Bulgarian Journal of Agricultural Science, 19 (No 3) 2013, 606-610
Agricultural Academy

BAY LAUREL (*LAURUS NOBILIS* L.) IN JAPANESE QUAILS FEEDING. 2. FATTY ACID CONTENT AND OXIDATIVE STABILITY OF BREAST MEAT

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Abstract

KARAALP, M. and N. GENC, 2013. Bay laurel (*Laurus nobilis* L.) In Japanese quails feeding. 2. Fatty acid content and oxidative stability of breast meat. *Bulg. J. Agric. Sci.,* 19: 606-610

In this study, the antioxidant effect of dietary supplementation with bay laurel (*Laurus nobilis* L.) leaves (LN) to a conventional diet on fatty acid content and oxidatif stability of breast meat was studied. For that purpose, a *total number* of 60 Japanese quails aged 56-d *were* fed diets supplemented with 0 (LN0), 2 (LN2) and 4 g (LN4) leaves/kg of feed, respectively. Quails were housed individually in cages during ten weeks. Fatty acid composition was determined for fresh breast meat. Malondialdehyde (MDA) values were determined in breast meat samples stored at +4°C (2, 5 and 8 d) and at -20°C (3 and 6 mo).

Supplementation of LN had no effect on any fatty acid content of meat. However, addition of LN lowered ($P<0.01$) MDA content of the refrigerated meat samples only at 8 d. On the other hand, supplementation with LN had no significant effect on reducing the MDA values of meat samples during the other stored times. Besides, the amount of MDA increased ($P<0.01$) in all of the refrigerated and frozen meat samples with time. The results of this study demonstrated that supplementation of LN could reduce MDA content of refrigerated meat samples only at 8 d.

Key words: bay leave, laying quail, breast meat, fatty acid, malondialdehyde

Introduction

It is well known that poultry meat is particularly susceptible to oxidative deterioration due to its relatively high content of polyunsaturated fatty acids and the susceptibility to oxidative deterioration differs from its fatty acid compositions among poultry species. Because synthetic antioxidants are considered to have carcinogenic potential, natural herbs and spices have been used for many centuries to improve the sensory characteristics and to extend the shelf life of foods (Botsoglou et al., 2003a). The oxidative stability of poultry lipids has been found to be dependent on the α-tocopherol concentration present in cell membrane phospholipids and antioxidative activity substances are possibly responsible for stabilization, recycling, and retaining of α-tocopherol in poultry meat (Botsoglou et al., 2003b). For this purpose, dietary suplementation with herbs (Botsoglou et al., 2003c; Young et al., 2003; Florou-Paneri et al., 2005) and herb extracts (Lopez-Bote et al. 1998; Tang et al., 2000; Botsoglou et al., 2002; Botsoglou et al., 2003a,b,c; Florou-Paneri et al., 2005) demonstrated to be a simple and convenient application to increase oxidative stability of meat in poultry species.

Among them, bay laurel *(Laurus nobilis* L.) is evergreen tree and cultivated particularly in the Mediterranean area (Derwich et al., 2009). Methanolic extracts of the plant contain polar compounds (such are phenols, flavones and flavonols), and show antioxidative activity against lipid peroxidation (Simić, et al., 2003; Elmastas et al., 2006). The essential oil from the leaves (0.8 to 3%) contains mostly 1,8-cineol (up to 50%), also eugenol, acetyl and methyl eugenol, α- and β-pinene, phellandrene, linalool, geraniol and terpineol (Zeković et al., 2009). It has been reported that supplementation of bay laurel leaves (2 or 4 g/kg feed) had no effect on any performance and egg quality parameters of quails. Besides, the study demonstrated that supplemented a conventional diet with bay laurel leaves could change some biochemical parameters of quail egg yolk (Karaalp et al., 2011).

Although Japanese quails are slaughtered economically at 35 days of age (Karaalp, 2009), laying quails are consumed by humans after the laying period or earlier. The biochemical

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pathway of fatty acid biosynthesis or tissue deposition may be influenced by the diet, age and strain and type of the bird. Breast meat is the most valued part of the bird and less susceptible to oxidation compared to thigh muscle (Botsoglou et al., 2003b). Malondialdehyde (MDA) is one of the secondary products of lipid oxidation, detrimental to health, formed because of the oxidation process of fatty acids containing at least three double bonds. Measurement of MDA content is a generally accepted method for determining the degree of lipid oxidation in food (Fenaille et al., 2001).

It has not been reported in the scientific literature that the antioxidant effect of bay laurel leaves on meat tissue of laying Japenese quails. Therefore, the objective of this study was to investigate the effect of supplemented bay laurel leaves to a conventional diet of laying Japanese quail on the fatty acid content and oxidatif stability of breast meat samples during refrigerated or long-term frozen storage.

Materials and Methods

Animals, diets and husbandry

A total number of 60 quails, 56-d-old, were distributed one by one into individual cages (20x25x25 cm) for three different treatments. All birds were kept under similar and standard hygienic, environmental and managerial conditions according to the Turkey Experimental Animal Care Guidelines. Diets of the three groups had 185 g crude protein and 2800 kcal ME per kilogram (Table 1). Bay laurel *(Laurus nobilis* L.) leaves (LN) harvested from Black Sea Region of Turkey were dried in the shade and ground into a fine powder in a mill. The control diet did not contain LN leaves (LN0) and the other diets were supplemented with 2 g leaves (LN2) and 4 g leaves (LN4) per kg, respectively. During the feeding period that lasted 70 days, feed and water were offered *ad libitum*, whereas photoperiod was 17 hours daily.

Sample prepar1ation

At the end of feeding period (126 d of age), 10 quails randomly selected from each treatment group were slaughtered. Individual carcases were trimmed for breast meat muscles by removing skin, bones and connective tissue. The muscles were ground, mixed and separated to six portions, vacuum packaged and stored. A portion of the meat was immediately analyzed for fatty acids. The remaining five samples were stored for 2, 5 and 8 d (+4^{o}C), and 3, 6 mo (-20^{o}C) until determination of MDA (the latter two were stored overnight at +4 ^{o}C at the time of analysis).

Fatty acid methyl esters of breat meat samples

The samples were extracted for analysis fatty acid methyl esters according to Folch et al. (1957). The samples (about 1 g) were homogenized by ultra mixer in a chloroform/methanol (2:1 v/v) mixture using a high-speed blender, and filtered through Whatman paper. The filtrate was evaporated for dryness with a rotary evaporator (Heidolph EssLab, Essex, UK) at 50ºC under reduced pressure, and quail breast meat lipids were obtained. Then, the fatty acid methyl esters were prepared from lipid extracted from samples by esterification reaction with 14% boron trifluoride-methanol complex according to the modified method of the AOAC (1990). Methanolic NaOH (0.5 N) was added to the lipid of samples. The mixture was heated at 100ºC during 5 min under refluxed conditions and cooled to 25ºC. The cooled mixture was esterified with 14% boron trifluoride at 100ºC during 15 min. After adding hexane, the hot solution was shaken and then saturated sodium chloride was added. The methyl ester phase was separated from the hexane phase in a separatory funnel and was placed in a vial. One microliter of fatty acid methyl esters from the extracted lipids was injected to gas chromatography. Subsequent fatty acid profiles were analyzed by gas chromatography. The fatty acid methyl esters were analysed using a

Table 1
Ingredient and chemical composition of basal diet

Ingredient	g/kg
Maize	484.57
Soybean meal	275.85
Wheat	150.00
Sunflower oil	10.00
Limestone	60.45
Dicalcium phosphate	11.45
NaCl	3.50
Vitamin premix[1]	1.00
Mineral premix[2]	1.00
DL-Methionine	1.74
L-Lysine HCl	0.44
Calculated contents (per kg)	
Metabolizable energy, kcal	2.800
Crude protein, g	185.00
Methionine, g	4.50
Lysine, g	10.00
Threonine, g	6.90
Ca, g	26.00
Available P, g	3.50
Na, g	1.60

[1] Vitamin premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 1,500 IU; vitamin E, 50 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; niacin, 25 mg; Ca-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apocarotenoic acid ester, 2.5 mg; choline chloride, 400 mg.
[2] Trace mineral premix provided per kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.

30 m (with 0.25 mm film thickness), Quadrex 30.0m x 250µm 0.25 mm ID capillary GC column installed on a Perkin Elmer (Auto System) gas chromatograph with a flame ionization detector (FID). The gas chromatograph temperature-programme was oven: Initial temp 100°C for 0 min, ramp 10°C/min to 180°C, hold 6 min, ramp 1.5°C/min to 220°C, hold 6 min, InjAauto=250°C, Volume=1 µL, Split=30:1, Carrier Gas=He, Solvent Delay=0.00 min, dedector Temp=250°C.

Malondialdehyde (MDA) analysis

Lipid oxidation was assessed based on the MDA formed during storage. MDA, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method (Botsoglou et al*.,* 1994). Breast meat samples (about 1 g) were homogenized in the presence of 4 mL of 5% aqueous trichloroacetic acid and 5 mL of 0.8% butylated hydroxytoluene in hexane, and the mixture was centrifuged at 2000xg for 10 minute. The top layer was discarded, and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of 0.8% aqueous 2-thiobarbituric acid, to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under ice water bath and submitted to conventional spectrophotometry (Perkin-Elmer). The concentration of MDA in analyzed samples (mg/kg meat) was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane, the precursor of MDA. Butylated hydroxytoluene, 2-thiobarbituric acid, and 1,1,3,3-tetraethoxypropane were obtained from Sigma Chemical Co. (St. Louis, MO), whereas trichloroacetic acid was obtained from Merck (Darmstadt, Germany).

Statistical analysis

The means of groups were compared through Duncan's Multiple-Range Test after all data were subjected to analysis of One Way-ANOVA (Norusis, 2002).

Results and Discussion

Supplemented LN leaves (2 or 4 g/kg feed) had no effect (P>0.05) on fatty acid composition of quails' breast meat samples (Table 2). There is no published report on antioxidant effects of LN or other natural plants on meat tissue of Japanese quails. In egg yolk of Japenes quail, Karaalp et al. (2011) reported that 4 g LN supplementation per kg feed decreased palmitic acid and increased linoleic acid after two months use. In the present study, however, the effect of storage time on the change of fatty acids of breast meat was not investigated. Koreleski and Świątkiewicz (2007) observed a decrease for saturated fatty acids content and an increase for polyunsaturated fatty acids (n-3) content in the initial month and after 6 months of cold storage. *These changes were thought to be associated with* changes in meat fat content, direction of oxidation and degree of fatty acid saturation or desaturation perhaps when meat was stored, unfrozen and prepared for analyses. However, this issue remains controversial in the published reports. For example, Coetzee and Hoffman (2001) did not find any changes of fatty acids proportions over storage time of chicken meat.

Dietary suplementation with herbs and their extracts demonstrated to be a simple and convenient application to increase oxidative stability of chicken (Botsoglou et al., 2003c) and turkey meats (Botsoglou et al., 2003a). Dietary *oregano supplementation (30 g/kg)* protected against stres-induced increase of MDA in different muscles of chicken (Young et al., 2003). Florou-Paneri et al., (2005) fed turkeys for 4 weeks with diet containing oregano herb (5 and 10 g/kg) versus oregano essential oil (100 and 200 mg/kg) and they found that both additives were effective in increasing the oxidative stability of the fresh meat. On the other hand, supplementation of oregano (10 g/kg diet) did not affect MDA in pork meat (Vichi et al., 2001). In the present study, the effect of dietary LN supplementation on lipid oxidation of quail breast meat stored at +4°*C for 2, 5 and 8 days, and at* -20°*C 3 and 6 months is presented in Table 3.*

The extent of lipid oxidation, as measured by MDA formation, did not differ (P>0.05) among the dietary treatments in refrigerated (+4°C) breast meat for 2 or 5 days. However, only at 8 day, feeding with diet containing 2 and 4 g LN/kg (the most effective) decreased (P<0.001) the amount of MDA in breast meat compared to control. These results suggest that the antioxidant property of LN on the breast meat emerges in a longer period. However, there is no available report on the determination of antioxidant effects of LN on meat tissue in poultry species. Poultry breast meat contains α-tocopherol and a higher percentage of fatty acids containing at least three double bonds in the fat. The susceptibility of breast meat to lipid oxidation has been attributed to these fatty acids (Jensen et al., 1997). The oxidative stability of poultry lipids has been found to be dependent on the α-tocopherol concentration present in cell membrane phospholipids (Wen et al., 1997), although it was not determined in the present study. The lower MDA contents found in meat of the LN consumed groups indicated lower *in vivo* production and deposition of MDA in breast tissues at 8 d. In addition, antioxidative activity substances of LN might be responsible for by stabilization, recycling, and retaining of α-tocopherol in breast meat as in turkey meat (Botsoglou et al., 2003b). This could not be attributed to consumption and subsequent deposition of the MDA. It is possible that effective substances in LN are responsible

for decreasing of *in vivo* MDA production (Florou-Paneri et al., 2005) by the quails and lowering MDA in meat through decreasing α-tocopherol oxidation (Botsoglou et al., 2003b).

Dietary supplementation with LN had no significant effect on the amount of MDA in the breast meat samples during 3 or 6 months frozen storage. Higher quantities of α-tocopherol could be consumed for protecting the polyunsaturated fatty acids of the membrane phospholipids from oxidation during the early stages of frozen storage. It was claimed that α-tocopherol levels in turkey meat decreased during the frozen storage with time (Botsoglou et al., 2003b). At -20°C, because some catalysts and antioxidants may be trapped in the frozen solid phase, the antioxidant activity of the cytosolic phase may no longer function (Wen et al., 1996). Free radicals may escape the antioxidants in the frozen aqueous phase and diffuse into the membrane cell phospholipids, where they initiate and promote lipid oxidation. When this happens, α-tocopherol becomes the first line of antioxidant defense and thus may be rapidly depleted (Wen et al., 1996). Botsoglou et al. (2003b) stated that the greater rate of α-tocopherol depletion occurred between 1 and 3 months of frozen storage in turkey breast meat and after this, it remained almost unchanged. They also added that the concentration of MDA was quite stable during the same period, whereas the main increase in MDA occurred during 3-9 months of storage.

The amount of MDA in breast meat of all groups increased both by storing in refrigerator and deepfreeze with time (P<0.001). In all groups, the MDA amounts occurring as a result of storing in the deep freeze for 3 months were lower (P<0.001) than those refrigerated storage *for 5 and 8 days and*

Table 3
MDA amount (mg/kg) of breast meat (N= 10) at different storage time and temperature

Storage	LN0	LN2	LN4	SEM	P-value
Refrigerature (*+4°C)*					
2 d	^{E}0.62	^{E}0.66	^{E}0.65	0.03	NS
5 d	^{C}3.02	^{C}3.06	^{C}3.03	0.06	NS
8 d	^{A}7.98 ^{a}	^{A}5.76 ^{b}	^{B}4.65 ^{c}	0.30	0.001
Deepfreeze (-20°*C)*					
3 mo	^{D}1.31	^{D}1.36	^{D}1.25	0.07	NS
6 mo	^{B}5.14	^{B}4.86	^{A}5.17	0.07	NS
SEM	0.46	0.32	0.27		
P-value	0.001	0.001	0.001		

* SEM, standard error of the mean; P, probability; NS, not significant (P>0.05)
$^{a, b}$ Means within a row with no common superscript differ significantly (Duncan, P<0.05).
$^{A, B}$ Means within a column with no common superscript differ significantly (Duncan, P<0.05).

Table 2
The fatty acids composition of total lipid in quail breast meat (% of fatty acid methyl esters, N= 10)

Fatty Acids	LN0	LN2	LN4	SEM	P-value
C14:0	0.94	1.00	1.03	0.03	NS
C16:0	25.02	24.08	23.40	0.42	NS
C16:1	3.92	4.05	4.19	0.08	NS
C18:0	8.63	8.04	8.48	0.13	NS
C18:1n9c	33.26	34.31	34.64	0.68	NS
C18:2n6c	22.55	23.17	22.83	0.44	NS
C18:3n3	1.61	1.60	1.46	0.04	NS
C20:3n6	1.66	1.54	1.71	0.05	NS
C20:4n6	1.89	1.71	1.79	0.05	NS
C24:0	0.18	0.19	0.18	0.01	NS
C24:1	0.24	0.20	0.21	0.01	NS
C22:6n3	0.11	0.10	0.09	0.005	NS
Σ Saturated	34.77	33.22	33.09	0.40	NS
Σ Unsaturated	65.23	66.68	66.91	0.40	NS
Σ Monounsaturated	37.41	38.56	39.03	0.72	NS
Σ Polyunsaturated	27.82	28.18	27.87	0.44	NS

* SEM= standard error of the mean; NS= not significant (P>0.05).

were higher than for 2 days'. All diets gave higher the MDA values (P<0.001) during frozen storage for 6 months compared to refrigerated storage for 2 and 5 days. Besides, freezing for 6 months produced lower MDA amounts (P<0.001) in both LN groups except for LN4 than refrigerated storage *for 8 days.*

Conclusion

The results of the present work demonstrated that addition of LN upto 4 g/kg to a conventional diet did not alter fatty acid contents in breast meat of laying Japenese quail. However, only at 8 d, LN supplementation decreased MDA content of the refrigerated meat samples. Besides, the amount of MDA in breast meat increased in both storing refrigerator and deepfreeze with time. Further studies should be carried out to evaluate the possible antioxidant effects of bay laurel leaves and its essential oil in birds.

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Received April, 22, 2012; accepted for printing December, 2, 2012.