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Do Rearing System and Free-range Stocking Density Affect Meat Quality of Chickens Fed Feed Mixture with Rapeseed Oil?

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ABSTRACT

Skřivanová V., Tůmová E., Englmaierová M., Chodová D., Skřivan M. (2017): **Do rearing system and free-range stocking density affect meat quality of chickens fed feed mixture with rapeseed oil?** Czech J. Anim. Sci., 62, 141–149.

Recently, consumers have paid an attention to animal-friendly meat. The aim of the study was to evaluate the meat quality of breast muscles in indoor and free-range chickens with respect to the stocking density of outdoor chickens (8.3 or 4.15 birds per m^2) fed feed with rapeseed oil. The free-range chickens reached 96 and 97% of the final live weight of the indoor chickens and their feed : gain ratio was by 7 and 9% higher. The pasture intake had a small effect on the breast meat quality and was presumably associated with rapeseed oil in the basal diet which strongly influenced meat monounsaturated and polyunsaturated fatty acid content and reduced the effect of pasture. The free-range system and lower stocking density led to an increase in muscle fibre cross-sectional area and diameter and a higher meat shear force (P = 0.003). Concerning the meat sensory properties, the total acceptability of the meat of free-range chickens with a lower stocking density was found to be lower compared with the other groups. Results indicate a minor effect of the rearing system and free-range stocking density on chicken meat quality, and also that meat quality in experiments with free-range housing can be affected by a fat source in basal diet.

Keywords: chicken; housing; fat source; breast meat

Chicken meat plays an important role in human meat consumption. Recently, consumers have shifted attention from the meat origin to animal-friendly rearing systems. It is assumed that free-range and organic poultry production is beneficial to meat quality because of the prohibition of pesticides and chemicals. Recent studies on alternative rearing systems in meat chickens have demonstrated that reduced stocking densities increase the possibility of movement in both indoor and outdoor housing and that access to a pasture modifies the meat quality (Castellini et al. 2008). Pastures may constitute a source of energy and proteins for chickens, making available a range of bioactive compounds, such as antioxidants, hypocholesterolemic and anticarcinogenic compounds (Ponte et al. 2008). In terms of physical meat parameters, colour is important to consumers. Yellowness (CIE b^*) was observed to be dramatically higher in free-range breast meat (Fanatico

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et al. 2007; Funaro et. al. 2014). This meat colour can be affected by pigments from the pasture (Sales 2014). Pastures also significantly increased the redness (CIE *a**) of breast meat (Skrivan and Englmaierova 2014; Skrivan et al. 2015). Colour differences may occur in the lightness (CIE *L**) of breast, but they mainly occur in thigh meat (Funaro et al. 2014). However, Sales (2014) in his meta-analysis did not determine differences in the lightness of breast meat in free-range and conventional chickens. Meat colour is related to ultimate pH, which was observed to be significantly higher in free-range chickens (Funaro et al. 2014; Sales 2014). Sales (2014) reported that the breast comprises glycolytic fibres and that movement would improve oxidative metabolism and increase the number of mitochondria in glycolytic fibres, consequently transforming them into oxidative fibres (Ouhayoun and Dalle Zotte 1993).

An effect of pastures on meat chemical composition has not been observed (Skrivan et al. 2015). On the other hand, Funaro et al. (2014) found lower moisture and lipid but higher protein content in breast meat of free-range chickens. In birds with pasture access, Sales (2014) reported higher quantities of polyunsaturated (PUFA) n-3 fatty acids and less n-6 fatty acids. In contrast, a higher n-6/n-3 ratio was observed in free-range chickens (Funaro et al. 2014). The fatty acid (FA) composition may affect the lipid oxidation level. In free-range chickens, lower lipid oxidation levels but higher thiobarbituric acid reactive substances were found (Funaro et al. 2014; Skrivan et al. 2015). Fatty acids, α-tocoferol, and meat oxidation stability have been affected by a source of fat in basal diet. For example, in free-range chickens Ponte et al. (2008) observed a higher content of n-6 FA which was affected by a higher level of corn in the feed mixture. Liu and Zhou (2013) found a higher content of linolenic acid, eicosapentaeonic acid (EPA), reduced n-6/n-3 ratio, greater thiobarbituric acid reacting substances in meat of geese on pasture but fed a diet with corn and soybean oil. A positive effect of pasture on α-linolenic acid and α-tocopherol in eggs was reported by Lopez-Bote et al. (1998) in eggs but basal diet contained full fat soybean and lard. These data showed that FA composition depends on the source of oil in feed mixture. The positive effect of rapeseed oil (RO) on increasing of PUFA and n-3 FA in chicken meat was documented (Haugh et al. 2011). Similarly, Jankowski et al. (2012) observed higher concentration of linolenic acid, PUFA, and improved n-6/n-3 ratio in turkeys fed RO in comparison with soybean oil.

Pasture intake and its effect on meat quality may depend on stocking density. Skrivan et al. (2015) reported that the surface area per chick varies from one to tens of m^2. Presumably, the stocking density affects pasture intake because Ponte et al. (2008) reported that pasture intake was 2.5% (4 g dry matter (DM) per chick daily), Lorenz et al. (2013) observed that grazing can account for 10–15% of the feed intake (2–5 g DM/chick/day), and Skrivan et al. (2015) found that chickens consumed only 1 g DM/chick/day. The present study was oriented not only to compare indoor and free-range systems but also to evaluate two stocking densities in the free-range system on meat quality parameters of chickens fed basal diet with RO.

MATERIAL AND METHODS

Animals and sample collection. The experiment was performed with 270 one-day-old Hubbard JA757 cockerels. The chickens were split into three groups of 90 chickens. The first group (I) was kept in one indoor litter pen under intensive conditions with a floor density of 13.50 chickens per m^2. The second group (FRI) comprised free-range chickens with a higher stocking density (8.30 chickens per m^2), and the last one (FRII) was free-range with half the density of group FRI (4.15 chickens per m^2). Chickens of all groups were kept until 28 days of age in indoor pens, and then the free-range groups were moved to floorless portable pens (Skrivan et al. 2015). The portable pens were moved daily to restrict grassland damage. The experiment lasted until the age of 55 days. The environmental conditions in indoor pens were kept in accordance with the requirements for chickens. The lighting regime consisted of 16 h light. The experiment was conducted in spring from April to June. The experimental area and experimental conditions were described by Skrivan et al. (2015). The experiment was approved by the Ethical Committee of the Institute of Animal Science.

Throughout the experiment, the chickens were fed and watered *ad libitum*. Three feed mixtures were fed during the experiment: a starter until 28 days of age, a grower between 29 and 42 days

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of age, and a finisher from 43 to 55 days of age (Table 1). Pasture intake was indirectly assessed by a modified method of Dal Bosco et al. (2014). Pasture samples were collected in each of square areas (50 × 50 cm) and then calculated for the whole area of each pen. Measurements were taken at 12 locations and evenly distributed throughout the pasture plot to achieve an average difference. The sward was first measured before the placement of the portable pen and once again after the portable pen was relocated to another position (Skrivan et al. 2015).

At the end of the experiment, 16 chickens of average weight from each group were selected for slaughtering. The carcasses were cooled for 24 h, and then the breast muscles were dissected for physical, chemical, and sensory analyses.

Physical analysis. The ultimate pH values were detected 24 h *post mortem* using a 330i pH meter (WTW, Germany) with a glass probe introduced 1 cm deep into the transversal section of the *pectoralis major* muscle. The meat colour was measured on a transversal section of the breast muscle 24 h *post mortem* using the Minolta SpectraMagic™ NX analyser (Konica Minolta Sensing, Inc., Japan) with the CIELAB System (1976). The instrumental meat colour was expressed as *L**, *a**, and *b**. Meat tenderness was determined by the Warner-Bratzler shear test in the breast muscle. The right part of the *pectoralis major* muscle was frozen to –20°C, and the samples were later defrosted at 4°C for 24 h. Samples of the *pectoralis major* muscle were packaged in plastic bags with zip ties and heated in a water bath at 75°C for 1 h. The meat samples were cut into 2 × 1 cm cuboids with the cuts running parallel to the muscle fibres. Tenderness was measured using an Instron Model 3342 (Instron, USA) with a Warner-Bratzler shear blade with a triangular hole. The load cell was 20 N with a crosshead speed of 100 mm/min and a sampling rate of 20 points/s. The maximum shear force (N) was determined.

Histochemical analysis. To determine the histochemical parameters (Tumova et al. 2015; Chodova et al. 2016) of the *pectoralis major* muscle, samples were collected immediately after slaughter, frozen in 2-methylbutane cooled by liquid nitrogen (–156°C), and stored at –80°C until histochemical analysis. For each sample, 12 µm thick cross-sections were cut at –20°C using Leica CM1850 (Leica Microsystems Nussloch GmbH, Germany).

Table 1. Composition and nutrient content in the diet and pasture

Ingredient (g/kg)	Starter	Grower	Finisher	Lyophilized pasture
Wheat	290.0	420.0	486.7	
Maize	277.5	210.0	210.0	
Soybean	360.0	248.0	215.0	
Wheat bran	–	50.0	39.6	
Rapeseed oil	30.0	30.0	18.0	
Monocalcium phosphate	13.0	11.0	7.5	
Sodium chloride	3.0	3.0	3.0	
Limestone	17.0	18.5	12.5	
L-Lysine hydrochloride	1.3	2.1	1.0	
DL-Methionine	2.9	2.1	1.7	
L-Threonine	0.3	0.3	–	
Vitamin-mineral premix[1]	5.0	5.0	5.0	
Analyzed composition				
Dry matter (g/kg)	883.7	902.6	890.9	937.9
Crude protein (g/kg)	216.6	183.9	180.5	150.8
Ether extract (g/kg)	59.3	52.5	40.4	43.6
Crude fibre (g/kg)	43.0	44.3	43.1	241.9
AME (by calculation MJ/kg)	11.8	12.0	11.8	5.4
ALA (mg/100 g)	498	382	178	1270
EPA (mg/100 g)	6.1	4.5	2.7	3.0
DHA (mg/100 g)	3.2	2.5	1.8	2.0
SFA (mg/100 g)	1599	1386	1142	475
MUFA (mg/100 g)	2913	2258	1630	182
PUFA (mg/100 g)	2748	2062	1337	1645
Vitamin E (mg/100 g)	57.8	31.9	20.2	34.8
Vitamin A (mg/100 g)	3.0	2.7	1.7	–
Zeaxanthin (mg/100 g)	0.75	0.69	0.91	162.9
Lutein (mg/100 g)	1.04	1.00	1.28	187.2

AME = apparent metabolizable energy, ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

[1]vitamin-mineral premix provided per kg of diet: retinyl acetate 3.6 mg, cholecalciferol 13 µg, α-tocopherol acetate 30 mg, menadione 3 mg, thiamine 3 mg, riboflavin 5 mg, pyridoxine 4 mg, cyanocobalamin 40 µg, niacin 25 mg, calcium pantothenate 12 mg, biotin 0.15 mg, folic acid 1.5 mg, choline chloride 250 mg, copper 12 mg, iron 50 mg, iodine 1 mg, manganese 80 mg, zinc 60 mg, selenium 0.3 mg

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Subsequently, staining by haematoxylin and eosin for the basic histological characteristics of the muscle fibres was performed. Image analysis NIS Elements AR 3.1 (Laboratory Imaging s.r.o., Czech Republic) was used to detect the number of muscle fibres per 1 mm^2, fibre cross-sectional area (CSA) and diameter.

Chemical analyses. Chemical composition of the meat was analyzed using the left breast. Until analyses the meat samples were stored in plastic bags at –20°C. Dry matter of the meat was determined by oven drying at 105°C to constant weight. The ether extract was obtained by extraction with petroleum ether in a Soxtec 1043 apparatus, the protein content using a Kjeltec Auto 1030 Analyser (both FOSS Tecator AB, Sweden). For determination of the cholesterol lipids were saponified, and the unsaponified matter was extracted with diethyl ether. The fatty acid composition of the diet and the breast meat was determined after chloroform-methanol extraction of the total lipids (Folch et al. 1957). Nonadecanoic acid (C 19:0) was used as an internal marker to quantify the FA in the samples. The alkaline trans-methylation of the FA was performed (Raes et al. 2003). The gas chromatography of the methyl esters was performed using an HP 6890 chromatograph (Agilent Technologies, Inc., USA) with a programmed 60 m DB-23 capillary column (150–230°C) and a flame-ionisation detector; the split injections were performed using an Agilent autosampler. One-microlitre samples of FAME in hexane were injected at a 1 : 40 split ratio. The separation was achieved using the following column temperature program: initially, the column was operated at 60°C for 7 min, and then the temperature was programmed at 20°C/min to 110°C and held for 4 min, programmed at 10°C/min to 120°C and held for 4 min, programmed at 15°C/min to 170°C and held for 4 min, programmed at 2°C/min to 210°C and held for 13.5 min, and finally programmed at 40°C/min to 230°C and held for 7 min. The fatty acids were identified by the retention times compared with the standards. The PUFA 1, PUFA 2, PUFA 3, and 37 Component FAME Mixes (Supelco, USA) were used as standards.

Next, the following calculations were performed:

Atherogenic index

AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + n-3 PUFA + n-6 PUFA)
(Ulbricht and Southgate 1991)

Thrombogenic index

TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6 PUFA)
(Ulbricht and Southgate 1991)

Peroxidation index

PI = (0.025 × % monoenoics) + (1 × % dienoics) + (2 × % trienoics) + (4 × % tetraenoics) + (6 × % pentaenoics) + (8 × % hexaenoics)
(Cortinas et al. 2003)

Hypocholesterolemic/hypercholesterolemic index (the ratio between hypocholesterolemic and hypercholesterolemic fatty acids)

h/H = (C18:1 + C18:2 + C20:4 + C18:3 + C20:5 + C22:5 + C22:6)/(C14:0 + C16:0)
(Santos-Silva et al. 2002).

To determine the α-tocopherol and retinol contents of the feed, lyophilized pasture vegetation, and skinless breast muscle, each sample was homogenized. The European Standards for high-performance liquid chromatography (HPLC) (EN 12822, 2000; EN 12823-1, 2000) using a diode-array detector (VP series; Shimadzu, Japan) were followed. The samples were subjected to alkaline saponification with 60% potassium hydroxide followed by appropriate extraction with diethyl ether. The standard used was α-tocopherol (purity ≥ 97.0%) (Sigma-Aldrich, USA).

The contents of lutein and zeaxanthin were measured by HPLC (Froescheis et al. 2000). One gram of homogenized samples was placed in a plastic tube together with 20 ml of acetone. After vortex mixing (2 min), the sample was cooled in ice for 10 min and centrifuged at 13 000 *g* for 10 min at 4°C. The supernatant was transferred to a glass tube, and the pellet was extracted a second time as previously described. The combined acetone phases were evaporated to dryness under a stream of N_2 at 50°C, redissolved in 2 ml ethanol–water (1:1, v/v), and extracted twice with hexane (4 and 2 ml). Each extraction step was performed by vortex mixing for 2 min followed by centrifugation at 13 000 *g* (10 min, 4°C). After evaporating the combined organic phases to dryness under a stream of N_2, the residue was reconstituted in a final volume of 1 ml hexane/dichloromethane (1:1, v/v). An aliquot of 60 µl was subjected to HPLC

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(VP series; Shimadzu) analysis. A Kinetex C18 column (100 × 4.6 mm; 2.6 µm) (Phenomenex, USA) was used. A gradient system was applied with acetonitrile : water : ethylacetate (88 : 10 : 2) as eluent A and acetonitrile:water:ethylacetate (88 : 0 : 15) as eluent B.

The oxidative stability was measured with the TBARS test in the skinless breast muscle using the thiobarbituric acid (Piette and Raymond 1999). The results are expressed in mg of malondialdehyde (MDA) per kg of meat. The meat samples were vacuum-packed and stored at 4°C for zero or five days.

Sensory analysis. For the sensory evaluation, the procedure described by Bures et al. (2014) was applied. Chicken breasts without skin were evaluated by a panel of ten trained assessors. The evaluation was performed in a sensory laboratory equipped with booths. The samples were cooked at 180°C for 1 h without any spices and other ingredients. The samples were cut into approximately 2 × 2 × 2 cm cubes, placed in covered glass containers marked with three-digit random numbers, and served at 50°C to the sensory panel. To prevent cooling, the samples were served on preheated plates. There was a 10 min interval between each sample. Room temperature water and bread were provided to the panel members to neutralize their sensory precepts. The appearance and odour of the samples were scored, followed by tenderness, juiciness, flavour, and overall acceptability. A nine-point scale was used for the assessment (1 – very undesirable, 9 – very desirable).

Statistical analysis. The data from the experiment, meat physical measurements, muscle fibre characteristics, meat chemical composition were processed with one-way analysis of variance using ANOVA method of the SAS software (Statistical Analysis System, Version 9.3., 2013). Differences between the groups were tested by the Duncan test. The sensory data were evaluated using a GLM procedure of the SAS with group and panellist as fixed effects. Differences between groups were evaluated with Tukey's range test. A P-value of $P < 0.05$ was considered significant for all measurements.

RESULTS AND DISCUSSION

Performance. The final body weight (BW) was lower in both groups of free-range rearing systems ($P = 0.005$) compared with indoor chickens (Table 2). Free-range chickens reached 96% and 97% of the indoor chickens' BW. These results agree with Almasi et al. (2015); however, Sales (2014) did not observe significant variability in BW between indoor and free-range systems. The stocking density did not affect the final BW. The feed : gain ratio was by 7% and 9% higher in free-range chickens than in indoor ones. Free-range chickens pasture represented 2.3–3.5% of feed intake (g DM/chick/day), which was similar to the findings of Lorenz et al. (2013). Regarding to stocking density, negligible differences in pasture intake were observed whereas chickens at a lower density did not use the pasture properly.

Meat physical measurements and muscle fibre characteristics. The results of pH, meat colour, and tenderness are presented in Table 3. The ultimate pH of the breast muscles was higher in free-range chickens but significantly only in the group with higher stocking density, whereas the chickens with lower stocking density did not differ from indoor chickens as much as from the chickens with higher density. It is possible to assume that pH was more likely related to the muscle energy metabolism of free-range chickens. Muscle pH corresponds with meat colour; however, in the current study, none of the breast meat colour parameters was affected by the rearing system or stocking density. The results correspond with the findings of Almasi et al. (2015) but are in contrast with the data of Funaro et al. (2014), Sales (2014), and Skrivan et al. (2015), who found higher meat yellowness in

Table 2. Growth traits

Characteristic	I	FRI	FRII	SEM	P
BW (day 28; g)	1065[a]	1009[b]	1052[a]	7.1	0.003
BW (day 55; g)	3194[a]	3071[b]	3109[b]	15.9	0.005
Feed intake (g/day/pcs)	107.0	111.2	113.0		
F : G (g/g)	1.89	2.03	2.07		
Pasture intake (g DM/day/pcs)					
Day 36	–	2.7	2.7		
Day 42	–	2.6	3.7		
Day 49	–	3.5	3.8		

I = indoor housing, FRI = free-range 8.30 chickens per m^2, FRII = free-range 4.15 chickens per m^2, BW = body weight, F : G = feed : gain, DM = dry matter

[a,b]means with different superscripts differ significantly

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Table 3. Physical characteristics of breast meat

Characteristic	I	FRI	FRII	SEM	*P*
pH_{45}	6.6	6.4	6.6	0.06	ns
pH_{24}	5.6^{b}	5.9^{a}	5.7^{ab}	0.04	0.043
Colour of raw meat					
*L**	55.6	56.0	53.6	0.71	ns
*a**	–2.0	–2.7	–2.8	0.16	ns
*b**	4.0	4.2	3.6	0.46	ns
Shear force of boiled meat (N)	34.2^{a}	29.2^{b}	27.1^{b}	0.82	0.003

I = indoor housing, FRI = free-range 8.30 chickens per m^2, FRII = free-range 4.15 chickens per m^2, pH_{45} = pH 45 min after slaughter, pH_{24} = pH 24 h after slaughter, ns = not significant

^{a,b}means with different superscripts differ significantly

free-range chickens. Yellowness is associated with the carotenoid content of the diet, which can also be deposited in intramuscular and intracellular lipids (Funaro et al. 2014). The variability in meat colour could also be explained by the length of the experiment and the free-range period (Sales 2014), which was 27 days in this experiment and much shorter than in the studies of Funaro et al. (2014) and Almasi et al. (2015).

The breast muscle shear force was significantly lower in free-range chickens without the effect of stocking density (Table 3). The results are in contrast with Funaro et al. (2014), who did not find differences between free-range and indoor chickens in muscle shear force. The discrepancy between both studies could be explained by the age of chickens at slaughter in their study because the indoor chickens were slaughtered earlier than the free-range chickens and could have lower collagen content. However, the lower shear force of free-range chickens in the present study might be associated with the muscle fibre characteristics (Table 4). The breast muscles in all groups were composed only by glycolytic αW fibres with higher CSA ($P = 0.001$) and diameter ($P = 0.001$) in free-range chickens. Both these characteristics were significantly affected by the stocking density. The higher CSA and fibre diameter in the group with lower density supports the assumption of the effect of movement on muscle fibre area suggested by Branciari et al. (2009) in which the greater CSA could be due to their adaptability to motor activity. The lower number of muscle fibres and their larger CSA may be associated with the lower collagen content and, therefore, the lower shear force in free-range chickens. The lowest diameter was observed in indoor chickens the meat of which scored best for sensory juiciness.

Chemical composition. Meat nutritional value is important for consumers and may be affected by many factors. The basic breast chemical composition (Table 5) was not significantly affected by the rearing system except dry matter. In the literature, Fanatico et al. (2007) and Skrivan et al. (2015) did not observe any effect of the rearing system on the meat chemical composition. The indoor chickens had significantly ($P = 0.003$) higher dry matter compared with both free-range groups. Presumably, the lower breast dry matter content in free-range chickens was associated with numerically lower fat content in these groups and is similar to the data of Funaro et al. (2014), who found a significantly lower fat content in breast. In the current study, the fat content in free-range chickens was approximately by 7% lower compared with indoor chicken breast meat, and it is assumed that insignificant differences were affected by a higher variability of the measurement. The stocking density of free-range chickens did not affect the fat content, and it can be assumed that chicken movement was similar in both stocking densities.

The rearing system and stocking density had little effect on the FA composition of the breast meat (Table 5). There was no effect on the most abundant breast meat FA such as oleic and palmitic acid. Free-range chickens had a lower content of linoleic ($P = 0.031$) and α-linolenic acid (ALA) ($P < 0.001$), which corresponds with Ponte et al. (2008) and Funaro et al. (2014). The stocking density did not affect the linoleic acid content, but the ALA content was significantly lower in the

Table 4. Muscle fibre[1] characteristics of *pectoralis major*

Characteristic	I	FRI	FRII	SEM	*P*
Number of fibres (per 1 mm^2)	295	268	245	9.4	ns
Cross-sectional area (μm^2)	2303^{c}	2662^{b}	2886^{a}	36.5	< 0.001
Diameter (μm)	51.4^{c}	55.4^{b}	57.7^{a}	0.45	< 0.001

I = indoor housing, FRI = free-range 8.30 chickens per m^2, FRII = free-range 4.15 chickens per m^2, ns = not significant

[1]muscle fibre type IIB

$^{a–c}$means with different superscripts differ significantly

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Table 5. Chemical analysis of breast meat

Characteristic	I	FRI	FRII	SEM	*P*
DM (g/kg)	270[a]	265[b]	269[b]	0.8	0.003
Crude fat (g/kg DM)	21.1	17.6	17.5	0.90	ns
Crude protein (g/kg DM)	879	885	885	1.4	ns
Cholesterol (mg/kg)	444	396	446	13.3	ns
Palmitic acid (mg/100 g)	215.1	215.7	213.3	7.53	ns
Stearic acid (mg/100 g)	88.0	81.7	88.9	4.21	ns
Oleic acid (mg/100 g)	352.8	301.7	313.2	13.42	ns
LA (mg/100 g)	167.2[a]	134.4[ab]	119.8[b]	7.80	0.031
ALA (mg/100 g)	38.2[a]	30.2[b]	25.2[c]	1.38	< 0.001
EPA (mg/100 g)	4.5	4.2	4.1	0.20	ns
DHA (mg/100 g)	5.6	5.8	7.1	0.29	ns
SFA (mg/100 g)	316.5	314.0	319.3	11.30	ns
MUFA (mg/100 g)	417.0	351.1	369.2	15.67	ns
PUFA (mg/100 g)	264.7[a]	224.1[ab]	203.2[b]	9.97	0.029
n-3 (mg/100 g)	57.1[a]	48.6[b]	44.9[b]	1.58	0.002
n-6 (mg/100 g)	206.7	174.6	157.4	8.61	ns
n-6/n-3	3.6	3.6	3.5	0.10	ns
Zeaxanthin (mg/kg DM)	0.44	0.47	0.54	0.026	ns
Lutein (mg/kg DM)	0.53	0.55	0.64	0.030	ns
Vitamin E (mg/kg DM)	20.5[a]	16.5[b]	19.6[a]	0.61	0.009
Vitamin A (mg/kg DM)	0.14	0.13	0.12	0.004	ns
MDA 0 (mg/kg)	0.343[b]	0.372[a]	0.366[a]	0.005	0.020
MDA 5 (mg/kg)	0.371[b]	0.452[a]	0.434[a]	0.012	0.003
PI	51.8	52.8	49.0	1.12	ns
TI	0.64[b]	0.75[a]	0.79[a]	0.019	< 0.001
AI	0.35[b]	0.44[a]	0.45[a]	0.012	< 0.001
h/H	2.77[a]	2.28[b]	2.27[b]	0.066	< 0.001

I = indoor housing, FRI = free-range 8.30 chickens per m^2, FRII = free-range 4.15 chickens per m^2, LA = linoleic acid, ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, PI = peroxidation index, TI = thrombogenic index, AI = atherogenic index, h/H = hypocholesterolemic/hypercholesterolemic index, DM = dry matter, ns = not significant values of thiobarbituric acid-reactive substances are presented in mg malondialdehyde (MDA) per kg

[a–c]means with different superscripts differ significantly

groups with lower stocking density. This result is opposite to expectations because the pasture is a source of this FA. The lower content of ALA can be explained by the data of Ponte et al. (2008) who observed a different seasonal effect on the ALA with increasing content during autumn. Lower ALA content in our study is assumed to be linked with a fat source in feed mixtures because in literature, corn and soybean oil (Liu and Zhou 2013), full fat soya and lard (Lopez-Bote et al. 1998) were used and therefore pasture more affected the FA composition. Ponte et al. (2008) reported that the lower content of ALA in free-range chickens may be associated with greater conversion of the acid to its derivatives, EPA, and docosahexanoic (DHA) fatty acids. The EPA and DHA contents were not affected by the rearing system and stocking density in the current study. The DHA content numerically increased in free-range chickens and was higher at lower stocking density. It can be speculated that DHA was more effectively converted from ALA because its content decreased in a similar way. This assumption is related to the observation of Ponte et al. (2008) and Dal Bosco et al. (2014) that low levels of pasture intake did not contribute to increasing the ALA in breast meat, whereas the desaturation and elongation of this fatty acid precursor may contribute to the synthesis of its long-chain fatty acids derivatives. Regarding the FA group, the PUFA content was significantly lower in the free-range chickens with lower stocking density. The reduction in PUFA was associated with lower n-6 and significantly lower n-3 fatty acid content. However, stocking density did not significantly affect PUFA and n-3 FA. In the present study, a higher content of n-3 FA in indoor chickens was affected by RO in basal diet.

Table 6. Sensory analysis of breast meat

Characteristic[1]	I	FRI	FRII	SEM	*P*
Odour	6.0	6.0	5.7	0.10	ns
Tenderness	5.6	5.7	5.2	0.11	ns
Juiciness	5.1	4.8	4.5	0.12	ns
Flavour	5.8	5.7	5.6	0.09	ns
Total acceptability	5.6[a]	5.3[a]	4.8[b]	0.10	0.002

I = indoor housing, FRI = free-range 8.30 chickens per m^2, FRII = free-range 4.15 chickens per m^2, ns = not significant

[1]all traits were assessed by a 10-member panel on a scale: 1 – very undesirable, 9 – very desirable

[a,b]means with different superscripts differ significantly

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Pastures are a good source of natural antioxidants and may be beneficial to meat oxidative stability. In the current study, the rearing system did not affect the zeaxanthin, lutein, and vitamin A content in the breast meat. Vitamin E was the lowest (P = 0.009) in the group FRII with higher stocking density, whereas the indoor group and free-range chickens with lower stocking density did not differ. Ponte et al. (2008) did not find an effect of pasture on vitamin E; however, seasonal variations were observed. In contrast, Skrivan et al. (2015) reported a nearly doubled vitamin E concentration in breast muscle after 19 days in the pasture. In their experiment, fast-growing broiler chickens were used and presumably deposited more intramuscular fat which could affect the vitamin content in the breast meat.

The meat oxidative stability (Table 5) was better in the breast meat of indoor chickens and was confirmed by other oxidation measurements. The peroxidation index was not affected by either the rearing system or stocking density; however, a higher thrombogenic index (P < 0.001), atherogenic index (P < 0.001), and lower hypocholesterolemic/hypercholesterolemic index (P < 0.001) were found in free-range chickens without the effect of stocking density. The free-range system led to a significantly higher MDA content. The results are in correspondence with the findings of Funaro et al. (2014) and their assumption that higher TBARS in the meat of free-range chickens could be due to the content of metallic ions that catalyze peroxidation and to the greater degree of unsaturation of intramuscular fat. On the other hand, lipid oxidation was not affected by the stocking density. Presumably, better oxidation stability of indoor chickens in the present study was affected by RO in the diet.

Sensory characteristics. Sensory traits can be affected by many factors, such as breed, sex, carcass weight, diet, genetic variation, and biochemical changes that occur during further processing. Individual sensory characteristics, odour, tenderness, juiciness, and flavour were not affected by the rearing system and stocking density. However, total acceptability scored significantly lowest (P = 0.002) in the free-range chickens with lower stocking density whereas indoor and higher free-range stocking density groups did not differ (Table 6). The sensory total acceptability is mainly affected by tenderness and juiciness (Tumova et al. 2016), and these characteristics were numerically the lowest in the groups of free-range chickens with lower stocking density. Meat tenderness and juiciness are negatively correlated with intramuscular fat (Renand et al. 2001), which was the lowest in this group. Meat tenderness is also negatively correlated with muscle fibre CSA (Grashorn 2010), and the group had the significantly largest CSA. It is speculated that in the current study, the total acceptability was more likely affected by the muscle fibre characteristics (CSA).

CONCLUSION

In conclusion, the rearing system had a minor effect on chicken breast meat quality. For fatty acid composition, only α-linolenic acid significantly decreased in free-range chickens which is assumed to be affected by rapeseed oil in basal diet. The movement of free-range chickens was manifested in the large cross-sectional area and diameter of breast muscle fibres and, presumably, decreased instrumental tenderness. The muscle fibre characteristics were affected by the stocking density. However, the stocking density in free-range chickens had no impact on pasture intake and meat quality. Results also indicate that meat quality in experiments with free-range housing can be affected by a fat source in basal diet. This relationship between the fat source and the effect of pasture on meat quality has been neglected.

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