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Effect of genetic background, pen size and outdoor access on meat quality in two slow growing broiler hybrids

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ABSTRACT

The aim was to evaluate the effect of two different rearing systems, indoor small pens (S) and big pens with outdoor access (B), on meat quality and fatty acid (FA) composition of two slow growing broiler hybrids (Rowan Ranger [RR] and Hubbard CYJA57 [H]). In addition, changes in the phospholipid FA composition, due to the possibility of more movement, were investigated. The results regarding meat quality were exclusively influenced by genotype, where RR chickens had higher breast weight, higher Warner-Bratzler shear force and higher pH than H chickens. Differences in lipid composition were found both due to hybrid and to the rearing system. The H birds were slightly leaner and had, therefore, higher proportions of phospholipids. Rearing in a big pen resulted in lower concentrations of 16:0 and higher concentrations of both total n-3 and n-6 fatty acids and of individual long chain polyunsaturated FA.

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Meat quality; phospholipids; fatty acid composition; physical activity; alternative broiler production

Introduction

There is a growing interest for organic poultry products (Fanatico et al., 2005; Fanatico et al., 2007). In Sweden, organic broiler production has increased from 0.2% to 0.9% of the total broiler production from 2013 to 2017 (JO 27 SM 1801 (Swedish Board of Agriculture, 2017)).

In organic production, fast growing broilers must reach 81 days of age before slaughter (EG 889/2008 (Official Journal of the European Union, 2008)), while they can reach slaughter weight in 35–42 days in conventional production (Zampiga et al., 2018). At 81 days, fast-growing chicken can be too heavy, which causes more frequent leg- and cardiovascular problems for the animals, and generally fast growing hybrids do not adapt as well to organic production systems as slow-growing hybrids do (Castellini et al., 2002a; Wallenbeck et al., 2016). Hence, in organic production broilers should preferably be of a slow growing hybrid which is defined by the Swedish Board of Agriculture as hybrids who have the maximal average growth rate of 45 grams per day (SJFS 2017:42 (Swedish Board of Agriculture, 2017)) and reach their slaughter weight usually after 60–65 days. The slow-growing hybrids/breeds are less effective energy converters, but they have a lower mortality and reduced lameness (Castellini & Dal Bosco, 2017). There may be a difference in meat quality compared with the fast-growing hybrids (Sirri et al., 2010; Chen et al., 2013; Yang et al., 2015; Comert et al., 2016).

Differences in broiler meat quality properties can be caused by a number of different factors, such as genetic background, feeding, physical activity and also the foraging of a varied diet on an outdoor pasture and exposure to light (Castellini et al., 2002a; Castellini et al., 2002b; Chen et al., 2013; Aksit et al., 2017).

Even though differences in meat quality between different slow growing breeds or hybrids have been shown (Castellini et al., 2002b), there is a need to evaluate the potential and performance of new hybrids' prior commercialization. As organically produced broilers also need to have outdoor access (Official Journal of the European Union, 2008), leading to increased activity, we speculated that this will most likely also affect meat quality parameters, as seen earlier by Castellini et al. (2002a). A well-cited study on human leg muscle showed that increased activity resulted in higher proportion of n-3 fatty acids (FA) in skeletal muscle phospholipids (PL) (Andersson et al., 1998). If a similar effect would be found in broilers, increased activity caused by outdoor access, would be advantageous from a nutritional point of view for the consumers, as higher intake of n-3 FA is recommended (Simopoulos, 2000).

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The aim of the present study was to investigate possible differences in meat quality between two broiler hybrids raised in two different housing systems: small pens indoor and big pens with outdoor access. In addition, the effect of activity, due to outdoor access, on lipid composition in one leg muscle was investigated.

Material and methods

Chicken and treatments

The experiment was approved by the Ethics Committee on Animal Experiments in Gothenburg, Sweden (issue no 112-2015). In total, 400 broilers of mixed sex, 200 Rowan Ranger (RR) and 200 Hubbard CYJA57 (H) were reared to an age of 73 days in either small pens without outdoor access (S; 25 birds/hybrid) or in one large pen, free range with outdoor access (B; 175 birds/hybrid) resembling the commercial situation. They were, therefore, assumed to move around and exercise more than birds in the small pens. However, movement was not recorded. The four treatments are as follows: Rowan Ranger big pen (RRB); Rowan Ranger small pen (RRS); Hubbard big pen (HB); Hubbard small pen (HS).

The facility had windows along three walls, allowing good levels of daylight. Ceiling lights were on between 04:00 and 22:00 h daily. Wood shavings were used as litter material covering all floor area. The big pen was 20 × 7.5 m (0.86 m^2/bird), furnished with 10 straw bales (40 × 45 × 90 cm) and multi-levelled wooden perches (at 20, 40 and 70 cm height, 15 cm/bird) (Figures 1 and 2). Two pop-holes leading to the outdoor area were open between 08.00 and 18.00 h. The outdoor area was a 1400 m^2, fenced yard with mixed grass. Four 60 × 80 cm camouflage nets and four 150 × 140 cm wood boards were placed as protection between the outer wall and fence. The small pens had the size of 1 × 1.5 m and housed 5 birds per pen (0.3 m^2/bird) and were all furnished with perches (at 20 and 40 cm height, 15 cm/bird). All birds had free access to feed (B: 3.2 cm/bird, S: 12.5 cm/bird) and water (B: 4.8 cm/bird, S: 17.6 cm/bird). The distance between feed and water was approximately 2 m in B and 40 cm in S. During days 1–18, a starter diet was fed and thereafter a standard diet was used (Table 1, Table 2). In addition, 14 L chopped Alfalfa (*Medicago sativa*) as roughage was scattered on the floor once per day as environmental enrichment for all birds.

The broilers were slaughtered at 73 days of age. Birds were stunned individually with a dry electric stunner and killed by bleeding. For meat quality measurements, breast muscles were randomly sampled from 12 female birds from each treatment and of these, 6 whole thigh muscle from each treatment was sampled for lipid analyses. Total carcass weight, weight, pH and temperature in the breast muscle (*Pectoralis major and minor*) were recorded. Breast muscle proportion was calculated as percentage of total carcass. Left side breast muscles were vacuum packed, and left side legs were wrapped in aluminium foil in addition to vacuum packing to minimize lipid oxidation and stored at −20° C upon analyses. All cutting was performed by the same person, to ensure consistency.

Analyses

Ph

Immediately after slaughter, pH and temperature were measured in the left breast muscle in duplicate (Testo

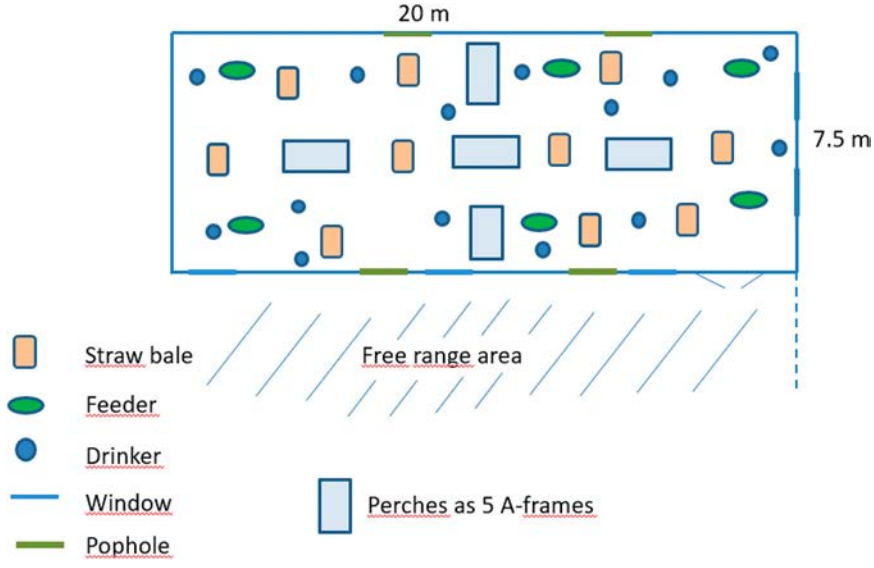

Figure 1. Plan of the big pen.

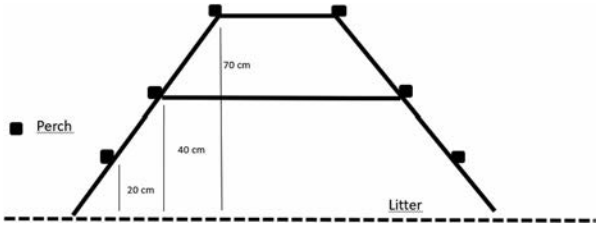

Figure 2. Sketch of the perches in the big pens.

205, Nordtec Instrument AB, Gothenburg, Sweden). Calibration of the instrument was performed with standard pH buffers of 4.00 and 7.00 at 20°C.

Drip loss

Directly after slaughter 5 g of the right breast muscle (cranial part) were taken for the evaluation of drip loss. The sample was weighed (W1) and placed hanging in a closed beaker for 24 h at 4°C, weighed again (W2) and drip loss was calculated as the difference between W1 and W2 and expressed in percentage.

Thawing loss

Determination of thawing loss was done according to Honikel (1998). The frozen vacuum-packed breast muscles were weighed and later corrected by subtracting the weight of the vacuum bag (A). After thawing for 24 h at 4°C and 4 h equilibration at room temperature, muscles were taken out of the bags, blotted dry with a paper towel and weighed (B). The thawing loss was calculated as the difference between A and B and expressed in percent.

Colour

Colour was measured in triplicates using a Minolta Chroma Meter CR-300 (Tokyo, Japan) calibrated against a white tile ($L* = 93.30$, $a* = 0.32$ and $b* = 0.33$). The aperture was 8 mm, and illuminant D65 and 10° Standard Observer were used. On the *P. major*, measurements were done at the anterior and interior (cranial side) surfaces, while on the *P. minor,* only the anterior surface was measured. Since there was no significant difference between the three sites, an average of all measurements was calculated and used for further statistical analysis.

Table 1. Nutritional composition of the diets HARMONI Slaktkyckling Start and HARMONI Slaktkyckling Final RR (Spannfod AB, Sweden).

Values per kg	Start	Final RR
KRAV %	95.0	95.0
Energy MJ/kg	12.5	11.9
Raw protein g	245.0	170.0
Raw fat g	80.0	60.0
Lysine g	15.0	9.5
Metionine g	4.6	3.5
M + C g	9.0	7.0
Calcium g	11.0	11.5
Phosphorus g	8.0	7.2
Water g	12.0	12.0
Feed additives		
Vitamin A* µg	36.6	36.6
Vitamin D** µg	82.5	82.5
Vitamin E*** mg	150.0	150.0
Selenium mg	0.6	0.6
Copper mg	7.6	7.6

*retinyl acetate; **cholecalciferol; ***tocopherol acetate.

Table 2. Fatty acid composition of the feed Harmoni slaktkyckling Final RR (% of total identified FA) (analysed in duplicate (means and stdev).

Fatty acid	
14:0	0.22 ± 0.01
16:0	12.36 ± 1.34
16:1(n-7)	0.30 ± 0.00
18:0	3.66 ± 0.64
18:1(n-9)	41.69 ± 0.34
18:1(n-7)	2.66 ± 0.01
18:2(n-6)	29.88 ± 1.52
18:3(n-3)	7.38 ± 0.17
20:1(n-9)	1.05 ± 0.03
22:6(n-3) (DHA)	0.40 ± 0.01
SFA	16.63 ± 2.03
MUFA	87.40 ± 0.68
PUFA	37.67 ± 1.69
n-3	7.78 ± 0.16
n-6	29.88 ± 1.52
n-6/n-3	3.84 ± 0.11

Abbreviations: FA: fatty acid SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, DHA: docosahexaenoic acid.

Cooking loss

Vacuum packed samples were cooked in a water bath at 70°C for 75 min to reach an inner temperature of 70°C. After cooking, samples were removed from the bags, dried with a paper towel and weighed (C). The cooking loss was calculated as the difference between weights B (see above) and C and expressed in percent.

Texture analysis

The cooked samples (see above) were cut along the fibre direction, in standardized 10 × 10 mm strips, 50 mm long (Figure 3). Freshly cut samples were stored in a plastic container with lid until analyses to prevent evaporation of water and drying. The tenderness of the meat was evaluated using Warner-Bratzler shear force (WBSF) measurement, as described by Honikel (1998). A TA.HDi Texture analyser (Stable Micro Systems, Surrey, United Kingdom) equipped with a 50 kg load cell was used with a penetration speed of 3.3 mm/s. Pre-test and post-test speed was set to 4.0 mm/s. Each sample was analysed in triplicate.

Lipid analyses

Lipid extraction. For lipid analysis, the red leg muscle *M. flexor hallucis longus* from six birds, per treatment

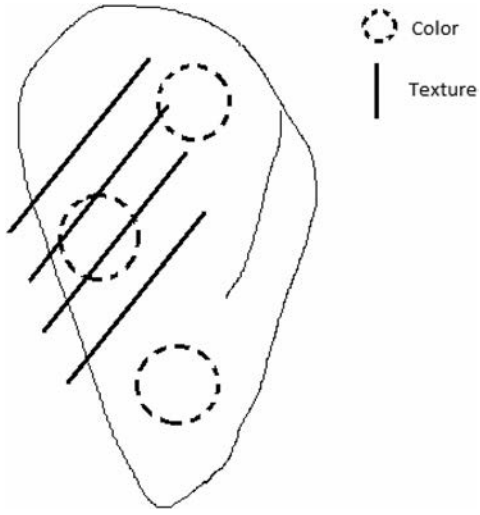

Figure 3. Areas of texture sampling for Warner-Bratzler Shear Force test and the site of colour measurements on the chicken breasts.

group, was collected. Visible connective tissues and fat were removed and minced, and sub-samples of 2 g were extracted for lipid, according to Pickova et al. (1997). Lipid content was determined gravimetrically. Lipid extraction of feed was performed in duplicate; 5 g dry feed and 5 ml water were mixed. From this, 1 g was taken for lipid extraction.

Thin Layer Chromatography (TLC)-for lipid class composition. Lipid class composition was determined according to (Olsen and Henderson, 1989) with slight modifications. Samples were diluted to a concentration of 1 µg/µl in hexane, and 5 µl of each was applied with a CAMAG TLC Sampler ATS4 (Camag Switzerland) 2 cm from the base edge of the TLC plates (20 × 10 cm; Silicagel 60; 0.20 mm layer, Merck, Darmstadt, Germany) in 2 mm bands with an application speed of 250 nl/sec. Nitrogen was used as spray gas. All samples were applied in duplicate, and the distance between tracks was 9.8 mm. Separation of the lipid classes was executed with a CAMAG Automatic Developing Chamber 2 (ADC 2) (Camag Switzerland). Hexane:diethyl ether:acetic acid (85:15:2; v/v/v) was used as a mobile phase. After the separation, plates were dipped in a solution of 3% cupric acetate in 8% phosphoric acid and then charred for 20 min at 140°C. Quantitative analysis was done with a CAMAG TLC Scanner 3 (Camag, Switzerland). The scanning was performed at a speed of 20 mm/sec and a data resolution of 100 µm/step, with a slit dimension of 6.00 × 0.45 mm at a wavelength of 350 nm. Identification of the lipid classes was done by comparison with an external standard (TLC 18-4A, Nu-Chek Prep, Elysian, USA). For data filtering, the mode Savitsky-Golay 7 was used. Manual baseline and peak correction were used, if necessary.

Fatty acid composition in total lipids, phospholipids and triacylglycerols. Total lipids were separated with TLC, as described in Pickova et al. (1997). As a stationary phase, TLC plates (20 × 20 cm; Silicagel 60; 0.20 mm layer, Merck, Darmstadt, Germany) were used. After identification by comparison with the standard, the PL and triacylglycerol (TAG) areas on the plates were scraped off the TLC plates. The PL fraction was extracted with chloroform:methanol (2:1, v:v); (1:1, v:v) and chloroform, while TAG was extracted three times with chloroform. FAs from the total lipids, PL and TAG fractions were methylated (Pickova et al., 1997).

Gas chromatography, GC. The FAME were analysed as described earlier (Pickova et al., 1997) by using a gas chromatograph (GC) (CP3800, Varian AB, Stockholm, Sweden), equipped with a FID detector and a BPX 70 column (SGE, Austin Texas) with 50 m length, id 0.22 mm and a film thickness of 0.25 µm. FAs were identified by comparison with a standard mixture (GLC- 68A, Nu-Chek Prep, Elysian, USA).

Statistical analysis

Data were analysed with the Mixed procedure in SAS (SAS 9.4, SAS Inst. Inc., Cary, NC, USA), using the following model:

$$Yij = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

where μ is the population mean, α_i is the fixed effect of hybrid, β_j is the fixed effect of housing system, $\alpha\beta_{ij}$ is the interaction between hybrid and housing system, and e_{ijk} is the error term.

Differences were considered significant at $P < 0.05$ and as a tendency for significance at $0.05 < P < 0.10$. Fatty acids and lipid classes are presented as mean and standard deviation, respectively.

In addition, a principal component analysis (PCA), using The Unscrambler × 10.1 (Camo Process A/S, Oslo, Norway), was performed for the FA from total lipids. Full cross-validation was used as the validation model.

Results

Carcass characteristics

There were no significant differences in slaughter weight, neither between hybrids nor due to the rearing system. There was, however, a tendency ($P = 0.066$) for RR birds to be heavier than H birds (Table 3). There was also an interaction ($P = 0.041$) between

Table 3. Slaughter weight, breast muscle weight and meat quality parameters in breast muscle (*M. pectoralis major*) at 73 days of age, of two slow growing broiler hybrids raised in either small pens indoor or big pen with outdoor access ($n = 12$).

	Hubbard		Rowan Ranger			Significances		
	Big pen	Small pen	Big pen	Small pen	s.e.m.	Hy	Ps	Hy x Ps
Slaughter weight (g)	2454 ^{ab}	2396 ^{a}	2440 ^{ab}	2659 ^{b}	65.20	0.066	0.226	0.041
pH after slaughter	5.89	5.89	5.97	6.05	0.052	0.025	0.486	0.466
Breast weight (g)[1]	242.2 ^{a}	240.0 ^{a}	271.5 ^{ab}	306.8 ^{b}	11.50	0.0001	0.157	0.110
Breast weight (%)[2]	19.73 ^{a}	19.50 ^{a}	22.25 ^{b}	22.13 ^{ab}	0.652	0.0003	0.770	0.912
TexPeak (N)	16.73 ^{a}	17.28 ^{a}	29.28 ^{b}	23.03 ^{ab}	2.469	0.001	0.254	0.176
TexTotal area (Nmm)	90.85 ^{a}	93.30 ^{a}	151.6 ^{b}	128.5 ^{ab}	11.56	0.0001	0.377	0.275
Drip loss %	1.91	1.49	1.53	1.97	0.318	0.888	0.975	0.179
Thawing loss %	9.93	10.19	10.43	9.43	0.711	0.856	0.612	0.381
Cooking loss %	14.53	13.21	13.99	14.03	0.560	0.800	0.256	0.230
Colour L*[3]	44.20	44.16	43.82	44.28	0.451	0.769	0.645	0.586
Colour a*	−4.19	−3.56	−3.99	−4.20	0.194	0.261	0.295	0.037
Colour b*	20.53	20.45	20.00	20.05	0.319	0.155	0.975	0.830

Abbreviations: Hy: hybrid, Ps: pen size, s.e.m.: standard error mean. Different superscript letters in a row indicate significant difference ($p < 0.05$).
[1]Weight of the left breast muscle.
[2]Calculated as ((weight of the left breast muscle*2)/slaughter weight)*100.
[3]Colour L* = lightness, Colour a* = redness and Colour b* = yellowness.

hybrid and rearing systems, where HB birds had a higher slaughter weight than HS, while the opposite was true for RR birds. Breast muscle weight was heavier in RR than in H ($P = 0.0001$) (Table 3). The pH of the breast muscle was higher in RR than H but was within normal range for all treatments (Table 3).

Colour, texture and water holding capacity

Meat colour, measured as lightness (L*), redness (a*) and yellowness (b*), did not show significant differences, neither between the rearing system nor between hybrids (Table 3). Nor were there any effects of rearing or hybrids on water holding capacity (WHC) measured as drip loss, thawing loss and cooking loss (Table 3).

WBSF values for both maximum and work were significantly higher ($P = 0.001$ and $P = 0.0001$, respectively) in RR compared to H chickens (Table 3).

Fat content, lipid classes and fatty acid composition in the leg muscle M. flexor hallucis longus

Fat content in the leg muscle *M. flexor hallucis longus* did not differ significantly between hybrids or treatments, but there was a tendency for higher fat content in leg muscles from RR chickens ($P = 0.096$; Table 4). Proportions of PL and cholesterol were higher in H chickens compared to RR ($P = 0.027$ and 0.023, respectively) while there was a tendency for higher proportion of TAG in RR birds ($P = 0.050$). There was a tendency for higher proportion of PL in muscles from birds reared in the big pen ($P = 0.070$), but no other differences in lipid class composition, due to the rearing system, were found (Table 4).

The FA composition of total lipids differed due to hybrid only in 14:0 with H having a higher proportion than RR ($P = 0.049$). There were, however, tendencies for H having higher proportions than RR of 20:4n-6 (arachidonic acid; AA), 22:4n-6, 22:5n-3 (docosapentaenoic acid; DPA) and 22:6n-3 (docosahexaenoic acid; DHA) ($P = 0.092$, 0.089, 0.074 and 0.062, respectively; Table 4). The ratio n-6/n-3 tended to be higher in RR than in H ($P = 0.075$). Rearing broilers in a big pen with outdoor access resulted in lower proportion of 16:0 and higher proportions of 22:4n-6, DHA and subsequently higher proportion of polyunsaturated FA (PUFA) and sum of both n-3 and n-6 PUFA compared to rearing in small pens for both hybrids (Table 4). A PCA correlation loadings plot was used to visualize the relationship between the FA in TL (Figure 4). There is a clear correlation between the n-3 and n-6 FA, while the saturated FAs except for 14:0 are negatively correlated to the unsaturated FA on PC1.

In the PL, H showed higher proportions of AA and EPA, while RR tended to be higher in 18:1n-7 (Table 5). When it comes to the rearing system, B resulted in lower proportions of 16:0 and 18:1n-9 and higher proportions of AA and DPA (Table 5) compared to S.

In TAG, H showed lower proportions of 16:1n-7 and monounsaturated FA (MUFA) than RR (Table 6). The rearing system had no effect on FA composition of TAG.

Discussion

The meat quality data (Table 3) indicate no differences between the two rearing systems investigated in this study. The differences were mainly due to hybrid. Since slaughter weight was not affected by pen size we conclude that access to outdoor pasture did not reduce growth, which is in line with results by Yang

Table 4. Fat content (g/100 g), lipid class- and fatty acid composition (% of total identified) of leg muscle (*M. flexor hallucis longus*) of two slow growing broiler hybrids raised in either small pens indoor or big pens with outdoor access ($n = 6$) (means ± stdev).

	Hubbard		Rowan Ranger		Significances			
	big pen (HB)	small pen (HS)	big pen (RRB)	small pen (RRS)	s.e.m.	Hy	Ps	Hy x Ps
Fat %	2.41 ± 0.48	2.39 ± 0.29	2.53 ± 0.21	2.89 ± 0.53	0.178	0.096	0.357	0.296
phospholipids	15.7 ± 1.76 ^{a}	15.0 ± 1.48 ^{ab}	14.7 ± 1.26 ^{ab}	13.1 ± 0.67 ^{b}	0.610	0.027	0.070	0.472
DAG	1.61 ± 0.16	1.84 ± 0.06	1.76 ± 0.33	1.74 ± 0.17	0.091	0.814	0.255	0.201
cholesterol	9.96 ± 0.99 ^{ab}	10.1 ± 0.94 ^{a}	9.72 ± 0.62 ^{ab}	8.48 ± 0.84 ^{b}	1.360	0.050	0.371	0.135
free fatty acids	7.55 ± 1.25	8.71 ± 1.44	7.98 ± 1.49	7.45 ± 2.07	0.386	0.023	0.191	0.081
TAG	65.2 ± 3.74	64.3 ± 2.53	65.9 ± 2.63	69.3 ± 3.15	0.711	0.564	0.688	0.250
14:0	0.65 ± 0.15	0.54 ± 0.06	0.49 ± 0.09	0.39 ± 0.28	0.075	0.049	0.156	0.964
16:0	23.2 ± 5.12	24.2 ± 5.00	19.3 ± 1.44	29.1 ± 8.67	2.530	0.852	0.046	0.097
16:1n-7	2.49 ± 0.63	2.86 ± 0.72	2.12 ± 0.77	2.68 ± 0.60	0.306	0.386	0.144	0.765
18:0	9.29 ± 3.20	9.16 ± 3.07	7.96 ± 1.12	10.9 ± 4.29	1.400	0.875	0.324	0.281
18:1n-11	0.27 ± 0.22	0.46 ± 0.33	0.31 ± 0.19	0.15 ± 0.17	0.105	0.208	0.900	0.105
18:1n-9	30.1 ± 3.44	31.6 ± 4.16	35.6 ± 1.68	30.1 ± 6.45	1.920	0.306	0.320	0.084
18:1n-7	2.03 ± 0.20	2.08 ± 0.24	2.27 ± 0.13	1.94 ± 0.50	0.136	0.712	0.318	0.172
18:2n-6	19.9 ± 3.08	19.0 ± 2.39	21.0 ± 2.48	16.8 ± 3.67	1.320	0.680	0.071	0.235
18:3n-3	3.05 ± 0.56	2.66 ± 0.39	2.99 ± 0.51	2.50 ± 0.54	0.224	0.633	0.065	0.824
20:1n-9	0.42 ± 0.22	0.27 ± 0.19	0.45 ± 0.04	0.31 ± 0.35	0.102	0.736	0.158	0.936
20:3n-6	0.27 ± 0.20	0.30 ± 0.21	0.32 ± 0.15	0.25 ± 0.28	0.095	0.973	0.849	0.592
20:4n-6 (AA)	3.99 ± 1.36	3.45 ± 0.64	3.58 ± 0.58	2.50 ± 0.62	0.387	0.092	0.050	0.490
20:5n-3	0.24 ± 0.19	0.23 ± 0.24	0.31 ± 0.16	0.12 ± 0.17	0.085	0.787	0.240	0.304
20:6n-6	0.66 ± 0.16 ^{a}	0.42 ± 0.32 ^{ab}	0.58 ± 0.07 ^{a}	0.16 ± 0.23 ^{b}	0.097	0.089	0.003	0.381
22:5n-3 (DPA)	1.14 ± 0.29	1.08 ± 0.23	1.05 ± 0.11	0.80 ± 0.21	0.098	0.074	0.130	0.363
22:6n-3 (DHA)	1.89 ± 0.58 ^{a}	1.55 ± 0.23 ^{ab}	1.61 ± 0.14 ^{ab}	1.20 ± 0.32 ^{b}	0.159	0.062	0.030	0.816
SFA	33.1 ± 8.82	33.9 ± 8.69	27.7 ± 1.83	40.4 ± 13.9	3.820	0.887	0.095	0.134
MUFA	54.1 ± 12.6	46.1 ± 11.3	40.4 ± 2.28	52.9 ± 12.0	4.660	0.473	0.639	0.040
PUFA	31.1 ± 5.60	28.7 ± 4.29	31.4 ± 3.67	24.3 ± 6.26	2.110	0.345	0.036	0.291
n-3	6.43 ± 1.26 ^{a}	5.52 ± 0.81 ^{a}	5.95 ± 0.64 ^{a}	4.62 ± 1.15 ^{b}	0.446	0.138	0.020	0.642
n-6	24.8 ± 4.74	23.2 ± 3.51	25.4 ± 3.08	19.7 ± 5.03	1.700	0.418	0.044	0.248
n-6/n-3	3.87 ± 0.24	4.22 ± 0.35	4.28 ± 0.33	4.30 ± 0.33	0.129	0.075	0.172	0.240
DPA + DHA	3.03 ± 0.85 ^{a}	2.63 ± 0.40	2.66 ± 0.17	2.00 ± 0.52	0.245	0.054	0.044	0.605
DPA + DHA/AA	0.78 ± 0.06	0.77 ± 0.08	0.76 ± 0.09	0.80 ± 0.05	0.032	0.898	0.591	0.459

Abbreviations: DAG: diacylglycerols, TAG: triacylglycerols, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, AA: arachidonic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, Hy: hybird, Ps: pen size. Different superscript letters in a row indicate significant difference ($p < 0.05$).

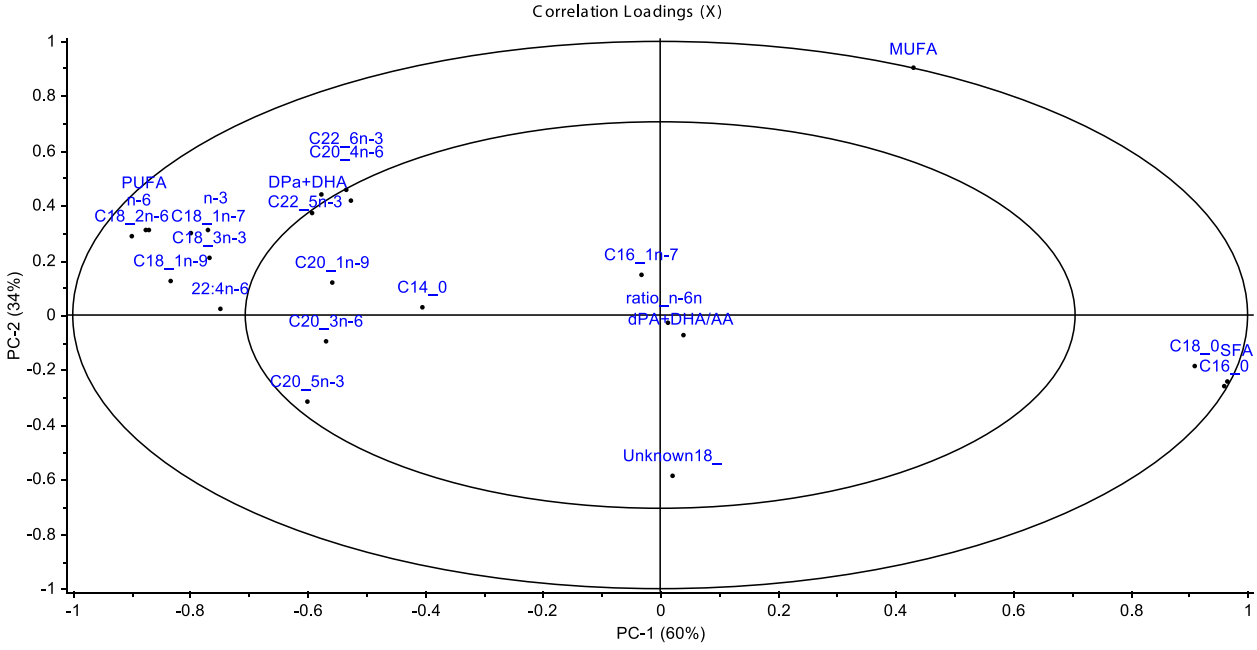

Figure 4. PCA plot showing the correlation loadings on PC1 and PC2 between the FA of the total lipids of leg muscle (*M. flexor hallucis longus*) of two slow growing broiler hybrids raised in either small pens indoor or big pens with outdoor access ($n = 6$).

Table 5. Fatty acid composition (% of total identified FA) in phospholipids from leg muscle (*M. flexor hallucis longus*) of two slow growing broiler hybrids raised in either small pens indoor or big pens with outdoor access ($n = 6$) (means ± stdev).

Fatty acid	Hubbard		Rowan Ranger		Significances			
	big pen (HB)	small pen (HS)	big pen (RRB)	small pen (RRS)	s.e.m.	Hy	Ps	Hy x Ps
16:0	19.3 ± 0.71^{ab}	20.1 ± 0.86^{ab}	18.4 ± 2.15^{a}	21.0 ± 0.90^{b}	0.590	0.939	0.012	0.111
18:0	17.7 ± 1.01	17.5 ± 0.60	18.3 ± 1.47	17.4 ± 0.98	0.480	0.671	0.371	0.365
18:1n-11	3.04 ± 0.35	3.05 ± 0.35	3.14 ± 0.57	3.20 ± 0.32	0.184	0.485	0.860	0.639
18:1n-9	12.0 ± 0.51^{a}	13.0 ± 0.49^{ab}	12.2 ± 1.05^{a}	13.8 ± 0.61^{b}	0.330	0.183	0.001	0.274
18:1n-7	2.40 ± 0.23	2.57 ± 0.12	2.62 ± 0.31	2.79 ± 0.29	0.111	0.074	0.117	0.955
18:1n-5	1.57 ± 0.16	1.42 ± 0.25	1.50 ± 0.22	1.56 ± 0.11	0.088	0.605	0.655	0.241
18:2n-6	17.2 ± 1.31	17.2 ± 0.68	17.6 ± 1.22	16.7 ± 0.91	0.470	0.948	0.655	0.193
20:2n-6	0.31 ± 0.22	0.43 ± 0.04	0.28 ± 0.20	0.10 ± 0.23	0.085	0.043	0.707	0.107
20:3n-6	0.44 ± 0.20	0.47 ± 0.21	0.41 ± 0.19	0.40 ± 0.28	0.101	0.626	0.949	1.000
20:4n-6 (AA)	15.4 ± 0.31^{a}	13.6 ± 0.77^{bc}	14.9 ± 1.27^{ab}	13.3 ± 0.73^{c}	0.40	0.534	0.001	0.859
20:5n-3	0.54 ± 0.09^{a}	0.55 ± 0.08^{a}	0.45 ± 0.36^{ab}	0.12 ± 0.23^{b}	0.101	0.017	0.137	0.098
20:6n-6	1.73 ± 0.13^{ab}	1.92 ± 0.10^{a}	1.63 ± 0.23^{b}	1.68 ± 0.16^{ab}	0.087	0.563	0.659	0.964
22:5n-3 (DPA)	2.72 ± 0.22	2.66 ± 0.20	2.72 ± 0.34	2.68 ± 0.20	0.089	0.791	0.012	0.049
22:6n-3 (DHA)	5.69 ± 0.40	5.31 ± 0.39	5.74 ± 0.80	5.22 ± 0.25	0.243	0.584	0.474	0.738
SFA	37.5 ± 0.65	37.2 ± 0.83	37.3 ± 0.72	37.6 ± 1.94	0.480	0.815	0.976	0.463
MUFA	16.0 ± 0.61^{a}	17.2 ± 0.34^{ab}	16.5 ± 1.11^{a}	18.2 ± 0.68^{b}	0.500	0.456	0.843	0.632
PUFA	42.5 ± 1.62	43.0 ± 1.25	42.5 ± 1.44	42.1 ± 3.60	0.010	0.552	0.831	0.507
n-3	8.96 ± 0.39	8.52 ± 0.56	8.91 ± 0.84	8.02 ± 0.45	0.292	0.848	0.193	0.311
n-6	34.4 ± 1.37	34.8 ± 1.58	33.9 ± 1.61	34.2 ± 3.13	0.830	0.408	0.468	0.691
n-6/n-3	4.09 ± 0.27	4.02 ± 0.39	3.81 ± 0.48	4.13 ± 0.19	0.143	0.506	0.120	0.543
DPA + DHA	8.42 ± 0.43	7.97 ± 0.56	8.46 ± 0.90	7.90 ± 0.43	0.255	0.841	0.017	0.678
DPA + DHA/AA	0.55 ± 0.03	0.59 ± 0.05	0.57 ± 0.07	0.60 ± 0.05	0.024	0.525	0.481	0.639

Abbreviations: FA: fatty acids, others see Table 3. Different superscript letters in a row indicate significant difference ($p < 0.05$).

et al. (2015) for slow growing chicken of the breed Hetian. Breast muscle percentage varied between 19.5 and 22.3%, a bit higher compared to the values found by Fanatico et al. (2005) (17.8–18.4% slow growing hybrid, comparing indoor and outdoor access and higher feeding intensity).

WHC measurements of fresh and cooked meat differ in methodology between studies, so the values are difficult to compare with previous research. However, cooking loss was lower in our study compared to Dogan et al. (2019) who used a method similar to ours. These authors did not find a difference

Table 6. Fatty acid composition (% of total identified FA) in triacylglycerols from leg muscle (*M. flexor hallucis longus*) of two slow growing broiler hybrids raised in either small pens or big pens with outdoor access ($n = 6$) (means ± stdev).

Fatty acid	Hubbard		Rowan Ranger		Significances			
	big pen (HB)	small pen (HS)	big pen (RRB)	small pen(RRS)	s.e.m.	Hy	Ps	Hy x Ps
14:0	0.72 ± 0.11	0.73 ± 0.12	0.66 ± 0.99	0.69 ± 0.06	0.042	0.251	0.680	0.846
16:0	25.3 ± 3.64	29.5 ± 5.26	25.6 ± 3.08	25.0 ± 1.06	1.610	0.203	0.265	0.150
16:1n-9	0.70 ± 0.08	0.54 ± 0.22	0.57 ± 0.32	0.48 ± 0.34	0.117	0.410	0.285	0.800
16:1n-7	2.44 ± 0.38^{a}	2.08 ± 0.92^{a}	2.68 ± 0.69^{ab}	3.75 ± 0.45^{b}	0.290	0.004	0.236	0.022
18:0	6.22 ± 0.55^{ab}	7.09 ± 0.51^{a}	6.89 ± 1.21^{a}	5.36 ± 0.55^{b}	0.341	0.130	0.343	0.002
18:1n-9	37.4 ± 2.65	35.0 ± 3.88	38.0 ± 1.14	38.7 ± 1.55	1.140	0.069	0.460	0.190
18:1n-7	2.01 ± 0.13	1.96 ± 0.15	2.02 ± 0.12	2.14 ± 0.20	0.067	0.192	0.623	0.192
18:2n-6	19.2 ± 1.73	17.8 ± 2.66	18.2 ± 1.72	18.0 ± 0.93	0.830	0.603	0.323	0.466
18:3n-3	3.13 ± 0.40	2.98 ± 0.49	3.03 ± 0.42	2.94 ± 0.26	0.180	0.692	0.505	0.873
20:1n-9	0.45 ± 0.06	0.41 ± 0.08	0.45 ± 0.07	0.40 ± 0.04	0.029	0.821	0.112	0.865
20:2n-6	0.25 ± 0.03	0.17 ± 0.10	0.15 ± 0.11	0.19 ± 0.08	0.038	0.312	0.651	0.183
20:3n-6	0.29 ± 0.05	0.18 ± 0.13	0.14 ± 0.14	0.32 ± 0.08	0.048	0.904	0.503	0.007
20:4n-6	0.63 ± 0.12	0.58 ± 0.18	0.68 ± 0.16	0.73 ± 0.25	0.082	0.250	0.976	0.518
20:5n-3	0.10 ± 0.11	0.08 ± 0.12	0.07 ± 0.10	0.20 ± 0.16	0.056	0.464	0.351	0.204
22:4n-6	0.26 ± 0.03	0.20 ± 0.15	0.09 ± 0.12	0.18 ± 0.14	0.055	0.101	0.776	0.156
22:5n-3	0.44 ± 0.08	0.36 ± 0.17	0.38 ± 0.09	0.46 ± 0.11	0.053	0.697	0.938	0.134
22:6n-3	0.45 ± 0.10	0.36 ± 0.17	0.44 ± 0.12	0.52 ± 0.18	0.066	0.289	0.940	0.195
SFA	32.2 ± 4.23	37.4 ± 5.70	33.1 ± 3.87	31.1 ± 1.54	1.840	0.158	0.417	0.065
MUFA	43.0 ± 3.05^{ab}	40.0 ± 3.40^{a}	43.7 ± 1.49^{ab}	45.4 ± 1.93^{b}	1.160	0.014	0.586	0.054
PUFA	24.8 ± 2.41	22.7 ± 3.22	23.2 ± 2.44	23.5 ± 2.05	1.150	0.717	0.450	0.300
n-3	4.12 ± 0.53	3.78 ± 0.70	3.91 ± 0.64	4.11 ± 0.68	0.286	0.829	0.803	0.355
n-6	20.7 ± 1.90	18.9 ± 2.66	19.2 ± 1.84	19.4 ± 1.38	0.894	0.594	0.374	0.301
n6/n3	5.04 ± 0.24	5.07 ± 0.63	4.98 ± 0.47	4.78 ± 0.44	0.210	0.428	0.690	0.590
DPA + DHA	0.89 ± 0.18	0.72 ± 0.34	0.81 ± 0.21	0.98 ± 0.29	0.117	0.441	0.978	0.164
DPA + DHA/AA	1.41 ± 0.10	1.17 ± 0.56	1.21 ± 0.14	1.37 ± 0.15	0.135	0.966	0.775	0.150

Abbreviations: FA: fatty acids, others see Table 4. Different superscript letters in a row indicate significant difference ($p < 0.05$).

in any of the measured WHC parameters between fast growing (Ross-308) and slow growing broilers (T2-Y2), while Kocer et al. (2018) found higher drip- and cooking loss in fast growing broilers (Ross308) compared to slow growing (Hubbard Red-JA87). However, the housing conditions and the feeding regimens were different in these two trials. Broilers with outdoor access (Kocer et al., 2018) showed higher WHC values in general compared to the broilers with only indoor access (Dogan et al., 2019). In our study, no difference related to outdoor access was found. We suggest that WHC is a result of several production traits and therefore affected by a combination of parameters.

WBSF values were within normal range in both hybrids and production systems (16.7-29.3 N; Table 3), indicating a tender meat. In comparison, in a study by Stadig et al. (2016), breast muscle from both indoor and outdoor raised chickens had WBSF values 22.5–23.1 and scored tenderness values from 5.4 to 6.7. In the present study, H birds showed lower WBSF values compared to RR. Yang et al. (2015) found higher WBSF values in free range broilers, while Stadig et al. (2016) did not find any difference in WBSF values in meat from indoor and outdoor raised broilers even if indoor density of birds was higher in the study by Stadig et al. (2016) (12.5 birds/m^2) compared to Yang et al. (2015) (5 birds/m^2) and our study. We did not find any differences in meat tenderness in our study with regard to pen size (density 3.3 and 1.2 birds/m^2) within hybrids, but significant higher WBSF values in RR compared to H, indicating H might be the better hybrid for outdoor rearing.

Contradictory to Stadig et al. (2016) we did not find any differences in breast muscle colour between chickens reared indoors or with outdoor access. L* and a* values in the present study were lower compared to Stadig et al. (2016) indicating a darker and less reddish meat, while b* values were higher indicating a more yellowish meat in our study. Colour is among other factors affected by feed and its content of carotenoids. As all birds were given Alfalfa as environmental enrichment, this may have masked an effect of foraging outdoors due to the carotenoids in it.

As already stated, pen size did not show any effect on meat quality (Table 3). Stadig et al. (2016) discussed the level of free-range in connection with inconsistent findings for meat quality parameters in chicken. Most probably in the present study the difference in activity, e.g. caused by the longer distances between resources in the big pen, of the broilers was not high enough to result in differences in meat quality.

Lipid composition in leg muscle M. flexor hallucis longus

The second aim of this study was to evaluate if higher level of activity would alter lipid composition. Muscles with higher activity have a higher amount of oxidative muscle fibres containing higher proportions of PL (Raes et al., 2004). It has been shown in leg muscle of human that PL FA, but not TAG FA, composition was affected by exercise (Andersson et al., 1998). In order to evaluate the maximum difference, we choose a red muscle, being the most active, in the thigh for this purpose (*M. flexor hallucis longus*).

In the present study, fat content in *M. flexor hallucis longus* was not affected by the outdoor access, but there was a tendency for higher fat content in RR birds (Table 4). Also others found differences in fat content between indoor and outdoor reared chicken for slow and fast growing breeds (Castellini et al., 2002b; Chen et al., 2013; Comert et al., 2016). It cannot be ruled out that this could be due to the fact that different muscles were studied. Chen et al. (2013) found higher fat content in thigh muscle from broilers reared indoors compared to broilers reared outdoors for 70 days. However, in their study the whole thigh was used for lipid determination. Also Yang et al. (2015) found lower fat content in thigh muscle from outdoor raised chickens compared to indoor cages.

Lipid class composition was only significantly affected by hybrid with H having higher PL content compared to RR (Table 4). However, PL proportion had a tendency to be higher in chickens with outdoor access compared to the ones raised indoors in small pens ($p = 0.070$).

In the present study, muscles from birds reared in a big pen with outdoor access tended to have lower proportions of SFA and showed a significantly higher proportion of PUFA and total content of n-3 and n-6 FA compared to those from indoor pens. This, together with the tendency of higher PL seen in chickens with outdoor access, indicates an effect of voluntary exercise. An additional reason for higher proportion n-3 FA could be the additional intake of grass, being a source of 18:3 n-3, in the outdoor group. Yet, if the higher content of 18:3n-3 would be due to the grass feed the 18:2 n-6 proportion then would be decreased. It is, however, increased and correlates positively with 18:3n-3 as can be seen in the PCA plot of the FA of the total lipids (Figure 4). The increase of n-3 FA is in line with Stadig et al. (2016), who found higher content of n-3 and DHA in total lipids of breast muscle of chicken reared with outdoor access compared to chicken reared indoors. However, Stadig et al. (2016) analysed breast muscle while we analysed

leg muscle, which has a different metabolic activity, making it difficult to compare these. Michalczuk et al. (2017) analysed FA composition in both breast and leg muscle and found a significant higher proportion of SFA and lower proportion of n-3 in both muscle types in indoor raised chicken (total lipids) which is in line with our results.

In PL, significantly lower proportions of 20:2n-6 and EPA were found in R compared to H chickens. Furthermore, lower proportions of 16:0 and 18:1n-9 and higher proportions of AA and DPA were found in treatment B compared to S (Table 5). These results indicate that unsaturation in PL is important when muscle cells are active. In opposite, Andersson et al. (1998) found increased 18:1n-9 due to exercise in PL, but in line with the results of the present study Andersson et al. (1998) found a significantly lower proportion of 16:0 in PL due to exercise.

Our results indicate changes due to voluntary exercise in FA composition in the leg muscle *M. flexor hallucis longus* in total lipids and PL but not in TAG. In addition, our results also show that breed/hybrid has an effect on muscle lipid metabolism and composition.

Conclusions

The obtained results indicate no differences in technological meat quality parameters between the rearing systems but an effect in FA composition. Voluntary exercise did increase the proportion of unsaturated FA in thigh muscle. Furthermore, according to our results, the choice of hybrid has an effect on both meat quality and FA composition where RR birds had both higher breast percentage and a higher WBSF value, while H birds had higher proportions of PL and cholesterol and higher proportions of 20:2n-6 and EPA within the PL fraction.

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Disclosure statement

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