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ARTICLE

Effects of Dietary Supplementation of Aqueous Extracts of *Liriopeplatyphylla* and *Akebiaquinata* on Breast Meat Qualities of Broiler Chickens

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Abstract

The present study investigated the effects of *Liriopeplatyphylla* extract (LPE) and *Akebiaquinata* extract (AQE) on breast meat properties when used as dietary supplements of broiler chickens. First, the identification and quantification of phenolic acids and flavonoids were carried out by HPLC. As a result, the total amount of phenolic acids and flavonoids was higher in AQE than LPE. These extracts were added at a rate of 0.2% to the broiler diets, and a feeding trial was conducted in battery cages for 35 d. At the end of the experiment (d 35), six carcasses from each treatments were used for evaluating meat quality. The experimental results indicate that color shades, pH levels, volatile basic nitrogen, thiobarbituric acid reactive substance (TBARS), cooking loss and drip loss of breast meat fed with 2 extracts were not different as compared with the controls at d 0 and d 10 of storage. However, TBARS values of breast meat fed with either the control diet or the LPE supplementation was increased as the storage period increased (from d 0 to d 10) (p<0.05), while AQE-fed groups were not different between d 0 to d 10 of storage. In textural properties, the addition of LPE and AQE decreased shear force values at d 10 of storage (p<0.05). Cohesiveness, gumminess and chewiness of breast meat were increased in AQE-fed groups when compared with the control at d 0 of storage (p<0.05). Dietary additions of AQE and LPE only increased the linoleic acid contents of chicken breast meat (p<0.05). In conclusion, supplementation of these extracts in broiler diets may potentially influence meat qualities including the TBARS, textural properties and linoleic acid levels in broiler chicken meats.

Key words: broiler chicken meat, meat quality, *Akebiaquinata*, *Liriopeplatyphylla*

Introduction

The livestock production industry has a growing interest in the use of new natural feed additives, and in all parts of the world, researchers are searching for new safe additives to replace conventional antibiotics. Among those, plant extracts represent a large class of alternatives. A variety of physiologically active substances isolated from medicinal plants have been reported for the prevention and treatment of diseases, and for antimicrobial and antioxidant effects (Cai *et al*., 2003; Sala *et al*., 2002). In poultry, many researchers indicated that medicinal plants supplementation results in increased productivity, health, immunity and stabilization of gut microflora (Chen *et al*., 2003; Guo *et al*., 2004; Hong *et al*., 2001; Ryu and Song, 1999).

In addition, many plants and their extracts have been used in a variety of foods to improve their sensory properties and prolong shelf-life (Shahidi *et al*.,1992). This was also confirmed in a broilers study,with positive effects determined in the meat quality characteristics (Sarraga and Garcia Regueiro, 1999). Thus, it seems that medicinal plants have a variety of features to improve the performance and meat qualities of poultry. These properties of plants were linked to chemical compounds such as phenols, flavonoids, lignans, alkaloids, and tannins etc., which have various physiological actions.

Liriopeplatyphylla is a wide spread perennial herb occurring throughout boreal and temperate regions. *Liriopeplatyphylla* has been used as a traditional medicine in asthma, bronchial and lung inflammation in Korea, China

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and Japan. In addition, the extract effectively prevents obesity, diabetes and neurodegenerative disease (Lee *et al*., 2012).

Akebiaquinata is a shrub that is widely distributed in hills and mountains in Korea, China and Japan. In traditional Chinese folk medicine, it is believed to be therapeutic as a diuretic, antiphlogistic and analgesic (Han *et al*., 2012; Ikuta, 1991). In addition, the principal use of the herb in China is as a traditional remedy for insufficient lactation in postpartum mothers.

The increasing consumer preference for natural foods has led to the inclusion of natural antioxidants in various products to improve quality and replace synthetic antioxidants.The use of plant extracts in animal diets is one of the most promising ways of improving meat quality because of its antioxidant properties. Although medicinal plant extracts have previously been added to animal diets to improve meat quality, the effects of addition of *Liriopeplatyphylla* and *Akebiaquinata* have not yet been tested in broiler chicken meats.

The objective of this study was to test the feeding effect of 2 medicinal plant extracts (LPE and AQE) on the quality properties of breast meat in broiler chickens.

Materials and Methods

Preparation of medicinal plantextracts

Akebiaquinata and *Liriopeplatyphylla* plants were purchased from a herbal medicine store in Seoul, Korea. The plant material was dried under dark conditions in the laboratory, and was then crushed and finely blended using a mill (IKA M 20, IKA, Germany). The ground powder samples were extracted with distilled water (1:10) at a temperature at 80°C in reflux for 6 h to give an initial extract. The residues were again extracted with distilled water (1:5) at a temperature at 80°C for 2 h. After cooling to room temperature, the insoluble material was filtered with Whatman No. 2 filter paper. The extracts were then completely dried in a freeze-drier, and were stored at -20 °C until further use.

Extraction and purification of phenolic acids and flavonoids

Powder samples (0.1 g) were mixed with 50 mL of 50% methanol acidified with formic acid. The mixture was shaken for 30 s and flushed with nitrogen for 2 min, and was then centrifuged at 2,000 rpm for 60 min at 4°C and the supernatant was collected. The pellet was submitted to a second extraction of 15 min with 50 mL of solvent. The supernatants were evaporated to dryness under vacuum using a rotary evaporator (RW-0525G, Heidolph, Germany) below 40°C, and the residue was dissolved in 10 mL of solvent containing 0.01 mg/ml *p*-coumarin, and filtered through a 0.45-μm membrane filter prior to HPLC-DAD analysis.

HPLC-DAD analysis

The extracted compounds were analyzed by HPLC (Agilent 1100 series) with a diode array detector (DAD), using a Nucleosil 100-5 C-18 column (250 mm × 4.0 mm i.d., 5 μm particle size) which was protected by a 10 mm guard column with a gradient elution system. The mobile phase consisted of two solvents: solvent A was composed of a mixture of water/formic acid (pH 3.29), and solvent B was 100% acetonitrile/formic acid (pH 3.29). Gradient elution was as follows: initially 7.0% of solvent B, followed from 0% to 15% B in 25 min, 30% B at 35 min, 40% B at 50 min, 100% B at 45 min, 100% B at 55 min, 50% B at 60 min, 50% B at 63 min, 0% B at 64 min, and 0% B at 73 min. The flow rate was 1.0 mL/min, and the column temperature was 30°C.

The acquisition wavelength for the DAD was 280 nm (for gallic acid, 4-hydroxy benzhydrazide, epigallocatechin, epicatechin, epigallocatechingallate, vanillic acid, (+)-catechin hydrate, syringin acid, phloroglucinol, catechingallate, gallocatechin, proticchuic acid, *p*-anisic acid, and 3-(3,5-dimethyl-pyrazol-1-yl) benzoic acid), 320 nm (for chlorogenic acid, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, naringin, 2-amino-3,4-dimethyl benzoic acid, coumarin, morin hydrate, luteolin, hesperetin, alizarin and biotin), 370 nm (for rutin hydrate, myricetin, quercetin hydrate, quercetindihydrate, 3-hydroxyflavone and kaempferol). The chromatographic peaks of phenolic acids and flavonoids were confirmed by comparing its retention times with those of reference standards.

Experimental design

One hundred and eighty broiler chickens of the strain Arbor Acres were reared from 1 to 35 d of age. One day old male birds were individually weighed and assigned to 12 battery cages with 15 birds per replicate cage, each based on similar body weights. The birds were provided fresh feed and water daily *ad libitum* and 24 h consistent light was maintained. The basal diet was formulated to meet nutrient requirements of broiler chickens (NRC, 1994) and consisted of a starter diet (ME: 3,200 kcal/kg, CP: 22%, methionine: 0.50%, lysine: 1.10%, Ca: 1%, available P: 0.45%) until 21 d and a finisher diet (ME: 3,200 kcal/kg,

CP: 20%, methionine: 0.38%, lysine: 1.00%, Ca: 0.9%, available P: 0.35%) until 35 d of age. Birds in the control group received these diets without additives (control). The other two treatment groups were received the same diets as fed to the control groups, but supplemented with 0.2% *Akebiaquinata* extract and *Liriopeplatyphylla* extract.

Sample collection

At the end of the experimental period (d 35), six birds from each treatment were killed via CO_2 inhalation, and breast meat samples of each individual bird were collected and stored individually in plastic bag at 4°C. Most of the physicochemical qualities of the meat were analyzed on storage d 0 and 10, respectively. The analysis of fatty acid was measured on storage 0 d.

Meat quality measurements

Color measurements

Color measurement of breast meat was performed using a Minolta colorimeter (CR-300, Japan) calibrated with a standard white plate. Meat samples were measured at five different locations across the center of the breast. The center of the cut was measured in triplicate to determine CIE color values for lightness (L*), redness (a*) and yellowness (b*) values. The whiteness (W) was calculated using the following formula: L* − 3b* (Park, 2005) and chroma (C*) and hue (h*) values were calculated from a* and b* values.

Meat pH

The pH values of raw breast meats were measured using a digital pH meter (Seven Easy pH, Mettler-Toledo AG, Switzerland) after blending 10 g of finely homogenized sample with 90 mL of double-distilled water.

Drip loss and cooking loss

Drip loss (%) was measured on a 2 cm × 5 cm × 5 cm thick sections removed from chicken breast, which was weighed and suspended in a zipperbag for 10 d at 4°C. The initial and final weight of each sample was used to calculate drip losses. For cooking loss estimation, raw meat samples (2 cm × 5 cm × 5 cm) were vacuum packaged after weighing and were then cooked in a water bath at 100°C for 30 min. The samples were cooled at room temperature for 1 h and weighted again. Cooking losses were calculated as the difference in weight between the initial raw and final cooked samples.

Volatile basic nitrogen and thiobarbituric acid reactive substances

Volatile basic nitrogen (VBN) values were measured using the Conway micropipette diffusion method by Pearson (1968). The amount of thiobarbituric acid reactive substances (TBARS) was measured according to the method of Buege and Aust (1978).

Shear force and textural properties of meat

The shear force values of raw breast meat cut into 1.8 cm × 2 cm size pieces were recorded using a Warner-Bratzler shear press (Instron 3343, US/MX50, A&D Co., USA) and were expressed as kg/cm^2. For the textural profile analysis (TPA), meat samples were cut into cylindrical shapes (2 cm thick, 2 cm in diameter) and were measured using a texture analyzer (TA-XTZ-5, Shimadzu Co., Japan) with a flat plate set in a 500 N load cell and a compression speed of 60 mm/min). To determine the TPA values, samples were compressed to 75% of their original height as a two-cycle compression; TPA values determined included hardness, cohesiveness, springiness, chewiness and adhesiveness.

Fatty acid composition of meat

Fatty acid contents of meat samples were measured by the direct methylation method of Lepage and Roy (1986), and the sample solutions were injected into an Agilent 6890 gas chromatograph equipped with an Agilent 7683 auto sampler, split/splitless injector, and an Agilent 5973 N mass selective detector (MSD). Separations of the fatty acid methyl esters were carried out by using a fused-silica capillary column (30 m × 0.25 mm × 0.25 μm; DB-Wax). The GC oven temperature was programmed from 180°C to 230°C at a rate of 1.5°C/min. Injector and detector temperatures were maintained at 240 and 260°C, respectively.

Statistical Analysis

All data collected was subjected to statistical analysis by the General Linear Model (GLM) procedure of SAS program. Duncan's multiple range test was used to test the significance of difference between means. Statistical difference of the values were determined at $p<0.05$.

Results and Discussion

Phenolic acid and flavonoids content of 2 medicinal plant extracts

Medicinal plants have been a source of a variety of active components, including flavonoids, phenolic compounds,

tannins, isoprene, saponins and terpenoids (Duthie and Crozier, 2000). Therefore, to confirm the flavonoids and phenolic compounds contents of the medicinal plant extract used in this study, HPLC separation and identification were conducted. Total amounts of phenolic acids of AQE and LPE were 2,453 and 25.78 mg/100 g, respectively. The total amount of flavonoids of AQE and LPEwere 5,105 and 459.86 mg/100 g, respectively. Because these structural features of phenolic compounds of these plants are reportedly responsible for antioxidant activity (Fukumoto and Mazza, 2000), the concentrations of phenolic acids and flavonoids may be related to their antioxidant properties.

Table 1. Phenolic acids and flavonoids contents and in *Liriopeplatyphylla* and *Akebiaquinata* (mg/100 g extract)

	Liriopepla-typhylla	*Akebia-quinata*
Benzoic acid		
Phloroglucinol	-[a]	-
4-hydroxy benzhydrazide derivative	-	-
Gallic acid	19.68	32.53
Vanillic acid	-	0.50
Protocatechuic acid ethyl ester	-	23.05
2-Amino-3,4-dimethyl-benzoicacid	-	1,806
p-Anisic acid	-	-
Alizarin	-	-
3-(3,5-Dimethyl-pyrazol-1-yl)-benzoic acid	-	203.37
Total of benzoic acid	19.68	2,065
Cinnamic acid	-	-
Chlorogenic acid	-	112.58
Caffeic acid	-	-
Syringic acid	-	67.90
p-Coumaric acid	6.09	61.22
Chlorogenic derivative	-	-
trans-Ferulic acid	-	50.57
Coumarin	-	22.65
Total of cinnamic acid	6.09	387.60
Total of phenolic acid	25.78	2,453
Flavanols		
(+)-Catechin hydrate	-	-
Catechingallate	-	130.01
Gallocatechin	-	389.92
(-)-Eipgallocatechin	-	3,603
Epicatechin	-	105.61
Epigallocatechingallate	-	-
Quercetin hydrate	-	687.35
Myricetin	-	-
Morin hydrate	-	-
Quercetindihydrate	386.87	-
Morin derivative	-	-
Kaempferol	-	-
3-Hydroxyflavone	72.99	-
Total of flavanols	459.86	4,916
Flavones		
Rutin hydrate	-	114.22
Luteolin	-	-
Total of flavones	-	114.22
Flavanones		
Naringin	-	74.98
Total flavanones	-	74.98
Total flavonoid	459.86	5,105

[a]-: Not detected.

Color and physicochemical properties of chicken breast meat

The addition effects of AQE and LPE on physicochemical properties of breast meat were measured with 35-d-old birds, and the results are summarized in Tables 2 to 5.

As shown in Table 2, breast meats fed with AQE and LPE did not show any significant change in lightness, redness, yellowness, whiteness, chroma or hue, compared with the controls at d 0 and d 10 of storage, respectively.

The inclusion of 2 medicinal plant extracts in the broiler diet did not affect breast meat pH, volatile basic nitrogen (VBN), thiobarbituric acid reactive substance (TBARS), cooking loss or drip loss at d 0 and d 10 of storage, respectively. However, TBARS values of breast meat fed with control diet and LPE gradually increased as storage period increased (from d 0 to d 10) ($p<0.05$), while that of AQE-fed groups did not differ between d 0 to d 10 of storage (Table 3). Therefore, this result indicates that feeding AQE

Table 2. Effect of dietary LPE and AQE supplementation on color of chicken breast meat

Item	Treatments	Storage (d)	
		0	10
CIE Lightness	Control	42.30±1.72	43.94±0.83
	LPE	42.91±2.30	44.64±1.77
	AQE	40.75±1.46	43.33±0.66
Redness	Control	1.89±0.40	1.61±0.26
	LPE	1.70±0.25	2.10±0.22
	AQE	1.37±0.29	1.70±0.31
Yellowness	Control	5.50±0.89	6.83±0.66
	LPE	4.73±0.75	5.80±0.93
	AQE	3.91±0.38	4.85±0.72
Whiteness	Control	25.81±1.44	23.44±1.37
	LPE	28.73±2.29	27.24±1.90
	AQE	29.02±1.10	28.77±1.56
Chroma	Control	7.91±1.13	9.56±0.81
	LPE	6.79±0.85	8.22±1.17
	AQE	5.61±0.53	6.92±0.87
Hue	Control	70.76±4.61	76.52±3.63
	LPE	68.52±6.00	69.61±3.80
	AQE	71.58±3.86	69.53±5.00

Standard errors of mean.

led to a delay in the lipid oxidation of breast meat. Phenolic compounds such as flavonoids limit the generation of polyunsaturated fatty acidoxidation products, thereby resulting in decreased oxidative injury in cell membranes (Ohta *et al*., 1999). Several investigations have shown that phytochemicals in plants cause the prevention of the formation of the strong peroxidation agents superoxide ions (O_2^-) and hydroxy radicals (OH) (Du *et al*., 2010; Facino *et al*., 1990; Kumaran and Karunakaran, 2007). Loperz-Bote *et al*. (1998) also suggested that the meat from broilers fed on the diet including rosemary and sage extracts had decreased concentrations of lipid oxidation products than meat from the control group. In addition, dietary oregano, rosemary, and sage essential oils addition appeared to retard lipid oxidation (malondialdehyde (MDA) formation) in meat stored at both 4°C and -20°C (Botsoglou *et al*., 2003; Simitzis *et al*., 2008). The fact that meat samples had lower MDA formation suggests that these samples contained antioxidant compounds, which might be absorbed into the circulatory system after ingestion, distributed, and retained in muscle and other tissues. Similar findings were reported by Jang *et al*. (2008) suggested that the breast meat from chickens fed a medicinal plant mixture (mulberry leaf + Japanese honeysuckle + gold thread) showed a significantly increased total phenols content, and consequently, the DPPH free radical scavenging activity in breast meat was more greater and TBARS was decreased. In the present study, phenolic acid accumulated in the meat during the feeding period of the broiler chickens might have also delayed this oxidation process during storage.

Table 3. Effect of dietary LPE and AQE supplementation on physicochemical properties of chicken breast meats

Item	Treatments	Storage (d)	
		0	10
pH	Control	6.11±0.05	6.11±0.06
	LPE	6.16±0.08	6.19±0.07
	AQE	6.35±0.08	6.23±0.06
VBN (mg%)	Control	11.37±0.38	11.68±0.35
	LPE	10.95±0.63	11.84±0.15
	AQE	10.82±0.17	12.12±0.85
TBARS (mg/100g)	Control	0.16±0.01^{z}	0.26±0.01^{y}
	LPE	0.15±0.01^{z}	0.25±0.02^{y}
	AQE	0.18±0.02	0.24±0.02
Cooking loss (%)	Control	74.27±0.97	71.55±1.13
	LPE	73.40±1.66	72.60±2.04
	AQE	73.25±1.19	70.44±0.75
Drip loss (%)	Control	-	3.76±0.69
	LPE	-	3.12±0.62
	AQE	-	3.94±1.15

Standard errors of mean.
^{y,z}Means are significantly different within the same row (p<0.05).

Textural properties and fatty acids of chicken breast meat

In textural properties, the addition of LPE and AQE decreased shear force values at d 10 of storage and shear force declined over time from d 0 to d 10 (p<0.05). Reduction of meat shear force is likely to be due to the action of the proteolytic enzymes on myofibrillar proteins by the addition of extracts (Fiems *et al*., 1990). As the breakdown of muscle protein occurred, small peptides or proteins (connective tissue and myofibrillar proteins) were generated, reducing the firmness of the meat structure (Kemp *et al*., 2010). The exact mechanism of its relationship to shear force is not fully understood, but Chen *et al*. (2008) suggested that alow value in firmness may be due to a decrease in water loss from the pork meat fed with diets containing plant extract (garlic powder) during refrigerated storage. Cohesiveness, gumminess and chewiness of breast meat were increased in the AQE-fed groups compared with the control at d 0 of storage (p<0.05) (Table

Table 4. Effect of dietary LPE and AQE supplementation on shear force and textural properties of chicken breast meats

Item	Treatments	Storage (d)	
		0	10
Shear force (kg/cm^2)	Control	3.88±0.26^{y}	2.75±0.25^{a,z}
	LPE	3.08±0.31^{y}	1.99±0.10^{b,z}
	AQE	4.15±0.80^{y}	1.91±0.12^{b,z}
Hardness (kg)	Control	0.57±0.04	0.72±0.08
	LPE	0.57±0.05	0.56±0.04
	AQE	0.63±0.08	0.55±0.05
Cohesiveness (%)	Control	0.38±0.02^{b}	0.39±0.02
	LPE	0.47±0.04^{ab}	0.42±0.01
	AQE	0.56±0.04^{a,y}	0.38±0.01^{z}
Springiness (mm)	Control	1.10±0.05	1.00±0.00
	LPE	1.12±0.08	1.00±0.00
	AQE	1.24±0.09^{y}	1.00±0.00^{z}
Gumminess (kg)	Control	0.21±0.00^{b}	0.29±0.04
	LPE	0.26±0.02^{ab}	0.24±0.02
	AQE	0.34±0.04^{a,y}	0.21±0.02^{z}
Chewiness (kg,mm)	Control	0.23±0.01^{b}	0.29±0.04
	LPE	0.29±0.02^{b}	0.24±0.02
	AQE	0.43±0.06^{a,y}	0.21±0.02^{z}
Adhesiveness	Control	0.06±0.01^{z}	0.11±0.01^{y}
	LPE	0.08±0.00	0.10±0.01
	AQE	0.08±0.01^{z}	0.11±0.01^{y}

Standard errors of mean.
^{a,b}Means are significantly different within the same column (p<0.05).
^{y,z}Means are significantly different within the same row (p<0.05).

Table 5. Effect of dietary LPE and AQE supplementation on fatty acids of chicken breast meats

	Control	LPE	AQE
	(% total fatty acid)		
C16:0	23.30±0.96	24.12±0.33	23.42±0.15
C16:1	1.82±0.29	2.22±0.55	2.36±0.40
C18:0	13.87±1.52	13.14±0.43	14.16±0.53
C18:1n9t	0.13±0.07	0.21±0.07	0.28±0.05
C18:1n9c	20.47±1.47	23.09±2.48	21.68±1.03
C18:2n6c	18.49±0.48^{z}	20.07±0.55^{y}	20.93±0.19^{y}
C18:3n6	0.00±0.00	0.03±0.02	0.00±0.00
C20:1	0.86±0.37	0.36±0.12	0.44±0.05
C18:3n3	0.94±0.47	0.45±0.17	0.61±0.03
C20:2	1.83±0.51	0.91±0.11	1.05±0.05
C20:4n6	11.68±0.93	8.89±1.61	8.55±1.06
C20:5n3	1.33±0.20	1.18±0.09	1.18±0.33
C22:6n3	5.29±0.13	5.32±1.16	5.35±0.41
SFA	37.17±0.94	37.26±0.50	37.58±0.39
UFA	62.83±1.03	62.74±0.52	62.42±0.33
PUFA	39.56±0.77	36.85±2.90	37.66±1.21
MUFA	23.28±1.47	25.89±3.17	24.76±1.54
UFA/SFA	1.69±0.05	1.68±0.03	1.66±0.02
n-3 fatty acids	7.56±0.31	6.95±1.21	7.13±0.22
n-6 fatty acids	30.17±0.67	28.99±1.97	29.48±1.25
n-6/n-3 fatty acids	3.99±0.21	4.17±0.81	4.14±0.26

SFA (saturated fatty acid), UFA (unsaturated fatty acid), PUFA (polyunsaturated fatty acid), MUFA (monounsaturated fatty acid) Standard errors of mean.

^{y,z}Means are significantly different within the same row ($p<0.05$).

4). However, the addition of AQE led to a decrease in cohesiveness, gumminess and chewiness, and in increase in adhesiveness as the storage period increased from d 0 to d 10. Therefore, the result of textural properties of this study showed that the addition of AQE affected an increase of cohesiveness, gumminess and chewiness at d 0 of storage, while shear force was decreased through the addition of AQE and LPE at d 10 of storage.

As shown in Table 5, most fatty acids of breast meat were not influenced by the addition of dietary extracts. However, concentrations of linoleic acid were increased in both the extract-fed groups compared with the control ($p<$ 0.05).As far as we know, there are currently no reports of fatty acid compositions due to LPE or AQE supplementation in chicken meats, and whether these extracts contributed to the increase in linoleic acids in breast meat is unknown. However, it is possible that the two extracts could reduce the susceptibility of PUFA, linoleic acid to oxidation, resulting in increasing concentrations of meat linoleic acids. Moreover, our results support the findings of a previous study (Ji *et al*., 2011) which indicated that dietary peppermint (*Menthapiperita* L.) powder affects some changes in PUFA contents in broiler chicken meat. The use of plant extracts in modulating PUFA metabolism in poultry needs to be further investigated.

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