Korean J. Food Sci. An.
Vol. 33, No. 1, pp. 83~88(2013)
DOI http://dx.do.org/10.5851/kosfa.2013.33.1.83

ARTICLE

Effect of Dietary Supplementation of Wild Grape on the Antioxidative Potential of the Breast and Leg Meat of Broilers

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Abstract

This study investigated the effect of wild grape (*Vitis coignetiae*) dietary supplementation on the antioxidative potential and quality of the breast and leg meat of broilers. A total of 36 one-day-old male Cobb broiler chicks were obtained from a commercial hatchery, and randomly assigned to 9 pens with 4 birds per pen. Then, broilers were fed 3 different dietary supplementations, including 0%, 0.25%, or 0.5% wild grape, for 2 wks at the finishing period. After slaughtering, the total phenolic content, α,α'-diphenyl-β-picryl-hydrazyl (DPPH) radical scavenging activity, 2-thiobarbituric acid reactive substances (TBARS), and total cholesterol content of broiler breast and leg meat were measured. Higher total phenolic content was recorded in the leg meat of broilers fed the wild grape when compared with the control, while breast meat did not show any difference. Dietary supplementation of 0.25% and 0.5% wild grape significantly increased DPPH radical scavenging activity of both breast and leg meat. TBARS values of both breast and leg meat were decreased by supplementation of 0.5% wild grape during storage when compared to the control, except for the leg meat at day 7. However, there was no significant difference found in total cholesterol content in both breast and leg meat. The results indicate that the antioxidative potential of broiler meat is improved by supplementing the diet with wild grape.

Key words: wild grape, antioxidative potential, lipid oxidation, total cholesterol content

Introduction

Since ancient times, meat has played a vital role in the human diet, mainly as an excellent source of protein with high biological value. In addition, meat and meat products are important sources of fat, essential amino acids, minerals, and vitamins (Biesalski, 2005). Chicken meat is well recognized as a nutritional and healthy animal food, due to its relatively low fat, calorie, and cholesterol content, as well as its relatively high concentration of polyunsaturated fatty acids and protein content (Lee *et al.,* 2012; Liu *et al.*, 2012). However, oxidation in meat and meat products is a major problem in the meat industry (Kang *et al.*, 2012). Furthermore, chicken meat is more liable to lipid oxidation, and thereby to the development of "off-flavors," because it contains higher levels of unsaturated fatty acids compared to red meat. This issue presents a major problem with respect to retaining the quality of chicken meat for longer periods of time.

For this reason, antioxidants are added to fresh and processed meat to delay the onset of oxidative processes and loss of meat quality. In effect, antioxidants extend the storage period of meat by inhibiting the initiation or propagation of oxidative chain reactions (Xiong *et al.*, 1993). In general, natural antioxidants are preferentially used in many products in the food industry over synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which may be carcinogenic to consumers (Branen, 1975; Huang *et al.*, 2011; Reishe *et al.*, 1998). Therefore, research in this field is now primarily focused on natural antioxidants, which ultimately provide higher consumer acceptability, palatability, safety, and potential to improve the functional aspects of meat (Brenes *et al.*, 2008; Jo *et al.*, 2009; Jung *et al.*, 2010).

Recent studies have demonstrated the beneficial effect of plant originated phenolic compounds, which contain antioxidant potential via redox properties, in addition to having several beneficial actions on human health (Catherine *et al.*, 1997; Fraga *et al.*, 2010; Kafakaya, 2004). Grape and tea are of special interest as natural polyphenol

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antioxidants, due to their high phenolic compound content (Banon *et al.*, 2007). These polyphenols are well known for their beneficial functions, such as the inhibition of lipid oxidation, cancer, or microbial growth, in addition to the suppression of blood pressure or atherosclerosis, prevention of diabetes, and the reduction of allergenicity (Byun *et al.*, 2004; Catherine *et al.*, 1996; Fraga *et al.*, 2010; Mazza, 1998). Furthermore, the antioxidant potential of grape polyphenols has been confirmed in studies conducted using fish oil, frozen fish, cooked pork patties, and cooked turkey stored under retail display conditions (Banon *et al.*, 2007). Previous studies have demonstrated that the negative outcome of lipid oxidation in chicken meat may be reduced by supplementing the diet of live chicken with antioxidants, such as medicinal herb mix and grape pomace (Jung *et al.*, 2010).

Wild grape (*Vitis coignetiae*) is considered to be a rich source of mineral, dietary fiber, organic acids, water-soluble vitamins, and phenolic compounds, including resveratrol, epicatechin, catechin, procianidin, and anthocyanin (Cheon, 1999; Jeong *et al.*, 2007; Kim *et al.*, 2006). In a study conducted by Yoon and Kim (2007) on total phenolic compounds and antioxidant activity of fruits (including strawberry, kiwi, apple, and wild grape), wild grape contained the highest amount of phenolic compounds, and exhibited over twice the antioxidant activity of a grape cultivar (*Vitis labrusca*). In addition, feeding fermented wild grape by-products to pigs decreased the 2-thiobarbituric acid-reactive substances (TBARS) values and cholesterol content of pork, as well as increasing its color, taste, flavor, and juiciness (Park and Jung, 2005). Won (2009) reported that wild grape juice increased the antioxidative activity of the blood and liver of rats that were fed high oxidized lipids. Furthermore, Yong *et al.* (2012) recently reported an improvement in the quality and freshness of eggs from layers that were fed wild grape powder.

Thus, the objective of this study was to investigate the effect of providing wild grape as a dietary supplement on the antioxidative potential of broiler breast and leg meat.

Materials and Methods

Preparation of animals and samples

A total of 36 one-day-old male Cobb broiler chicks (Cobb strain) were obtained from a commercial hatchery, and randomly assigned to 9 pens with 4 birds per pen. During the entire experiment, broilers were housed under 24 h fluorescent lighting, standard temperature, humidity, and ventilation conditions, and had *ad libitum* access to water and food. The broiler chicks were fed a commercial broiler starter diet (0-6 d), then, fed grower diets (7-21 d). At the end of week 3, broilers were reassigned to 3 different dietary treatments, and reared for a further 2 wks. Each treatment had 3 replicates, with 4 broilers in each replicate (total n = 36). Dietary treatments consisted of a control (commercial finisher diet with no supplementation), and finisher diets supplemented with 0.25% (WG-0.25), and 0.5% wild grape powder (WG-0.5), respectively.

At the end of the feeding trial, broilers from each pen were slaughtered, and the feathers and entrails (evisceration) were removed from the carcasses. Breast and leg meat were then dissected from each carcass, vacuum packaged, and stored in a deep freezer at -50°C until the analysis.

Measurement of antioxidative activity

The meat samples (3 g) were homogenized (T25b, Ika Works (Asia), Sdn, Bhd, Malaysia) in 15 mL of distilled water at 16,000 rpm for 20 s. The samples were centrifuged (Union 32R, Hanil Co., Ltd., Korea) at 3,000 rpm for 10 min, and then filtered through Whatman No. 1 filter paper (Whatman Ltd., England). Chloroform (10 mL) was added to the homogenates to remove fat, and the mixture was shaken 3 times. The mixture was then separated into lipids and aqueous supernatant by centrifugation (Union 32R, Hanil Co., Ltd., Korea) at 3,000 rpm for 10 min. The supernatant was used for the analysis of total phenolic content and α,α'-diphenyl-β-picryl-hydrazyl (DPPH) radical scavenging activity.

Total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method (Subramanian *et al.*, 1965). A 0.1-mL aliquot was added to 0.2 mL Folin-Ciocalteu reagent and allowed to react for 1 min. Sodium carbonate (5%, 3 mL) was added to the mixture and vortexed. The mixture was then incubated at 23°C in the dark for 2 h. The absorbance was measured using a spectrophotometer (DU 530, Beckman Instruments Inc., USA) at 765 nm. The natural phenolics were quantified using a standard curve generated for gallic acid, and were expressed as gallic acid equivalents.

DPPH radical scavenging activity

DPPH radical scavenging activity was estimated according to the method described by Jung *et al.* (2010). A 0.2-mL aliquot was mixed with 0.8 mL distilled water

and 1 mL of 0.2 mM methanolic DPPH solution. For the control, the aliquot solution (0.2 mL) was replaced with distilled water. The mixture was vortexed and maintained at room temperature for 30 min. The absorbance of the solution was measured using a spectrophotometer (Beckman Instruments Inc., USA) at 517 nm. The percentage of DPPH radical scavenging was obtained from the following equation:

DPPH radical scavenging activity
= [1 − (absorbance of sample/absorbance of control)] × 100

2-Thiobarbituric acid-reactive substances (TBARS) value

TBARS values of meat samples were analyzed after storage at 4°C for 0, 3, and 7 d, according to the method of Jung *et al*. (2011). Nine milliliters distilled water and 50 μL BHT (7.2% in ethanol) were added to each meat sample (3 g). The mixture was homogenized (T25b, Ika Works (Asia), Sdn, Bhd, Malaysia) at 16,000 rpm for 20 s. The homogenate (1 mL) was transferred to a test tube, and then thiobarburic acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA, 2 mL) was added. The test tubes were heated in a water bath at 90°C for 15 min, cooled in cold water, and then centrifuged (Union 32R, Hanil Co., Ltd., Korea) at 3,000 rpm for 10 min. The absorbance of the supernatant was measured using a spectrophotometer (Beckman Instruments Inc.) at 532 nm. TBARS values were reported as mg malondialdehyde per kg meat.

Cholesterol content

Meat samples (1 g) were mixed with 20 mL Folch solution (chloroform:methanol = 2:1), and separated into 2 layers by centrifugation (Union 32R, Hanil Co., Ltd., Korea) at 3,100 rpm for 5 min. The chloroform layer containing total lipids was dehydrated using anhydrous Na_2SO_4, and the resulting solution was evaporated using nitrogen. Subsequently, 2 mL of 2 N ethanolic KOH was added to the sample, which was then placed in a 90°C water bath for 15 min for saponification. The aliquot was then cooled in cold water, and 1 mL of distilled water was added. Cholesterol in unsaponifiable fractions was extracted 3 times using 1 mL hexane. The resulting hexane aliquot was dried up to 1.5 mL using nitrogen, dehydrated using anhydrous Na_2SO_4, and injected into a gas chromatograph (GC-17A, Simazu, Japan). 5α-Cholestane (Sigma-Aldrich) was used as an internal standard. A split inlet (split ratio, 50:1) was used to inject the samples into a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm), and a ramped oven temperature was used (200°C for 5 min, increased to 300°C at 10°C/min). The injector temperature was 270°C, and the flame ionization detector temperature was 300°C. N_2 served as the carrier gas at a constant flow rate of 2.0 mL/min.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences among the mean were determined using Duncan's multiple range test with the significance defined as $p<0.05$.

Results and Discussion

Antioxidative activity and lipid oxidation

The total phenolic content of WG-0.5 was 0.75 mg/g for broiler leg meat, which was significantly higher compared to the other treatments (Table 1). The breast meat exhibited higher total phenolic content (1.13-1.14 mg/g) compared to the leg meat (0.69-0.75 mg/g), which was due to the low fat content of breast meat compared to leg meat (Jo *et al.*, 2009). However, no significant difference in the total phenolic content of breast meat was found among the 3 treatments. These results indicate that WG-0.5 increases the antioxidative activity of broiler leg meat.

To evaluate the antioxidative effect of wild grape on broiler meat, DPPH radical scavenging activity was determined (Table 2). The breast and leg meat of broilers fed WG-0.25 and WG-0.5 produced significantly higher DPPH values compared to the control. This finding indicates the utility of wild grape as a dietary supplement in which electron donors neutralize free radicals.

Table 3 shows the TBARS values of the breast and leg meat of broilers fed wild grape dietary supplements after

Table 1. Total phenolic contents (mg/g) of the meat from broilers fed wild grape

Treatment[1)]	Breast meat	Leg meat
Control	1.13	0.69[b]
WG-0.25	1.13	0.66[b]
WG-0.5	1.14	0.75[a]
SEM[2)]	0.014	0.013

[1)]Control, commercial finisher diet; WG-0.25, finisher diet with dietary supplementation of 0.25% wild grape; WG-0.5, finisher diet with dietary supplementation of 0.5% wild grape
[2)]Standard error of means (n=9)
[a,b]Means with different letters within the same column differ significantly ($p<0.05$).

Table 2. α, α'-Diphenyl-β-picryl-hydrazyl radical scavenging activity (%) of the meat from broilers fed wild grape

Treatment[1)]	Breast meat	Leg meat
Control	15.63[b]	23.66[b]
WG-0.25	19.51[a]	27.74[a]
WG-0.5	18.60[a]	27.95[a]
SEM[2)]	0.438	0.947

[1)]Control, commercial finisher diet; WG-0.25, finisher diet with dietary supplementation of 0.25% wild grape; WG-0.5, finisher diet with dietary supplementation of 0.5% wild grape
[2)]Standard error of means (n=9)
[a,b]Means with different letters within the same column differ significantly ($p<0.05$).

Table 3. 2-Thiobarbituric acid reactive substances (mg malondialdehyde/kg meat) values of meat from broilers fed wild grape

Treatment[1)]	Storage (d)			
	0	3	7	SEM[2)]
		Breast meat		
Control	0.39[cx]	0.52[bx]	0.65[ax]	0.011
WG-0.25	0.38[cx]	0.48[bxy]	0.62[ax]	0.025
WG-0.5	0.30[by]	0.43[ay]	0.44[ay]	0.010
SEM[2)]	0.010	0.016	0.022	
		Leg meat		
Control	0.55[cx]	0.82[bx]	0.88[ax]	0.012
WG-0.25	0.40[by]	0.82[ax]	0.88[ax]	0.071
WG-0.5	0.38[cy]	0.52[by]	0.75[ax]	0.025
SEM[2)]	0.012	0.061	0.044	

[1)]Control, commercial finisher diet; WG-0.25, finisher diet with die]tary supplementation of 0.25% wild grape; WG-0.5, finisher diet with dietary supplementation of 0.5% wild grape
[2)]Standard error of means (n=9)
[a-c]Means with different letters within the same row differ significantly ($p<0.05$).
[x,y]Means with different letters within the same column differ significantly ($p<0.05$).

storage at 4°C for 0, 3, and 7 d. Generally, TBARS values increased with storage time, due to the auto-oxidation of fat in the presence of oxygen. During the entire storage period, the TBARS value of the breast meat from broilers fed WG-0.5 was significantly lower compared to the other 2 treatments. The leg meat from the broilers fed WG-0.25 and WG-0.5 exhibited significantly lower TBARS values compared to the control during initial stage of storage. After 3 d of storage, the lowest ($p<0.05$) TBARS value was exhibited by leg meat from the WG-0.5 treatment. However, there was no significant difference among treatments for TBARS values of the leg meat after 7 d of storage. The WG-0.5 treatment was the most effective at preventing lipid oxidation, except for leg meat stored for 7 d.

The presence of polyphenols in meat is closely related to total antioxidant capacity (Prasad *et al.*, 2009). For instance, broilers fed a dietary medicinal herb extract mix exhibited higher total phenols and DPPH values at day 0 of storage compared to the control, indicating that the antioxidant activity of medicinal plants is transferred to broilers (Jang *et al.*, 2008; Jo *et al.*, 2009). Furthermore, the dietary supplementation of grape pomace was shown to improve antioxidant activity in chicken (Goni *et al.*, 2007).

Dietary phenolic sources, such as fermented wild grape, grape seed extract, and grape pomace also decrease TBARS values in pork, lamb meat, and chicken (Goni *et al.*, 2007; Jeronimo *et al.*, 2012; Park and Jung, 2005), with these results also being supported by the present study. This is because polyphenols donate hydrogen and electrons to free radicals, which results in oxidative chain reactions delaying lipid oxidation (Fraga *et al.*, 2010). Another reason why lipid oxidation is inhibited is related to corticosterone causing a decline in oxidative stress (Ohtsuka *et al.*, 1998). Grape leaf extract and tea polyphenol have been reported to induce oxidative stress in rats and broilers, with broilers exhibiting low TBARS values compared to the control (Eid *et al.*, 2003; Pari and Suresh, 2008).

The results of this study indicate that the dietary supplementation of wild grape might enhance antioxidant activity and delay lipid oxidation in broiler meat.

Cholesterol content

Cholesterol is an important component of human cell membranes; however, foods with high cholesterol contests might lead to cardiovascular diseases (Kang and Song, 1997; Park and Jung, 2005). Cholesterol is synthesized in the liver through acetyl-CoA, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), mevalonate, squalene, lanosterol, and over 30 intermediate substances, to maintain blood cholesterol concentrations (Kim, 1992; Park *et al.*, 1997). It has been reported that dietary fiber, vitamin C, and polyphenols including catechin and quercetin inhibit the synthesis of cholesterol or increase the extraction of bile acid, which is the only pathway for cholesterol excretion (Kang and Song, 1997; Kwon *et al.*, 1993; Paolisso *et al.*, 1995; Yokota *et al.*, 1996). Fermented wild gape by-product, Eosungcho powder, and onion peel contain large amounts of antioxidants, which have been shown to decrease the cholesterol content of pork meat by delaying lipid absorption (Joo *et al.*, 1999; Kang *et al.*, 2006; Park and Jung, 2005). However, the cholesterol

Table 4. Total cholesterol contents (mg/g) of the meat from broilers fed wild grape

Treatment[1)]	Breast meat	Leg meat
Control	0.50	1.33
WG-0.25	0.37	1.27
WG-0.5	0.36	1.34
SEM[2)]	0.034	0.078

[1)]Control, commercial finisher diet; WG-0.25, finisher diet with dietary supplementation of 0.25% wild grape; WG-0.5, finisher diet with dietary supplementation of 0.5% wild grape
[2)] Standard error of means (n=9)

content of breast and leg meat was not significantly affected by wild grape dietary supplementation (Table 4). Similar findings to our study have been reported for green tea by-products, wild grape juice, and wild grape, which were found to have no effect on the cholesterol content of chicken, blood cholesterol content of rats fed high oxidized lipid, or chicken eggs (Yang *et al.* 2003; Yong *et al*., 2012; Won, 2009).

The extraction of bile acid contributes towards reducing the amount of cholesterol in the body. However, this effect is not entirely related to the reabsorption of bile acids. Triglycerides, cholesterol, and other nutritional ingredients also have an additional effect (Kang and Song, 1997). Hence, further studies are required to better understand the synthesis of cholesterol and the factors that influence the cholesterol content of broiler meat.

In conclusion, the present study demonstrated the bioavailability of wild grape as an antioxidative dietary source for broilers.

Acknowledgement

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0081330), Rural Development Administration, Republic of Korea.

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(Received 2012.11.5/Revised 2013.1.24/Accepted 2013.2.18)