Research Note

Transfer of bioactive compounds from pasture to meat in organic free-range chickens

A. Dal Bosco,[*,1] C. Mugnai,[†] S. Mattioli,[*] A. Rosati,[‡] S. Ruggeri,[*] D. Ranucci,[§] and C. Castellini[*]

[*]*Dept. of Agricultural, Food and Environmental Science, University of Perugia, Borgo 20 Giugno, 74, 06100 Perugia, Italy;* [†]*Dept. Food Science, University of Teramo, Via C. Lerici 1, 64023 Mosciano S.A., Italy;* [‡]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, centro di ricerca per l'olivicoltura e l'industria olearia (CRA – OLI), via Nursina 2, 06049 Spoleto (PG), Italy; and* [§]*Dept. of Veterinary Medicine, Via san Costanzo, 4, 06126, Perugia, Italy*

ABSTRACT The aim of this study was to analyze the transfer of bioactive compounds from the pasture to the body and meat of organic free-range chickens and to verify the effect of these compounds on the oxidative processes of the meat. Starting at 21 d of age, 100 male naked-neck birds were divided into two homogeneous groups: an indoor group (0.12 m^2/bird) and an outdoor group (0.12 m^2/bird indoor and 10 m^2/bird of forage paddock). At slaughter (81 d of age), blood samples were collected, and the carcasses were stored for 24 h at 4°C (20 birds/group). The grass samples had higher values of carotenoids, tocopherols, and flavonoids respect to standard feed (based on dry matter comparison). The polyunsaturated fatty acid (**PUFA**) content was also greater in grass, especially the n-3 series (so named because its first double bond occurs after the third carbon atom counting from the methyl at the end of the molecule). The antioxidant profile of the grass improved the antioxidant status of the crop and gizzard contents in the outdoor chickens. The higher antioxidant intake resulted in a higher plasma concentration of antioxidants in outdoor birds; thiobarbituric acid reactive substances (**TBARs**) and the antioxidant capacity of the plasma were also better in the outdoor than the indoor group. The meat of the outdoor birds had higher levels of antioxidants, mainly due to the higher amount of tocopherols and tocotrienols. Despite the higher antioxidant protection in the drumstick of the outdoor group, the TBARs value was greater, probably due to the kinetic activity of birds, the higher percentage of PUFAs, and the peroxidability index. In conclusion, grazing improved the nutritional value of the meat (PUFA n-3 and the ratio between n-6 and n-3 PUFA) with a minor negative effect on the oxidative stability. Suitable strategies to reduce such negative effects (e.g., reduction of kinetic activity in the last days of rearing) should be studied.

Key words: chickens, organic system, antioxidants, pasture

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INTRODUCTION

In recent years, relevant efforts to improve the polyunsaturated fatty acid (PUFA) and nutritional properties of animal products have been made (Bourre, 2005; Palmquist, 2009). The main strategy to increase PUFAs in the meat is to add them to the feed. However, such enrichment increases the oxidative risk in the animal's tissues and requires suitable antioxidant protection (Lauridsen et al., 1999; Oliveira et al., 2012).

Free-range chickens acquire several bioactive compounds from the pasture (e.g., PUFA, vitamins, and pigments) (Sossidou et al., 2015) that could affect meat characteristics.

The effect of foraging in birds is complex, depending on the balance between anti- and pro-oxidant compounds and on the kinetic activity of the animal, which drives oxidation (Sossidou et al., 2011). Oxidation may be low with grass intake because green forage has high levels of tocopherols and tocotrienols (Kerry et al., 2000), carotenoids, vitamin C, and polyphenols (Mugnai et al., 2013). The current poultry rearing systems do not emphasize outdoor grazing areas for poultry (Horsted and Hermansen, 2007); however, studies on crop content indicate that slow-growing poultry strains have a considerable intake of pasture and other accessible foods (Mwalusanya et al., 2002; Dal Bosco et al., 2014).

It is therefore crucial to assess the intake and the nutritional relevance of pasture, the ability to transfer the above-mentioned compounds into poultry products, and the development of suitable feed for free-range birds.

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[1]Corresponding author. alessandro.dalbosco@unipg.it

Some studies have shown a high capacity of α-tocopherol and other bioactive compounds to transfer from the pasture to the eggs (Jiang et al., 1994; Lopez-Bote et al., 1998; Surai et al., 1998; Mugnai et al., 2013), but the contribution of grass to the oxidative stability of the meat from free-range chickens has not been established. The present study analyzes the transfer of bioactive compounds (antioxidants, PUFA) from the pasture to the muscle in free-range chickens and the effect on meat oxidative processes.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The experiment was carried out in the spring and summer on a farm near Spoleto, Italy, where 100 day-old male naked-neck birds (Avicola Berlanda, Italy) were reared according to the Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes.

The chicks were kept for 20 d in an environmentally controlled poultry house with temperatures ranging from 20 to 32°C and with a relative humidity ranging from 65 to 75%. An incandescent light (30 lux) placed at bird level was used for heating and illumination. The chicks were vaccinated against Marek and Newcastle disease and coccidiosis (Paracox®).

All chickens were fed the same starter (1 to 21 d) and a grower-finisher (22 to 81 d) diet containing 100% organic ingredients. The diets were formulated according to the NRC (1994) recommendations (Table 1). The feed and water were provided *ad libitum*.

Table 1. Formulation (%), chemical composition (% DM) and energetic value (MJ kg^{-1}) of the diets and pasture.

	Starter	Finisher	Pasture
Ingredients			
Maize	52.0	46.0	
Full fat soybean	30.5	12.5	
Wheat	-	20.0	
Soybean meal[1]	9.0	14.0	
Alfalfa meal	2.8	2.8	
Gluten feed	3.0	2.0	
Vitamin-mineral premix[1]	1.0	1.0	
Dicalcium phosphate	1.0	1.0	
Sodium bicarbonate	0.5	0.5	
NaCl	0.2	0.2	
a) Chemical composition			
b) Dry matter	90.8	90.8	25.3
c) Crude protein	22.3	18.0	13.2
Ether extract	7.95	4.98	5.88
Crude fibre	4.67	4.01	23.2
Ash	5.76	5.59	7.86
NDF - Neutral Detergent Fibre	10.7	10.1	43.2
ADF - Acid Detergent Fibre	5.58	5.06	31.2
Cellulose	4.22	3.56	26.4
ADL - Acid Detergent Lignin	1.03	1.11	4.82
d) Hemicellulose	5.16	5.05	12.0
Metabolizable Energy[2]	12.5	12.9	5.7

[1]Amount per kg: Vit. A 11,000 IU; Vit. D_3 2,000 IU; Vit. B_1 2.5 mg; Vit. B_2 4 mg; Vit. B_6 1.25 mg; Vit. B_{12} 0.01 mg; α-tocopheryl acetate 30 mg; Biotin 0.06 mg; Vit. K 2.5 mg; Niacin 15 mg; Folic acid 0.30 mg Panthotenic acid 10 mg; Choline chloride 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

[2]Estimated by Carre and Rozo (1990).

Table 2. Floristic composition of pasture.

Phleum sp.	*Avena fatua*
Dactylis glomerata	*Sanguisorba minor*
Santolina sp.	*Linaria sp.*
Agropyron sp.	*Picris hieracioides*
Calamintha nepeta	*Reichardia picroides*
Rubus sp.	*Daucus carota*
Chondrilla juncea	*Geranium sp.*
Cichorium intybus	*Euphorbia sp.*
Centaurea sp.	*Campanula rapunculus*
Convolvulus sp.	*Portulaca oleracea*
Plantago lanceolata	*Petrorhagia prolifera*

At 21 d of age, the chicks were divided into two homogeneous groups:

- Indoor group, housed in an indoor pen (0.12 m^2/bird) with a natural temperature (20.5 ± 4.7°C and the relative humidity 65 to 75%) and a photoperiod of 12 h;
- Outdoor group, organically reared according to EU Regulation 834/07 and housed in a similar indoor pen (0.12 m^2/bird) with access to a forage paddock (10 m^2/bird); birds were confined indoors during the night.

The floristic and chemical composition in each pasture pen (three samples per pen and per group) was assessed by sampling a 1 m^2 fenced area by cutting with garden scissors (at 2 cm above the soil) before the onset of the trial. The plants in the mixture were manually separated into groups, and the species were identified by macroscopic examinations (Table 2).

Analytical Determinations of Diets and Pasture

The analyses of the chemical composition of the diets were carried out according to the AOAC methods (1995), and the metabolizable energy was estimated (Carrè and Rozo, 1990). The fatty acid profile of the feed and pasture was determined by gas chromatography after lipid extraction according to Folch et al. (1957). In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatized by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80°C for 1 h, the methyl esters were extracted with petroleum ether, and 1 μL was injected into the gas chromatograph (Mega 2 - model HRGC; Carlo Erba, Milan, Italy), which was equipped with a flame ionization detector. The separation of the fatty acid methyl esters (FAMEs) was performed using an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions upon the column injection of the 1 μL sample were as follows: the temperatures of the injector and detector were set at 270°C and 280°C, respectively, and the detector gas flows were H_2

at 50 mL/min and air at 100 mL/min. The oven temperature was programmed to provide a suitable peak separation, as follows: the initial oven temperature was set at 130°C; this temperature increased at a rate of 4.0°C/min to 180°C and was held for 5 min; the temperature was then increased at a rate of 5.0°C/min to 230°C; and the oven was held at the final temperature for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL/min. The individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the fatty acid pattern of the feed and pasture was expressed as g/kg of dry matter (DM). The mean value of each fatty acid was used to calculate the sum of the polyunsaturated (PUFA) fatty acids of different series (n-3 and n-6).

The tocopherols (α-tocopherol and its isoforms β+γ and δ; α, β+γ-tocotrienol) and some antioxidant (retinol, lutein, zeaxanthin and β-carotene) levels were measured according to Schuep and Rettenmeier (1994). Briefly, 1 g of finely ground feed sample and pasture was weighed and homogenized in 1 mL of water and 4 mL of an ethanol solution of 0.06% BHT (butylated hydroxytoluene). The mixture was saponified with water KOH (60%) at 70°C for 30 min and extracted with hexane/ethyl acetate (9:1, v/v). Following centrifugation, 2 mL of the supernatant was transferred into a glass tube, dried using N_2 and re-suspended in 200 μL of acetonitrile. The pellet was re-extracted two times. A 50 μL volume of filtrate was then injected into the HPLC/FD (pump model Perkin Elmer series 200, equipped with an autosampler system, model AS 950-10, Tokyo, Japan) on an Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 μm particle size; CPS Analitica, Milan, Italy). The flow rate was 1.5 mL/min. All of the tocopherols and tocotrienols were identified using a FD detector (model Jasco, FP-1525: excitation and emission wavelengths of 295 nm and 328 nm, respectively) and quantified using external calibration curves prepared with increasing amounts of pure standard solutions (Sigma-Aldrich, Bornem, Belgium) in ethanol. The carotenoids were analyzed with the same HPLC column; the solvent system consisted of solution A (methanol/water/acetonitrile 10/20/70, v/v/v) and solution B (methanol/ethyl acetate 70/30, v/v). The flow rate was 1 mL/min, and the elution program was a gradient starting from 90% A in a 20 min step to 100% B and then a second isocratic step of 10 min. The detector was an UV-VIS spectrophotometer (Jasco UV2075 Plus) set at λ 325 nm and 450 nm for retinol and lutein/zeaxanthin/beta-carotene, respectively. The different carotenoids were identified and quantified by comparing the sample with pure commercial standards in chloroform (Sigma-Aldrich, Steinheim, Germany; Extrasynthese, Genay, France). The other carotenoids (violaxanthin, neoxanthin, anteroxanthin, cryptoxanthin) were extracted with acetone and then filtered through a Millipore filter (0.2 μm). The extract was injected into a HPLC system (Jasco PU-880) equipped with a reversed phase column (Hypersil ODS 5 mm, 250 × 4.6 mm; Tracer) on a pre-C18 column (Luna, Phenomenex). The solvent system consisted of solution A (methanol/water/acetonitrile 10/05/85, v/v/v) and solution B (methanol/ethyl-acetate 70/30, v/v). The flow was 1 mL/min and the elution program was a gradient starting from 90% of A at intervals of 20 minutes up to 100% of B. The detector consisted of a UV-VIS spectrophotometer (Jasco 875-UV) with λ set at 436 nm. The different carotenoids were identified and quantified by comparison with commercial pure standards (Sigma-Aldrich, Steinheim, Germany; Extrasynthese, Genay, France; Menghini at al., 1992). The total carotenoids, chlorophylls and flavonoids, after extraction with methanol and centrifugation, were determined spectrophotometrically according to the AOAC procedures (1995).

Blood Sample Collection, Analytical Determinations and Carcass Dissection

The blood samples were collected from the brachial vein of 20 chickens per group at sunset (8.00 PM) in heparinized vacutainers. After collection, blood samples were immediately taken to the laboratory, where they were centrifuged at 1,500 × *g* for 10 min at +4°C to measure the *in vivo* parameters and were then frozen at −80°C until analysis.

The extent of blood lipid peroxidation was evaluated by a spectrophotometer (set at 532 nm, Shimadzu Corporation UV- 2550, Kyoto, Japan), which measured the absorbance of thiobarbituric acid reactive substances (TBARs), using a tetraethoxypropane calibration curve in sodium acetate buffer (pH = 3.5) (Dal Bosco et al., 2009). The results are expressed as malondialdehyde (MDA) nmol MDA/mL of plasma.

The tocopherol, tocotrienol, retinol and carotenoid levels in the plasma were assessed according to Schuep and Rettenmeier (1994) using the HPLC method (Jasco, pump model PU-1580, equipped with an autosampler system, model AS 950-10, Tokyo, Japan) on a Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 μm particles size; CPS Analitica, Milan, Italy). Antioxidant compounds were identified using the same method described previously for feed and pasture.

After 12 h of feed withdrawal, the marked chickens were slaughtered (at 81 d of age) in the processing plant of the farm at 9 AM.

The chickens were stunned by electrocution (110 V; 350 Hz) before killing. After bleeding, the carcasses were immersed in the scalder (56.5°C for 1 minute), plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, esophagus and full crop) and stored for 24 h at 4°C. Carcasses were handled following the standard procedure of World's Poultry Science Association (1984), and later the breasts and drumsticks were isolated.

The crop and gizzard contents, without the grit, was weighed and analyzed to evaluate the antioxidant content and the PUFA profile, following the same methods used for the pasture samples.

Meat Antioxidants and Extent of Oxidation Processes

The fatty acid composition of the breast and drumstick was determined on lipids extracted from samples of approximately 5 g in a homogenizer with 20 mL of 2:1 chloroform/methanol (Folch et al., 1957), followed by filtration through a Whatman No. 1 filter paper. The fatty acids were determined as methyl esters using a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Milano, Italy) and a D-B wax capillary column (25 mm ø, 30 m long). The fatty acid percentages were calculated using the Chrom-Card software. Individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the fatty acid pattern of the meat was expressed as a percentage. The separation and quantification of each fatty acid were performed as described above. The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

$$\begin{aligned}\text{PI} = {} & (\%\ \text{monoenoic} \times 0.025) + (\%\ \text{dienoic} \times 1) \\ & + (\%\ \text{trienoic} \times 2) + (\%\ \text{tetraenoic} \times 4) \\ & + (\%\ \text{pentaenoic} \times 6) + (\%\ \text{hexaenoic} \times 8)\end{aligned}$$

The tocopherol (α-tocopherol and its isoforms β+γ and δ; α, β+γ tocotrienol), retinol and carotenoid content of the meat were quantified using HPLC (Hewavitharana et al., 2004).

β- and γ-tocopherol and tocotrienol co-eluted, showing a single peak in the intermediate position between that of the α-T and δ-T peaks on the chromatogram. For this reason, β-T and γ-T could not be quantified separately. Further, β-isoforms have rarely been found in human or animal plasma, and when present, they were frequently above the detection limit of 0.005 μg/L (Mourvaki et al., 2008).

In particular, 5 mL of distilled water and 4 mL of ethanol were added to a 2 g sample of meat and then vortexed for 10 sec. After mixing, 4 mL of hexane containing BHT (200 mg/L) was added, and the mixture was carefully shaken and centrifuged. An aliquot of the supernatant (3 mL) was dried under a stream of nitrogen and then dissolved in 300 μL of acetonitrile. Fifty μL was injected into the HPLC system (Jasco, pump model PU-1580, equipped with an autosampler system, model AS 950-10, Tokyo, Japan) as described for feed and plasma. The extent of muscle lipid oxidation was evaluated by a spectrophotometer set at 532 nm (Shimadzu Corporation UV-2550, Kyoto, Japan), according to the modified method of Ke et al. (1977), which measures the absorbance of TBARs. The oxidation products were quantified as malondialdehyde equivalents (mg MDA/kg muscle) using a 1,1,3,3-tetraethoxypropane calibration curve.

Table 3. Means ± standard deviation of carotenoids (mg/kg of DM), tocopherols and tocotrienols (mg/kg of DM), chlorophyll (mg/kg of DM), flavonoids (mg/kg of DM) and PUFA (g/kg of DM) amounts in feed and pasture.

	Finisher diet	Pasture
Violaxanthin	0.51 ± 0.08	29.8 ± 2.03
Lutein	3.15 ± 0.36	13.9 ± 1.21
β-carotene	0.69 ± 0.15	26.1 ± 1.98
Neoxanthin	0.11 ± 0.03	3.38 ± 0.28
Zeaxanthin	6.29 ± 1.25	1.88 ± 0.18
Anteroxanthin	0.08 ± 0.01	1.01 ± 0.16
Cryptoxanthin	0.20 ± 0.04	1.35 ± 0.31
Σ carotenoids	9.35 ± 1.03	59.9 ± 2.98
α-tocopherol	45.9 ± 3.02	113.6 ± 14.6
β, γ-tocopherol	22.8 ± 1.59	18.2 ± 1.58
δ- tocopherol	2.83 ± 0.67	1.36 ± 0.31
α-tocotrienol	5.20 ± 0.78	51.2 ± 3.47
β, γ-tocotrienol	3.92 ± 0.64	60.6 ± 2.90
Σ tocopherols and tocotrienols	80.65 ± 3.69	244.96 ± 24.3
Chlorophyll	0.02 ± 0.004	49.5 ± 3.16
Flavonoids	184.9 ± 12.0	605.4 ± 44.9
Σ PUFA n-6	13.6 ± 3.10	3.33 ± 0.38
Σ PUFA n-3	1.67 ± 0.27	11.6 ± 1.89
n-6/n-3	8.14	0.29

Each value represents the mean value of three determinations.

Statistical Analyses

A linear model (STATA, 2005, procedure GLM) was used to evaluate the fixed effect of the rearing system (Outdoor *vs.* Indoor). The differences between groups were assessed by ANOVA using a Bonferroni multiple *t*-test. The differences with $P < 0.05$ value were considered statistically significant.

RESULTS

The floristic composition of the pasture present in the outdoor pen is shown in Table 2. The total ground cover was 54%, with the most abundant species being *Phleum sp., Dactylis glomerata, Santolina sp., Agropyron sp., Calamintha nepeta,* and *Rubus sp.*, which represented 75% of the floristic composition.

Violaxanthin was the most represented carotenoid in the pasture, followed by β-carotene and lutein, whereas the finisher poultry diet contained mainly zeaxanthin and lutein (90% of the carotenoids; Table 3). The total carotenoids were almost six fold greater in the pasture samples compared to the feed. As expected, the pasture had a higher content of tocopherols, tocotrienols (mainly α-tocopherol, α-tocotrienol and β+γ-tocotrienol), chlorophyll and flavonoids compared to the feed.

The PUFAs of the n-3 series were higher in the pasture than the feed (11.6 vs. 1.67 g/kg of dry matter), whereas the PUFAs of the n-6 series were higher in

Table 4. Antioxidants and PUFA concentration of crop and gizzard content of chickens.

		indoor	outdoor	*P*	Pooled SE
Crop					
α- tocopherol	μg/g	2.79	32.88	**	1.70
β, γ- tocopherol	″	1.28	3.07	n.s.	0.40
δ- tocopherol	″	1.23	2.77	*	0.70
α-tocotrienol	″	0.37	1.00	*	0.28
β, γ-tocotrienol	″	1.42	1.68.	n.s.	0.66
Lutein + Zeaxanthin	″	1.43	2.69.	*	0.98
β-carotene	″	0.01	0.12	*	0.07
Σ antioxidants	″	8.53	44.21	**	2.80
Σ PUFA n-6	% of total FA	13.02	9.98	n.s.	3.06
Σ PUFA n-3	″	1.65	4.72	*	1.03
n-6/n-3	″	7.89	2.11	*	2.76
Gizzard					
α- tocopherol	μg/g	1.53	21.46	**	1.21
β, γ- tocopherol	″	1.37	3.23	**	0.59
δ- tocopherol	″	1.74	2.82	n.s.	0.51
α-tocotrienol	″	0.18	0.84	*	0.06
β, γ-tocotrienol	″	0.70	1.23	n.s.	0.43
Lutein + Zeaxanthin	″	0.74	2.13	*	0.71
β-carotene	″	0.01	0.16	*	0.02
Σ antioxidants	″	6.27	31.90	**	3.55
Σ PUFA n-6	% of total FA	11.99	8.58	*	3.01
Σ PUFA n-3	″	1.43	3.32	*	0.98
n-6/n-3	″	8.38	2.58	*	2.28

N = 20 per group. **: $P < 0.01$; *: $P < 0.05$.

FA: fatty acids; n.s.: not significant.

Table 5. Antioxidant content of plasma and oxidative status of chickens.

		indoor	outdoor	*P*	Pooled SE
α-tocopherol	nmol/mL	7.99	18.98	**.	1.90
β, γ- tocopherol	″	0.38	0.45	n.s.	0.17
δ- tocopherol	″	0.58	2.27	**	0.31
α-tocotrienol	″	0.26	0.35	n.s.	0.20
β, γ-tocotrienol	″	0.03	0.03	n.s.	0.01
Lutein + Zeaxanthin	″	2.25	3.75	**	0.82
Retinol		1.64	2.49	**	0.86
Σ antioxidants	″	13.13	28.32	**	8.21
TBARs	nmol MDA/mL	21.29	18.67	*	2.44
Antioxidant capacity	μmol HClO/mL	638.2	717.9	*	21.2

N = 20 per group. **: $P < 0.01$; *: $P < 0.05$.

MDA: Malonodialdehyde; n.s.: not significant.

the feed, and consequently the n-6/n-3 ratio was much higher in the feed.

The experimental groups greatly differed regarding the antioxidant levels in the crop and gizzard contents (Table 4). The outdoor group always had the highest values of antioxidants (44.21 vs. 8.53 and 31.90 vs. 6.27 μg/g in the crop and gizzard, respectively).

The PUFA profile reflected the dietary intake in both chicken groups. The PUFA n-3 was higher in the outdoor group in both the crop and gizzard compared to the indoor group (4.72 vs. 1.65 and 3.32 vs. 1.43% of total FA, respectively), whereas the PUFA n-6 was lower (9.98 vs. 13.02% of total FA in crop; 8.58 vs. 11.99 in gizzard).

Concerning the antioxidant content of the plasma (Table 5), pasture availability increased the concentration of the main antioxidant compounds, with an almost two-fold greater level of a-tocopherol (18.98 vs. 7.99 nmol/mL) and retinol (2.49 vs. 1.64 nmol/mL).

The high level of antioxidants in the outdoor birds improved the *in vivo* antioxidant capacity of the birds (717.9 vs. 638.2 μmol HClO/mL) and reduced the TBARS values (18.67 vs. 21.29 nmol MDA/mL).

Regarding the antioxidant compounds in the meat (Table 6), the outdoor group had higher amounts of tocopherols and tocotrienols, both in the breast and drumstick. In particular, very high levels of a-tocopherol, the major vitamin E homologue, were found in the two muscle groups (951.18 vs. 539.28 ng/g and 1358.11 vs. 652.54 ng/g, respectively, in the breast and drumstick). Only δ-tocopherol was lower in the breast meat of the outdoor group compared to the indoor group.

Although outdoor chickens had a higher concentration of carotenoids and/or their metabolic product (retinol) in each matrix analyzed (diet, crop and gizzard contents, and plasma), no significant differences were found in the meat.

Table 6. Antioxidant content, polyunsatured fatty acids profile and oxidative status of breast and drumstick muscles.

		indoor	outdoor	P	Pooled SE
Breast					
α-tocopherol	ng/g	539.28	951.18	**	68.28
β, γ-tocopherol	″	33.71	29.61	n.s.	5.05
δ-tocopherol	″	15.92	8.77	*	2.50
α-tocotrienol	″	38.81	73.47	**	5.10
Lutein + Zeaxanthin	″	36.75	41.57	n.s.	6.70
Retinol	″	18.31	17.92	n.s.	0.68
Σ antioxidants	″	685.78	1,122.52	**	58.10
TBARs	mg MDA/kg	0.16	0.20	n.s.	0.08
Σ PUFA n-6	% of total FA	31.63	27.48	*	1.29
Σ PUFA n-3	″	2.34	3.76	*	0.36
n-6/n-3	″	13.52	7.31	*	2.56
Peroxidation index	″	59.87	67.11	**	3.28
Drumstick					
α-tocopherol	ng/g	652.54	1,358.11	**	24.20
β, γ-tocopherol	″	46.94	39.35	n.s.	9.02
δ-tocopherol	″	17.74	24.14	**	7.24
α-tocotrienol	″	40.90	107.44	*	8.70
β, γ-tocotrienol	″	0.02	0.03	n.s.	0.01
Lutein + Zeaxanthin	″	43.04	53.30	n.s.	20.26
Retinol	″	33.08	30.53	n.s.	3.46
Σ antioxidants	″	840.26	1,612.90	**	91.02
TBARs	mg MDA/kg	0.15	0.19	*	0.03
Σ PUFA n-6	% of total FA	27.61	23.75	*	3.58
Σ PUFA n-3	″	1.75	2.86	*	0.39
n-6/n-3	″	15.78	8.30	n.s.	8.02
Peroxidation index	″	50.89	54.52	*	3.35

N = 20 per group. **: $P < 0.01$; *: $P < 0.05$.

FA: fatty acids; MDA: Malonodialdehyde; n.s.: not significant.

However, this greater antioxidant concentration was not able to completely counterbalance the oxidative processes of the outdoor group. Indeed, the extent of the oxidative processes was the same in the breast meat and significantly higher in the drumstick (0.19 vs. 0.15 mg MDA/kg) of outdoor birds.

The PUFA profile of the meat showed a higher level of n-6, both in the breast and drumstick of the indoor group (31.63 vs. 27.48 and 27.61 vs. 23.75% of total FA, respectively) whereas, the outdoor birds showed a higher n-3 (3.76 vs. 2.34, and 2.86 vs. 1.75%, respectively, in breast and drumstick). However, given the greater content of total PUFA, the PI was significantly higher in the outdoor than the indoor group (67.11 vs. 59.87, and 54.52 vs. 50.89, in breast and drumstick).

DISCUSSION

Pasture and poultry feed have different compositions (Castellini et al., 2006; Mugnai et al., 2009), and in particular, pasture has a low dry matter and high fiber content, as well as increased tocopherols, carotenoids, and flavonoids compared to feed. According to Lopez-Bote et al. (1998), fresh grass has approximately 6 times more α-tocopherols and 2 to 8 times more carotenoids than standard poultry feed. Accordingly, pasture consumption is crucial for organic chickens as a furnisher of antioxidant compounds because some national rules ban the addition of synthetic vitamins to the feed.

According to Ponte et al. (2008), the terpene content of grass is mostly characterized by α-tocopherol, γ-tocotrienol (diterpenes) and β-carotene (tetraterpene), which act synergistically to prevent radical formation in biological systems (Olmedilla et al., 1992).

Dietary intake of antioxidants increases the antioxidant content of the plasma in outdoor birds, confirming our previous studies (Castellini et al., 2002). On the contrary, Tagliabue et al. (2012) observed a higher TBARS value associated with a lower α-tocopherol content in outdoor chickens compared to indoor chickens. The discrepancy between our results could derive from the different genotype of the chickens (Ross), which are known to have low kinetic activity and poor foraging abilities (Dal Bosco et al., 2010).

Although it is difficult to precisely estimate grass ingestion because the more fibrous particles tend to move more slowly toward the crop, proventriculus and gizzard, free-range birds had a relevant intake of grass. Dal Bosco et al. (2014) found that in the summer, the grass ingestion ranges from approximately 15 to 43 g of DM/d per bird according to the environmental enrichment of the pasture (e.g., tall grass or olive trees).

It is interesting to note that β-carotene, a lipid-soluble provitamin, was found in the crop and gizzard contents but was under the detection limit in the plasma and meat samples, which is in agreement with Ponte et al. (2008). Carotenoids are normal constituents of the blood and tissues of humans, cows, birds, fish, and some crustaceans (Bendich and Olson, 1989). Some

carotenoids (e.g., α- and β-carotene) are converted to retinol (an isoform of Vitamin A), primarily in the intestinal mucosa but also, to some extent, in the liver and other organs (Olson, 1989), whereas lutein and lycopene do not have the same metabolism. Unlike mammals, fish and birds can also form vitamin A from the xanthophylls: astaxanthin, canthaxanthin, and zeaxanthin (Olson, 1989).

The prevalence of α-tocopherol in meat is well known and is due to the 10-fold preference of the tocopherol-binding protein for α- rather than γ-homologues, which are the most common vitamin E precursors in plant foods (Decker et al., 2000). Even Sun et al. (2012), comparing standard chickens with free-range chickens reared on alpine steppe (dominated by the grass species *Stipa* spp., *Agropyron cristatum*, and *Leymus secalinus*), observed that rearing conditions affected the α-tocopherol content of the meat with an important effect on lipid oxidation reduction and nutritional value during storage (Gatellier et al., 2004).

In our study, despite the improved antioxidant profile of the outdoor group, the oxidative processes of the meat were increased, especially in the drumstick muscle. This effect was probably due to the higher kinetic activity of the outdoor chickens and the resulting process of oxidation (Castellini et al., 2002). Furthermore, the oxidative status of the meat also depends on its lipid profile. Unsaturated fatty acids (mainly PUFA) are more easily oxidized than saturated ones; the outdoor chicken meat was richer in PUFAs, resulting in a higher peroxidability index (54.52 vs. 50.89) compared to indoor birds.

The balance between the oxidative stress due to the kinetic activity and antioxidant capacity is a very dynamic equilibrium wherein genetics, feed and grass characteristics play a relevant role.

Pasture availability improved the n-3 fatty acid content of the meat. A high dietary concentration of α-linolenic acid increases its concentration in the meat (Rymer and Givens, 2005). The production of meat with a higher nutritional value and a healthy fatty acid profile is therefore an important topic for the poultry meat industry. Great attention has been paid to the essential fatty acids (EFA), linolenic (LA) and α-linolenic (ALA) acids, which cannot be synthesized by humans and are therefore crucial components of the human diet. Both LA and ALA may be converted by elongation and desaturation into their long-chain metabolites (LC-PUFA), such as eicosapentaenoic (EPA, C20:5n-3), docosapentaenoic (DPA, C22:5n-3), docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (ARA, C20:4n-6). Several trials have demonstrated that it is possible to enrich poultry meat and eggs with LC-PUFA through dietary strategies (Rossi et al., 2013; Fraeye et al., 2012; Woods and Fearon, 2009) or with free-range/organic rearing systems (Mugnai et al., 2013).

This rearing strategy also leads to improvement of the n-6/n-3 ratio and the nutritional qualities of the products.

CONCLUSIONS

Raising free-range chickens modified the bioactive compound content both in the plasma and in the meat, confirming that grazing improves the lipid and antioxidant profile of the meat.

In particular, the higher availability of the PUFA n-3, through the pasture intake, improved the fatty acid profile of the meat. This also resulted in a higher level of oxidation, probably due to the greater kinetic activity of free-range animals. However, the difference was significant only for the drumstick, while in the breast meat, the major cut of the poultry carcass, there was no significant difference.

We may conclude that grazing improved the meat quality in terms of PUFA n-3 content, matching the expectations of consumers regarding the quality of organic poultry meat.

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