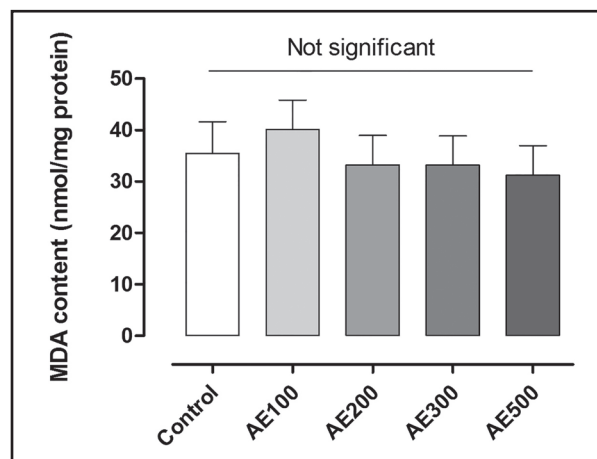


Figure 3. Means \pm SD of malondialdehyde (MDA) content in broiler thigh meat in control, AE 100 (100 mg/l), AE 200 (200 mg/l), AE 300 (300 mg/l) and AE 500 (500 mg/l) of Artichoke extract in liter drinking water. There was not significant differences among control and AE-treated groups ($p>0.05$).

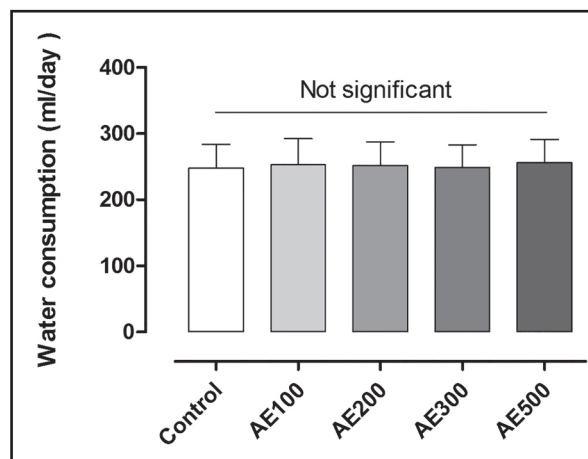


Figure 4. Means \pm SD of water consumption (ml/day/chicken) in 21 to 35 d of growing phase in control, AE 100 (100 mg/l), AE 200 (200 mg/l), AE 300 (300 mg/l) and AE 500 (500 mg/l) of Artichoke extract in liter of drinking water. There was not significant differences among control and AE-treated groups ($p>0.05$).

Table 1. Mono and dicaffeoylquinic acids in 100 gram of Artichoke extract. (*) It was taken with permission from Lattanzio et al., 1978; J Food Chem., 24:37-50.

| Caffeoylquinic acid derivatives* | mg/100 g dry weight AE |
|----------------------------------|------------------------|
| 1-o-Caffeoylquinic acid | 38.14 |
| 3-o-Caffeoylquinic acid | 47.22 |
| 4-o-Caffeoylquinic acid | 267.02 |
| 5-o-Caffeoylquinic acid | 1544.91 |
| ----- | |
| 1,3-o-Dicaffeoylquinic acid | 61.24 |
| 1,4-o-Dicaffeoylquinic acid | 142.91 |
| 4,5-o-Dicaffeoylquinic acid | 224.56 |
| 3,5-o-Dicaffeoylquinic acid | 347.05 |
| 1,5-o-Dicaffeoylquinic acid | 837.01 |
| 3,4-o-Dicaffeoylquinic acid | 428.71 |

had no effect on water consumption in broilers (Fig. 4).

Regarding growth performance, weight gain, average daily weight gain, percentage of weight gain in 21 to 35 d as well as final weight were similar in control and AE-treated groups ($p>0.05$), and AE indicated similar effect for all the treatments (Table 3).

Discussion

In the present study, the free radical scavenging potential of AE was 35%. In this regard,

Wang et al. (2003) reported a wide range of relative activities toward DPPH (8.3- 49.7%), which were correlated with their polyphenol contents (1.7-9.86%) of the different artichoke samples. Moreover, Llorach et al. (2002) showed a relatively high free radical scavenging activity and capacity to inhibit lipid peroxidation in artichoke byproducts. The phenolic values estimated for the edible portion of artichoke give a range of 31-58 mg/g dry matter (Lattanzio and Sumere van, 1987). Our results showed that phenolic content of AE was 3.3 ± 0.2 g per 100 g of dry extract. In this regard, Schutz et al. (2004) reported that AE had 1.2 g phenolic contents per 100 g dry matter.

Thus, in the present study, AE had relatively high content of phenolic compounds compared to the previous reports. Phenolic compounds are the secondary metabolites and one of the best sources of natural antioxidants (Balasundram et al., 2006). Cynarin is the main phenolic compound in artichoke (Llorach et al., 2002). Other flavonoids, such as the flavones (apigenin and luteolin) and anthocyanidins (cyanidin, peonidin, and delphinidin) have been found in Artichoke (Lattanzio et al., 2009). The scavenging activity against ROS and antiradical activity of Artichoke was lin-

Table 2. Ingredient composition (%) and nutrient content of the chicken's diet (0-42 day). ⁽¹⁾ One Kg Concentrate = Carbonate Calcium 174.06 g, Dicalcium phosphate 313.63 g, DL-Methionine 49.26 g, L-Lysine 21.35 g, Vitamin premix 41.05 g, Mineral premix 41.05 g, Salt 57.47 g, Soybean powder 240.56 g, Antioxidant 41.05 g, Colin colorid 20.53 g.

| | Pre-starter (0-10 d) | Starter (11-21 d) | Grower (22-35 d) | Finisher (36-42 d) |
|--------------------------|----------------------|-------------------|------------------|--------------------|
| Corn (%) | 45.3 | 47.9 | 46.7 | 47.8 |
| Soybean meal (44% CP) | 34.8 | 33.9 | 26.9 | 23.6 |
| Wheat (%) | 7 | 12 | 20 | 22 |
| Maize Gluten meal (%) | 6 | - | - | - |
| Concentrate ¹ | 6.9 | 6.2 | 6.4 | 6.6 |
| Calculated composition | | | | |
| ME (Mcal/kg) | 2.96 | 2.88 | 2.95 | 2.99 |
| Crude protein (%) | 24.3 | 21.2 | 18.8 | 17.6 |
| Calcium (%) | 1.10 | 1.00 | 1.00 | 1.00 |
| Available phosphorus (%) | 0.55 | 0.50 | 0.50 | 0.50 |
| Sodium (%) | 0.22 | 0.21 | 0.18 | 0.18 |
| Lysine (%) | 1.29 | 1.09 | 0.95 | 0.88 |
| Methionine (%) | 0.59 | 0.51 | 0.45 | 0.43 |
| Met+Cys (%) | 0.93 | 0.80 | 0.72 | 0.68 |
| Linoleic acid (%) | 1.27 | 1.30 | 1.29 | 1.30 |
| Tryptophan (%) | 0.24 | 0.23 | 0.20 | 0.18 |

Table 3. The Effect of Artichoke Extract (AE) on Weight Gain, Average Daily Gain and Weight Gain (%) in 21-35 d in broiler chickens. There were not significant differences among the groups for weight gain, average daily weight gain, percent of weight gain in 21-35 days and final weight of the treatments (p>0.05).

| Treatment | Period | Control | AE 100 | AE 200 | AE 300 | AE 500 | SEM | p-Value |
|------------------------|-----------------------------------|---------|---------|---------|---------|---------|------|---------|
| Intel Weight (g) | | 47.190 | 46.610 | 45.160 | 45.360 | 45.970 | 0.34 | 0.29 |
| Weight Gain (g) | 1 to 21 (Starter) | 638.14 | 649.94 | 654.14 | 656.32 | 662.08 | 4.37 | 0.54 |
| | 21 to 42 (Grower) | 1505.23 | 1485.28 | 1468.05 | 1503.23 | 1462.05 | 9.43 | 0.51 |
| | 1 to 42 (Total) | 2143.36 | 2135.22 | 2122.19 | 2159.54 | 2124.13 | 9.50 | 0.76 |
| | 21 to 35 (Experimental Period) | 744.73 | 721.93 | 740.00 | 722.88 | 712.13 | 5.60 | 0.33 |
| Average Daily Gain (g) | 1 to 21 (Starter) | 30.38 | 30.94 | 31.14 | 31.25 | 31.52 | 0.21 | 0.54 |
| | 21 to 42 (Grower) | 71.677 | 70.727 | 69.907 | 71.582 | 69.621 | 0.45 | 0.51 |
| | 1 to 42 (Total) | 51.032 | 50.838 | 50.528 | 51.417 | 50.574 | 0.23 | 0.76 |
| | 21 to 35 (Experimental Period) | 53.195 | 51.566 | 52.857 | 51.634 | 50.866 | 0.40 | 0.33 |
| Weight Gain (%) 21-35 | Total Weight | 34.75 | 33.82 | 34.89 | 33.47 | 33.53 | 0.69 | 0.46 |
| | Final Weight | 34.003 | 33.103 | 34.163 | 32.791 | 32.829 | 0.68 | 0.47 |
| Final Weight (g) | | 2190.55 | 2181.83 | 2167.35 | 2204.90 | 2170.10 | 9.54 | 0.76 |

early correlated to its phenolic concentration (Llorach et al., 2002). In fact, AE is known as a natural non-enzymatic antioxidant (Balasundram et al, 2006) because of its high phenolic contents.

As previously mentioned, for evaluation of the effects of AE on antioxidant status and meat quality, some antioxidant enzymes and

the concentration of MDA as a lipid peroxidation marker were measured. GPx and CAT are two key antioxidant enzymes that can decompose hydrogen peroxide to water (Kheradmand et al., 2010). With respect to antioxidant enzyme activities, our results showed a significant decrease in GPx and CAT activities in AE 200 mg/l-treated broilers. In this regards,

Bobek et al. (1998) performed a hypercholesterolemic study in which rats were fed with a diet supplemented with tomato, apple, or grape pomaces. In all of the supplemented diets, decreases were observed in SOD, CAT, and GPx enzymes (range 36-56%). Dietary supplementation with thyme oil and thymol, rich in antioxidant terpenoids, also prevented the age-induced decline of GPx activity in rat brain (Youdim and Deans, 2000).

Moreover, Breinholt et al. (1999) observed activity of GPx and Glutathione reductase (GR) decrease after gavage administration of natural flavonoids to rats. Experimental administration of low molecular weight antioxidant (such as poly phenolic components) has also been shown to cause compensatory changes in enzymatic antioxidant defenses (Sohal and Allen, 1986). In this setting, Masella et al. (2005) have expressed that "Antioxidant Responsive Elements (AREs) are present in the promoter regions of many of the genes inducible by oxidative and chemical stresses". Thus, it appears that in the present study, consumption of AE (as source of phenolic compounds) was able to decrease activity of the GPx and CAT by the compensatory mechanism via AREs. This effect presumably is due to quenching of free radicals by phenolic compounds in AE-treated broilers compared to the control group (Chu et al., 2000).

It is well-known that superoxide radicals and peroxides are continuously produced within the cell by various subcellular reactions. The first defense of the cell to protect against oxidation is non-enzymatic antioxidants (Sies, 1997). If the non-enzymatic antioxidants are not adequate to cope with continuously produced free radicals in the cells, then enzymatic antioxidant comes through. Therefore, reduction in GPx activity in 100 and 200 mg /l AE-treated groups probably occurs due to high content of phenolic compound in these groups. Generally, compounds like flavonoides and vitamin C destroy free radicals and trap ROS

(Chu et al., 2000). Considering that AE is rich in flavonoides and vitamin C, AE might reduce the concentration of ROS in the thigh meat. Reduction in ROS consequently postpones the need for enzymatic antioxidants (i.e. GPx and CAT). On the other hand, the AE may cause a decrease in GPx activity by down regulation of gene expression in the thigh muscles during growing period in broiler chickens. Although we were not able to measure gene-expression of antioxidant enzymes, a previous report indicated a decrease in GPx activity by non-enzymatic antioxidants in broiler muscles and a negative relationship between GPx activity and tissue α -tocopherol levels and a positive relationship between MDA and antioxidant enzyme activity has been observed by Marschiello et al. (1999).

Regarding lipid peroxidation, MDA concentration decreased with AE treatments, however, the differences between groups were not significant. In this regards, Dobbau et al. (2014) demonstrated that the supplementation of Artichoke brackets did not induce any effect on MDA content in rabbit meat. Moreover, Llorch et al. (2002) reported that the extracts from artichoke by-products showed a high capacity to inhibit linoleic acid (LA) peroxidation and prevented lipid peroxidation. The MDA results, probably due to small sample size of broilers or less specific TBA method, is unable to determine differences among the groups as significant.

Herein, weight gain, average daily weight gain; percentage of weight gain in 21 to 35 d as well as final weight were similar in control and AE-treated groups. In this regard, Yildiz et al. (2006) concluded that the supplementation of Jerusalem artichoke as an inulin source with or without Vetch had no adverse effect on laying hen performance. Findings of the current study are in agreement with those of Dora et al (2005), which reported that treatment using Echinacea cob produced a significantly lower body weight compared to the control group,

and was contrary to those reported by Lee et al. (2004), in which dietary additions of cinnamon to broiler diet improved weight gain. It seems, our results are correct since, AE dose not induce diet consumption or feed conversion ratio to enhance weight gain in the broilers.

Taken together, it seems that administration of 200 mg/l AE in drinking water during growing phase decreases GPx and CAT activities in chicken meat, presumably due to down-regulation of gene expression for antioxidant enzymes. However, further studies should be performed to evaluate antioxidant properties of AE with large sample size of broilers and more specific methods, such as Vyncke (1975), for evaluation of lipid peroxidation in meat of broilers.

Acknowledgements

We are most grateful to Dr. Rasoulian in Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences for his kindly cooperation.

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