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Effect of transport length on *in vivo* oxidative status and breast meat characteristics in outdoor-reared chicken genotypes

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ABSTRACT

The aim of the study was to evaluate the effect of transport length on *in vivo* oxidative status and breast meat characteristics in two chicken genotypes reared under free range conditions. A total of 200 male chicks, 100 from fast-growing (Ross 308) and 100 from medium-growing (Naked Neck) strain were used. Fifty-six of these, 28 for genotype, before slaughtering, were randomly allocated to 2 pre-slaughter conditions: absence (0 h) or 4 h of transport. The transport length significantly affected the *in vivo* oxidative status of broiler greatly reducing the α and δ-tocopherol, retinol and lutein + zeaxanthin content of plasma, and increased the oxidative stress (thiobarbituric acid reactive substances, TBARS) in both strains. Concerning meat quality, the pH (0, 2 and 24 h *post-mortem*) of breast muscles of chickens transported for 4 h, showed higher values, and respect to strains, Naked Neck had lower values. The pH values were negatively correlated with the lightness (2–24 h) and the shear force of meat. The transport length significantly affected the fatty acid profile of breast muscle, with a decrease in polyunsaturated fatty acids and an increase in TBARS value. Even the antioxidants content of breast was reduced by chicken transport (α-tocotrienol, α-, δ-tocopherol and lutein + zeaxanthin), especially in Naked Neck birds. In conclusion, the results indicate that transport for 4 h prior to slaughter, negatively affect the meat quality of poultry. Slow-growing chickens seem more sensible to stress transport due to the higher kinetic behaviour of these strains.

Introduction

The stress experience is one of the factors affecting the quality of meat; during the transportation from the farm to the slaughterhouse, broilers are exposed to a variety of potential stressors, including thermal imbalances, vibrations, withdrawal of water, social disruption and noise (Mitchell & Kettlewell 1998). A number of studies focused on the effects of transport stress on blood traits (Yalcïn et al. 2004; Nijdam et al. 2005). Zhang et al. (2009) reported that transportation of broilers caused an increase in glycogenolysis resulting in glycogen decrease in both breast and thigh muscles. In addition, transport stress was associated with an enhanced skeletal muscle energy metabolism, to mitochondrial superoxide production, acceleration of lipid peroxidation and to induction of cellular damages (Zhang et al. 2010).

In chickens, stress and kinetic activity before slaughter are also involved in pH variations in the early

stages of rigour (Debut et al. 2003), whereas the final pH of meat mainly depends on the glycogen content at the time of slaughter (Berri et al. 2005, 2007).

Time in transit to slaughter is a major concern involving welfare and meat quality. Several studies have considered the effect of transport length and distance on animal welfare and resultant meat quality of broiler, but data on the interaction (genetic strain vs transport length) are lacking.

Indeed, it is described that the effect of stress could be different in fast- and slow-growing strains (Rimoldi et al. 2015). Fast-growing strains tended to produce meat with a slower pH decline, higher ultimate pH and consequently higher water-holding capacity (WHC) (Le Bihan-Duval et al. 1999; Berri et al. 2007).

On the other hand, it is reported (Berri et al. 2007) that slow-growing strains suffer more the lag phase between the catching and the slaughter due to high kinetic activity (i.e. wing flapping) during transport and

slaughtering. Accordingly, when broilers are subjected to stressful conditions, the meat of slow-growing chickens could be different from that of standard broilers.

This study investigated the influence of pre-slaughter transport length in different genetic strains (fast- vs slow-growing); the physiological traits were studied in order to estimate the relationships between meat quality and *in vivo* oxidative status of the birds.

Materials and methods

Birds and farming system

This trial was conducted at the experimental section of University of Perugia (Italy) during Spring of 2015, using 200 male chicks, 100 from fast-growing (Ross 308) and 100 from medium-growing (Naked Neck) strain, furnished by a commercial poultry farm (Avicola Berlanda, Italy) at 20 days of age; birds were raised separately in an environmentally controlled poultry house (0.12 m^2/bird) with temperature ranging from 32 °C to 20 °C and relative humidity from 70% to 65%. At 21 days of age, chicks were transferred to straw-bedded indoor pens (0.10 m^2/bird), each equipped with feeders and drinkers and with free access to forage paddock (4 m^2/bird). Each strain was replicated in four pens containing 25 chicks each. Birds were confined to indoor pens during night. The chicks were vaccinated at hatch against Marek's disease and Newcastle disease. Chicks were fed starter (1–21 d) and finisher (22 d to slaughter: 81 d) diets. Access to feed and water was freely available, and all diets were formulated to contain adequate nutrient levels as defined by the NRC (1994).

Pre-slaughter and slaughtering conditions

Before slaughter, 28 birds per strain were taken and putted randomly in crates (7 birds per crate $-73 \times 53 \times 26$ cm) and allocated to 2 pre-slaughter conditions:

- absence of transport (0 h);
- 4 h of transport was chosen as examples of stressful pre-slaughter conditions because is commonly observed in commercial practice. For transport stress evaluation, bird crates were placed and driven for 4 h in a truck (temperature inside the crate ∼25°C to 28 °C). Chickens were weighted before loading and at the end of trip.

Birds not submitted to transport were taken out of the rearing room and placed in crates that were immediately brought to the slaughterhouse where were sacrificed within 30 min from the capture.

Animal submitted to transport was immediately slaughter at the arrival to the abattoir, without a resting phase.

All birds were slaughtered 12 h after feed withdrawal. The animals were electrically stunned (110 V; 350 Hz) before killed. After bleeding, the carcasses were placed in hot water (56.5 °C for 1 min) and then plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, oesophagus, and full crop), and stored for 24 h at 4 °C.

In vivo oxidative status of birds

Blood samples were taken at the slaughtering in 56 chickens (14 chicken/strain/transport length) and collected in heparinized vacutainers to measure the *in vivo* oxidative status. After collection, blood samples were immediately sent to the laboratory of Department of Agricultural, Food and Environmental Science where they were centrifuged at $1500 \times g$ for 10 min at +4 °C and 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), and a tetraethoxypropane calibration curve in sodium acetate buffer (pH = 3.5) (Dal Bosco et al. 2009). The results were expressed as nmol of malondialdehyde (MDA)/ml of plasma.

The tocopherols (α-tocopherol and its isoforms β, γ, δ and α-tocotrienol) and some antioxidant compounds (retinol, lutein + zeaxanthin) level was measured according to Schuep and Rettenmeier (1994). Briefly, 0.2 ml of plasma was mixed with 1 ml of water and 4 ml of ethanol solution of 0.06% BHT. 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 supernatant was transferred into a glass tube, dried under 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, Jasco, Tokyo, Japan) on a Sinergy Hydro-RP column (4 µm, 4.6 × 100 mm; Phenomenex, Bologna, Italy). The flow rate was 2 ml/min. All tocopherols and tocotrienols were identified using a FD detector (model Jasco, FP-1525 – excitation and emission wavelengths of 295 and 328 nm, respectively) and quantified using external calibration curves prepared with increasing amounts of pure

standard solutions (Sigma-Aldrich, Bornem, Belgium) in ethanol. Carotenoids (retinol, lutein + zeaxanthin) were analysed with the same HPLC column; the solvent system consisted of a solution A (methanol/water/acetonitrile 10/20/70, 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% 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 and 450 nm for retinol and lutein + zeaxanthin 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).

Physical and chemical characteristics of breast meat

After bleeding, carcasses were plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, oesophagus and full crop) and stored for 24 h at +4 °C. Head, neck, legs, edible viscera (heart, liver and gizzard) and fat (perivisceral, perineal and abdominal) were removed in order to obtain the ready-to-cook carcass (ASPA Commission 1996).

From the refrigerated carcasses (24 h at 4 °C), the breast muscles were excised.

Moisture, ash and total nitrogen were assessed by using the AOAC methods (1995 - N. 950.46B, 920.153 and 928.08, respectively). Total protein was calculated by Kjeldahl using a 6.25 conversion factor. Total lipids were extracted in duplicate from 5 g of each homogenized sample and calculated gravimetrically (Folch et al. 1957).

Ultimate pH (0, 2 and 24 h) was measured with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA) after homogenization of 1 g of raw muscle for 30 s in 10 ml of 5 M iodoacetate (Korkeala et al. 1986). The 0 h sample was collected immediately after feather removal and before evisceration.

The WHC was estimated by placing 1 g of whole muscle on tissue paper inside a tube and centrifuging for 4 min at 1500×g. The water remaining after centrifugation was quantified by drying the samples at 70 °C overnight. WHC was calculated as follows: (weight after centrifugation – weight after drying)/initial weight ×100 (Castellini et al. 1998).

The cooking loss (CL) was measured on samples as described by Honikel (1998). For CL determination, meat samples (6.0 × 6.0 × 2.5 cm; average weight: 20.0 ± 2.3 g) were held in plastic bags, then cooked in a water-bath at 80 °C for 1 h and finally cooled under running tap water for 30 min. The CL was estimated as the percentage of the weight of the cooked samples (cooled for 30 min to about 15 °C and dried on the surface with a paper towel) with respect to the weight of the raw samples.

At 2 and 24 h post-mortem, L* value (degree of lightness) was measured on the cut surface of each fillet using a tristimulus analyser (Minolta Chroma meter CR-200, Osaka, Japan), following the CIELAB colour system (1976).

The shear force was evaluated on three cylindrical cores (1.25 cm Ø; 2 cm length) obtained from the midportions of the CL samples. Three measures for each cores were performed by cutting samples perpendicular to the direction of the fibres, using an Instron, model 1011 (INSTRON Instrument, Norwood, MA; 50 kg loading range, shearing velocity 100 mm/min) equipped with a Warner-Blatzler meat shear apparatus. The peak force was expressed in kg/cm^2.

Fatty acids were quantified as methyl esters (FAME) by lipid extracted (Folch et al. 1957), with a Mega 2 Carlo Erba gas chromatograph (model HRGC, Milano, Italy), equipped with a flame ionization detector and D-B wax capillary column (0.25 mm ∅, 30 m long Agilent technologies, Palo Alto, CA). In particular, 1 ml of lipid extract was evaporated under a stream of nitrogen and the residue was derived by adding 3 ml of sulfuric acid (3% in methanol). Following incubation at 80 °C for 1h, the methyl esters were extracted with petroleum ether, and 1 μl was injected in the GC system. The operating conditions used during the column injection 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 good 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; the final temperature was held for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 ml/min. Individual FAME were identified by referring to the retention time to 37 FAME pure standards (Supelco, Bellefonte PA). The average amount of each fatty acid was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated (PUFA) fatty acids.

Tocopherols (α-tocopherol and its isoform $\beta + \gamma$ and δ), α-tocotrienol and retinol meat content were quantified by HPLC according to Hewavitharana et al. (2004). In particular, 5 ml of distilled water and 4 ml of ethanol were added to 2 g of meat sample and then vortexing

for 10 s. After mixing 4 ml of hexane containing BHT (200 mg/l) was added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (3 ml) was dried under a stream of nitrogen and then redissolved in 300 μl of acetonitrile. Fifty microlitres were injected into the HPLC system and quantified as previously described for plasma samples.

The meat lipid oxidation was evaluated according to Ke et al. (1977) by a spectrophotometer set at 532 nm (Shimadzu Corporation UV-2550, Kyoto, Japan), which measured the absorbance of TBARS. Oxidation products were quantified as milligram of malondialdehyde (MDA) per kilogram of muscle.

Statistical analyses

A linear model was used to assess the effects of strain and transport and their interaction (STATA 2015). The significance of differences was estimated by the multiple Student's t-test. Differences were considered significant for $p \leq 0.01$ and $p \leq 0.05$.

Results and discussion

The effect of bird strain and transport length on the oxidative parameters of plasma is given in Table 1. The genetic strain and transport both affected the *in vivo* oxidative status of plasma. (α-Tocopherol, retinol, lutein + zeaxanthin and TBARS) whereas δ-tocopherol and γ-tocopherol were affected only by transport and strain, respectively. In particular, the length of transport negatively affected the *in vivo* oxidative status of birds, reducing plasmatic α- and δ-tocopherol, retinol and lutein + zeaxanthin and increasing the TBARS value. The birds transported for four hours were exposed to a variety of potential stressors, including the thermal

demands of the transport micro-environment, acceleration, vibration, motion, impacts, fasting, withdrawal of water, social disruption and noise (Mitchell & Kettlewell 1998). Some reports suggested that different environmental stresses increase the superoxide free radical production in chicken skeletal muscle (Mujahid et al. 2005) simultaneously diminishing the *in vivo* antioxidant status (Klasing 1998).

Regarding the genotype effect, Ross 308 presented lower plasmatic levels of antioxidants with respect to Naked Neck birds. Accordingly, Ross 308 also showed a lower *in vivo* oxidative stability as demonstrated by the higher values of TBARS. Indeed, antioxidants play a major role in protecting cells from the actions of reactive oxygen species (ROS) by reducing chemical radicals and preventing the lipid peroxidation of muscle (Nishigaki et al. 1992). Many Authors (Cheng et al. 1990; Sahin et al. 2002) stated that low plasma concentration of antioxidant vitamins (C and E) has been correlated with higher oxidative damage in stressed broilers. To this aim, the different genetic aptitudes for foraging (Dal Bosco et al. 2014) partly explain the higher content of antioxidant in plasma of Naked Neck birds.

Fast-growing strains are characterized by a certain incidence of myopathy which can induce muscle abnormalities (Mitchell 1999; Sandercock et al. 2006; Branciari et al. 2014), alter mitochondrial function (Bottje & Carstens 2009) and change the ROS production. This fact may contribute to the reduction of antioxidant observed in Ross 308 broilers. It is possible to assume that muscle dysfunction may be further compounded during transport, when fast-growing birds could be more exposed to muscle discomfort or pain (Mitchell & Kettlewell 2009).

Table 2 presents the effect of genetic strain and length of transport on the live weight and the main

Table 1. Effect of genetic strain and transport length on the main plasmatic antioxidants and oxidative stress of chicken.

Strain	Ross 308		Naked Neck		Significance			
Transport (h)	0	4	0	4	S	T	S X T	Pooled SE
α-Tocopherol nmol/ml	569.4	329.66	620.77	487.10	*	*	ns	185.23
δ-Tocopherol nmol/ml	258.11	151.04	245.59	201.38	ns	*	ns	102.52
γ-Tocopherol nmol/ml	8.28	4.65	8.71	9.69	*	ns	*	2.91
α-Tocotrienol nmol/ml	54.89	42.56	67.67	50.66	ns	ns	ns	20.26
Retinol nmol/ml	91.71	42.71	142.78	106.63	**	*	ns	40.23
Lutein + Zeaxanthin nmol/ml	24.04	17.61	63.04	31.43	*	*	ns	26.42
TBARS nmol MDA/ml	3.32	3.89	2.53	3.30	*	*	ns	0.45

$n = 14$ for each strain and transport length. TI: Tonic immobility; S: strain; T: transport; ns: not significant.
*$p \leq 0.05$.
**$p \leq 0.01$.

Table 2. Effect of genetic strain and transport length on chicken productive performance and main physical and chemical breast meat characteristics.

Strain		Ross 308		Naked Neck		Significance			
Transport (h)		0	4	0	4	S	T	S X T	Pooled SE
Live weight	g	3811	3794	2897	2773	**	*	ns	64.6
Weight loss	%	–	1.05	–	4.05	**	–	–	1.05
Carcass	g	3304	3295	2467	2373	**	*	ns	67.2
Dressing percentage	%	86.6	86.8	85.1	85.5	*	ns	ns	5.10
Dressing percentage loss	%	–	0.27	–	3.81	**	–	–	0.92
pH									
0 h		6.84	6.92	6.8	6.83	**	**	ns	0.04
2 h		6.10	6.25	6.07	6.19	*	**	ns	0.05
24 h		5.93	6.18	5.85	6.13	*	**	*	0.08
pH decline		0.91	0.74	0.95	0.70	*	*	*	0.01
Moisture	% f.m.	75.5	75.0	76.2	76.0	*	*	ns	0.22
Protein	% f.m.	21.1	21.5	20.6	20.8	**	**	ns	0.25
Lipid	% f.m.	1.79	1.86	1.62	1.63	**	ns	ns	0.11
Ash	% f.m.	1.46	1.59	1.44	1.42	ns	ns	ns	0.12
L* value									
2 h		48.21	46.18	46.92	45.17	**	**	ns	1.18
24 h		50.28	47.51	48.57	45.92	**	**	ns	0.98
WB shear force	kg/cm^2	1.45	1.31	1.67	1.50	**	**	ns	0.07
Water-holding capacity	%	44.8	45.7	45.3	46.4	ns	*	ns	0.59
Cooking loss	%	27.8	27.1	27.6	26.8	ns	ns	ns	0.79

$n = 14$ for each strain and transport length. S: strain; T: transport; WHC: Water-holding capacity; CL: cooking loss; ns: not significant.
*$p \leq 0.05$.
**$p \leq 0.01$.

physico-chemical characteristics of breast meat. As expected, Ross 308 birds showed a significant higher live and carcass weight and dressing percentage. This was due to the genetic selection for improved muscle growth rate (Aviagen 2009) and the lower percentage of viscera in fast-growing strains. Anyway, in both the strains the length of transport significantly affected the live weight, in accordance with Bianchi et al. (2005) that found a greater weight loss in birds transported for >3.5 h. Nijdam et al. (2005) reported that greater weight loss in starved and transported broilers compared with chickens that were only starved for the same amount of time. Doktor and Poltowicz (2009) also found weight loss (4.2%) for Naked Neck birds that were starved and transported before slaughter similar to that observed in this trial. The same authors showed that the transport had a detrimental effect on the dressing percentage, confirming the results of the present study (−0.27% Ross 308 and −3.81% for Naked Neck).

Meat quality is defined as a combination of several factors, of which pH, WHC and tenderness are particularly important. WHC, pH and tenderness are crucial for the cooking value and technological properties of chicken meat. These traits can be shaped by short-term pre-slaughter factors such as fasting and transport (Lyon et al. 2004). Rough handling, over-crowding, social disturbances, heat stress during transportation, pain due to mini-fractures and bruises and the effect of the vehicle vibrations may all together lead to metabolic fatigue.

Accordingly, pre-slaughter stress can cause undesirable changes in meat quality by altering muscle metabolism; indeed, an altered rate of *post-mortem* changes is the adverse consequence of *ante-mortem* stress (Owens & Sams 2000).

In this study, the pH of breast muscles of 4 h transported chickens showed higher muscle pH at 0, 2 and 24 h *post-mortem*. The transportation for 4 h resulted in higher muscle pH probably due to higher glycogen depletion. Glycogen is converted to lactic acid during glycolysis resulting in a reduction of ultimate pH (Lawrie 1992). Such metabolism is affected by stress because transport increases plasma corticosterone (Kannan et al. 1997) and consequently the muscle glycogenolysis (Lehninger et al. 1993). Thus, long transport contributes to reduce glycogen stores in the liver and skeletal muscles by imposing an acute demand of energy.

Respect to strains, Naked Neck birds showed lower pH value (0, 2 and 24 h) than Ross 308 mainly when immediately slaughtered: on the contrary, the pH decline was lower (0.74 vs 0.70) for the longer transportation. Other authors stated that the pH decline and the *rigor mortis* occurs more rapidly in the breast of slow-growing strains (Culioli et al. 1990), mainly because they struggle more (Berri et al. 2005). Indeed, Naked Neck birds probably have a greater kinetic activity during pre-slaughter (shackling, etc.) with a greater depletion of glycogen stores; whereas in fast-growing chickens, *ante-mortem* conditions scarcely affect meat pH (Berri et al. 2005). The final pH also influences the

Table 3. Effect of genetic strain and transport length on the main fatty acid (% total fatty acids) of chicken breast meat.

Strain	Ross 308		Naked Neck		Significance			
Transport (h)	0	4	0	4	S	T	S X T	Pooled SE
Fatty acids								
C14:0	1.17	1.38	0.74	0.94	**	*	ns	0.25
C16:0	29.98	30.13	28.99	30.90	ns	*	ns	2.16
C18:0	10.39	12.15	8.76	10.78	**	*	ns	1.28
SFA	41.52	44.77	39.51	43.83	*	*	ns	1.46
C16:1n-7	1.56	1.74	3.15	3.13	**	ns	ns	0.48
C18:1n-9	28.17	27.16	26.41	25.90	ns	ns	ns	2.08
C20:1n-9	0.24	0.32	0.26	0.26	ns	ns	ns	0.07
MUFA	30.75	29.94	30.65	30.06	ns	ns	ns	2.05
C18:2n-6	19.23	17.72	21.49	18.94	**	**	ns	1.49
C20:4n-6	5.02	4.46	3.87	3.29	**	**	ns	0.50
Σ n-6	24.87	22.69	26.07	22.75	ns	**	ns	1.57
C18:3n-3	1.09	1.07	1.46	1.32	**	ns	ns	0.18
EPA	0.13	0.08	0.28	0.13	*	*	ns	0.05
DHA	1.12	1.07	1.13	1.12	ns	ns	ns	0.04
Σ n-3	2.84	2.59	3.80	3.35	**	**	ns	0.20
PUFA	27.71	25.52	29.86	26.05	ns	*	ns	1.65
n-6/n-3	8.78	8.76	6.86	6.81	ns	**	ns	0.82

$n = 14$ for each strain and transport length. S: strain; T: transport; ns: not significant.
*$p \leq 0.05$.
**$p \leq 0.01$.

structure of myofibrils and consequently the WHC, CL and colour of the meat.

According to Warris (2000), the low pH contributes to the reduction of muscle fibres integrity so decreasing the capacity to retain water. The effects of animal transportation on the capacity of muscular water retention are controversial with an increase in turkeys and in swine (McPhee & Trout 1995), no effect of 1.5 h of transport in the breast meat of Ross 208 chickens (Savenije et al. 2002) or an increasing in the drip loss values in turkey breast fillets (Owens & Sams 2000) was reported. The breast muscles from 4 h-transported chickens showed significantly lower L^* values at 2 and 24 h. This parameter is negatively correlated with muscle pH and WHC (Barbut 1993).

However, contrary to what expected, the transport length did not significantly affect CL; lower cook losses are associated with higher muscle pH and better protein functionality (Barbut 1993).

These findings are consistent with previous results on several poultry species (Le Bihan-Duval et al. 1999), showing a decrease in colour intensity and an increase in lightness in fast-growing genotypes compared with less selected strains. This difference in colour could be due to a decrease in heme pigment content. Indeed, iron which is characteristic of the total pigments has been shown to be highly related to the colour (redness and lightness) of broiler breast meat (Boulianne & King 1995). Furthermore, the higher kinetic activity of slow-growing chickens causes an increase in α-red fibre (oxidative) in breast muscle (Branciari et al. 2009), which, on the basis of previously described *post-mortem* modifications (pH decline, protein denaturation, water-

holding properties of myosin), increase the tenderness of meat. In our study, meat tenderness (shear force value) was affected by genotype and transport: meat from Naked Neck birds was tougher, whereas after transport, in both genotypes, higher tenderness was observed.

In Table 3, presented results are relative to main fatty acids in breast meat. Transport length and genetic origin affected the fatty acid profile; in particular, Ross 308 chickens showed higher values of C14:0, C18:0, C20:4n-6 and n-6/n-3, whereas unsaturated fatty acids (C16:1n-7, C18:2n-6, C18:3n-3, EPA, DHA, Σ n-3 and PUFA) were lower. These results are in agreement with Sirri et al. (2010) which compared the lipid composition of chicken strains with different growing rates reared under organic conditions. According to Dal Bosco et al. (2012), the differences in the fatty acid profiles of breast meat may be attributed to genetic and epigenetic effects which could affect lipid metabolism and fatty acid deposition with different conversions of dietary fatty acid into long-chain derivatives. Indeed, it is well known that chickens are expected to eat variable amounts of forages that could modify fatty acid profiles in their meat, but it is not clear how much the chicken is able to elongate and desaturate the LNA of the pasture into eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Rymer & Givens 2005).

The length of transport significantly increased the proportion of saturated fatty acids of breast, in particular: C16:0, C18:0 and decreased PUFA (C18:2n-6, C20:4n-6, Σ n-6, EPA and DHA) probably due to greater formation of peroxides, as showed by higher TBARS

Table 4. Effect of genetic strain and transport length on the main antioxidants and oxidative stability of breast meat.

Strain		Ross 308		Naked Neck		Significance			
Transport (h)		0	4	0	4	S	T	S X T	Pooled SE
α-Tocotrienol	ng/g	28.46	4.76	33.21	4.58	ns	**	ns	1.12
δ-Tocopherol	ng/g	17.55	11.80	18.39	6.83	ns	*	ns	1.69
γ-Tocopherol	ng/g	13.36	10.94	11.10	10.69	ns	ns	ns	2.01
α-Tocopherol	ng/g	555.45	410.70	587.94	420.77	*	*	ns	52.62
Retinol	ng/g	5.35	3.45	13.35	12.48	**	*	ns	1.68
Lutein + Zeaxanthin	ng/g	16.83	8.13	23.42	9.54	*	*	ns	2.60
TBARS	mg MDA/kg	0.17	0.20	0.13	0.18	*	*	*	0.04

$n = 14$ for each strain and transport length. S: strain; T: transport; ns: not significant.
*$p \leq 0.05$.
**$p \leq 0.01$.

value. It is presumable that the oxidative stress triggered by 4 h of transport, reducing the *in vivo* antioxidant defences of birds, increased their *post-mortem* susceptibility to lipid oxidation, also affecting the fatty acids profile of meat. The level of PUFA in poultry meat can play an important role in the susceptibility of poultry meat to lipid oxidation which leads to discolouration, increase in drip loss during storage and a decrease in *shelf-life* and development off flavour. The ratio between *n*-6 and *n*-3 fatty acids is considered an important index for nutritional evaluation of fat (Griffin 2008). Rymer and Givens (2005) reported that higher concentration of *n*-3 PUFA, particularly alpha-linoleic acid, is associated with higher oxidative instability.

In Table 4, presented results are related to the main antioxidant and TBARS contents in breast meat with respect to the genetic strain and transport duration. Ross 308 showed the lower value for α-tocopherol, retinol and lutein + zeaxanthin, whereas the TBARS value was higher, with respect to the Naked Neck.

Antioxidants protect against free-radical damage all the living system, but in the birds their levels widely vary in response to potential stressor (Cohen et al. 2008), i.e. fear associated with the strange noises, vibrations and jolts encountered during transport.

As expected, the transport reduced antioxidants compounds of breast meat. In particular, 4 h of transport lowered in breast: α-tocotrienol, δ-tocopherol, α-tocopherol and lutein + zeaxanthin contents. Retinol and tocopherols are fat-soluble vitamins acting as antioxidants via the prevention of lipid oxidation. Retinol is one of the active forms of vitamin A which promotes vision, participate in protein synthesis and cell differentiation, support reproduction and growth (Persson et al. 2008), and inhibit lipid peroxidation (Rózanowska et al. 2005).

The two main forms of vitamin E are α- and γ-tocopherol; the concentration of α-tocopherol is reported to be 4–10 times higher than those of γ-tocopherol in the plasma (Behrens & Madère 1986). They both exert an antioxidant action via the prevention of

lipid oxidation, but α-tocopherol is considered to be more powerful relative to γ-tocopherol for inhibiting lipid peroxidation (Kamal-Eldin & Appelqvist 1996). However, it has been suggested that γ-tocopherol is superior in detoxifying reactive nitrogen oxide species (Jiang et al. 2001). Furthermore, Serbinova et al. (1991) reported that the activity of α-tocotrienol, in scavenging peroxyl radicals is 1.5-fold higher compared with α-tocopherol in rat liver microsome. Such greater antioxidant power of tocotrienol could be the cause of its firm reduction in chicken meat after 4 h of transport.

Conclusions

In conclusion, the results of the present study suggest that transport for 4 h prior to slaughter negatively affect meat quality resulting in higher muscle pH, lower $L*$ values, and higher water retention. Even the meat oxidative status is affected by transport (TBARS value), with a decreased of antioxidant compounds (vitamins E, A and xanthophylls) important in terms of product *shelf-life*.

To this aim slow-growing lines, being animals more active, seem more sensible to stress transport due to their higher movement before slaughter. Future research is needed to evaluate various transportation times to determine the effects on meat quality.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article

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