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To cite this article: Dorota Cygan-Szczegielniak, Giuseppe Maiorano, Bogdan Janicki, Mateusz Buzala, Karolina Stasiak, Magdalena Stanek, Aleksandra Roślewska, Gabriela Elminowska-Wenda, Joanna Bogucka & Siria Tavaniello (2019) Influence of rearing system and sex on carcass traits and meat quality of broiler chickens, Journal of Applied Animal Research, 47:1, 333-338, DOI: 10.1080/09712119.2019.1634076

To link to this article: <https://doi.org/10.1080/09712119.2019.1634076>



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Published online: 26 Jun 2019.



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Influence of rearing system and sex on carcass traits and meat quality of broiler chickens

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ABSTRACT

The trial was conducted in order to evaluate the effect of the rearing system and sex on live body weight, daily weight gain, carcass traits and meat quality of broiler chickens. Sixty 1-d-old Ross 308 chickens (males and females) were randomly divided, according to the sex, into 2 experimental groups based on rearing system: intensive system (IS; $n = 30$), birds reared till 42 d of age and semi-intensive system (SIS; $n = 30$), birds reared till 56 d of age. Compared with SIS group, IS group had higher ($P < 0.01$) daily weight gain, weight and yield of pectoral muscle (PM). Differently, SIS group had higher ($P < 0.05$) carcass weight and carcass yield. Males have usually a better performance than females. In comparison with IS, SIS exhibited a lower ($P < 0.01$) breast meat pH and higher ($P < 0.01$) lightness and yellowness values. Pectoral muscle from IS broiler chickens showed a greater ($P < 0.01$) fibre diameter and significantly higher shear force values than PM from SIS chicken. PM from IS chicken group had higher ($P < 0.01$) dry matter and protein content and slight fat content than PM from SIS chickens. The sex influence was not observed on physicochemical characteristics of PM in chickens reared in both system.

ARTICLE HISTORY

Received 2 July 2018
Accepted 12 June 2019

KEYWORDS

Chicken; meat quality; performance traits; rearing systems; sex; fibre diameter

Introduction

In the last decades, the growing demand for poultry meat has forced the producers to seek methods for increasing the productivity of live birds (e.g. growth rate, dry matter intake efficiency, size of pectoral muscle) and respond to the consumers' desire for healthier meat. Nowadays, consumers are more and more interested in the sustainability, health and nutritional aspects of meat products; they are willing to pay higher price for meat products that they perceived as naturally produced and with high standard of animal welfare. Thus, in many countries, rearing systems alternate to conventional ones were established for poultry meat production (Fanatico et al. 2007). However, in Europe and United States, organic farming frequently uses fast-growing broilers receiving a specially selected low-density diet, due to the high production efficiency of these birds, but also to the low availability of indigenous breeds (Fanatico et al. 2005; Sirri et al. 2011). However, with its characteristics specifically selected for production in controlled conditions, this genotypes may be unsuitable for systems with less controlled conditions (Sirri et al. 2011); furthermore, their growth profile does not follow the one for 81-d production, representing the statutory minimum slaughtering age for organic chickens (cited by Sirri et al. 2011). However, the suitability of these fast-growing broilers for natural and organic production systems has not been well researched with regard to performance, meat quality, histological characteristics of

muscles and sensory acceptability. Intensive selection, feeding system, genotype, age and sex may influence various parameters contributing to overall quality of poultry products, including their nutritional value and functional characteristics (Elminowska-Wenda et al. 1997; Mikulski et al. 2011; Tavaniello et al. 2014). Moreover, in recent years, great attention has been paid not only to the physicochemical and nutritional properties of meat, but also to the histological features of pectoral muscles, also due to the increasing occurrence of new histopathological changes in pectoral muscle, such as white striping and wooden breast (Maiorano et al. 2012; Elminowska-Wenda et al. 2014; Sihvo et al. 2014).

The main trial was conducted in order to evaluate the effect of the rearing system (intensive and semi-intensive) and sex on live body weight, daily weight gain, carcass traits, meat quality (physicochemical properties), as well as histological parameters in pectoral muscle (PM) of broiler chickens.

Material and method

Animals and experimental design

The experiment was conducted during the Spring season, from March to May, in a conventional poultry farm located in the central region of Poland (Kujawsko-Pomorskie Voivodeship). A total of 60 one-d-old Ross 308 chickens (half males and half females) were randomly divided, according to the sex, into 2

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experimental groups based on rearing system: intensive system (IS; n=30), birds reared till 42 d of age and semi-intensive system (SIS; n=30), birds reared till 56 d of age. Birds from IS and SIS groups were reared according to the Polish Local Ethical Commission (No 22/2012. 21.06.2012) and in accordance with the animal welfare recommendations of European Union Directive 86/609/EEC. The IS group was reared indoor at high stocking density (0.28 m²/bird; for 5 males and 5 females in each 3 pens: two replications for experimental groups). The SIS group had an indoor area (0.6 m²/bird; for 5 males and 5 females in each 3 pens: two replications for experimental groups) with an outdoor run availability of 1.6 m²/bird. Birds were provided with proper rearing conditions, with constant monitoring of stocking density, litter quality, ventilation and lighting. For the SIS group, outdoor access from the pens was provided after 4 weeks of age during daylight hours (from 8.00 am to 3.00 pm) and were exposed to the natural environment, therefore, they had access to open-air green, grassy pens (the average air temperature in the whole experimental period ranged from 13°C to 15°C). All birds of IS group were provided with a multiphase diet (Table 1).

Additionally, in the case of the SIS group, starting from the 14th day of breeding the birds obtained, aside from the compound feed presented in Table 1, also farm fodders in a form of cereal middlings (15% wheat, 10% barley, 5% corn), as well as wheat wholegrain (Table 2).

All birds had an unlimited access to water. During the whole time of the experiment, the birds' health was under constant supervision.

Table 1. Composition and calculated analysis of feeds (g/kg as fed-basis) supplied to the chickens reared under intensive system (IS).

	Starter (1–21 d)	Grower (22–35 d)	Finisher (36–42 d)
Ingredients (g/kg)			
Wheat	267.3	291.9	306.6
Corn	300.0	300.0	300.0
Soybean, extracted meal	325.0	282.0	253.3
Soybean oil	21.0	13.3	18.0
Canola	50.0	60.0	70.0
Lard	–	20.0	25.0
NaCl	3.0	3.0	2.8
Limestone	10.9	9.5	8.5
Monocalcium phosphate	11.5	9.4	6.3
DL-methionine	2.5	1.8	1.3
L-lysine	3.2	3.2	2.7
L-threonine	0.6	0.9	0.5
Vitamin-mineral premix ^a	5.0	5.0	5.0
Calculated nutrient level ^b (g/kg)			
CP	220.0	205.0	195.0
Crude fat	60.9	77.0	90.4
Lysine	13.5	12.5	11.5
Methionine + cystine	9.5	8.5	7.8
Ca	9.0	8.0	7.0
P	4.0	3.5	2.8
Na	1.4	1.4	1.3
ME (kcal/kg)	2980	3100	3200

^aProvided the following per kg of diet: vitamin A, 12,500 IU; vitamin D₃, 4,500 IU; vitamin E, 45 mg; vitamin K₃, 3 mg; vitamin B₁, 3 mg; vitamin B₂, 6 mg; vitamin B₆, 4 mg; pantothenic acid, 14 mg; nicotinic acid, 50 mg; folic acid, 1.75 mg; choline, 1.6 g; vitamin B₁₂, 0.02 mg; biotin, 0.2 mg; Fe, 50 mg; Mn, 120 mg; Zn, 100 mg; Cu, 15 mg; I, 1.2 mg; Se, 0.3 mg; phytase, 500 FTU; diclazuril, 1 mg (only in starter and grower diets).

^bEstimation based on the tables of Polish feedstuff analysis (Smulikowska and Rutkowski 2005).

Slaughter Surveys

On the last day of rearing (42 d or 56 d of age), each bird (males and females) was individually evaluated in terms of its weight (after a 12-hour fasting period) and then carefully caught, loaded and delivered to a commercial poultry abattoir, where they were unloaded, hung up at random, stunned by electric shock and then slaughtered all the tested birds (i.e. 60 birds, 15 males and 15 females from each group). After evisceration, the hot carcass weight was recorded, and carcass yield was calculated. At slaughter, the PM was removed from all carcasses and its percentage based on hot carcass weight was calculated. Afterward, it was vacuum packaged and stored frozen (–20°C) until analyses their chemical composition and shear force.

Physico-Chemical properties

The PM pH was measured using a portable pH-meter (pH-Star Matthäus GmbH, Pöttmes, Germany) at 15 min (pH₁₅) and 24 h (pHu) post mortem according to Polish Standard PN-77/A-82058 (1977). Colour measurements were performed at 24 h post mortem using the CIE system (L*, lightness; a*, redness; b*, yellowness), according to the method given by Litwińczuk et al. (2006), using a spectrophotometer Shimadzu UV-1800 Spectrophotometer Shimadzu UV-1800, Series No. A11635170868 US, Canby, OR, USA (Shimadzu Corporation, Kyoto, Japan). The tenderness of PM was assessed using a multi-functional machine Instron (Instron 3342, Series No. J 8528, Instron Corporation, Norwood, MA, USA, 2005) with Bluehill Application for tensile tests with Warner-Bratzler shear device (Instron force transducer, Model 2519-104, Series No. 47452, Capacity 500N, S/N 47452), which allowed to register the maximum shear force at crosshead speed of 150 mm/min. Five cores (10 mm² cross-sectional area and 50 mm length) were cut parallel to the muscle fibres and each core was sheared 3 times. The average of 15 shears was expressed in N/cm.

Water-holding capacity (WHC) was measured on the right pectoral muscle (PM) 24 h after slaughter. The sample was minced and analysed by a method of Grau and Hamm (1952) modified by Pohja and Niinivaara (1957). The measurement was performed using Whatman No. 1 filter paper. The obtained value was expressed as % hygroscopicity.

The protein content was calculated using the Kjeldahl method, while the content of fat was determined by Soxhlet method (AOAC International 2000).

Table 2. Chemical composition of feed supplied to chickens reared under semi-intensive (SIS) system used from 14 to 56 d of rearing.

Rearing system	SI
ME (kcal/kg)	1492.79
DM (%)	89.21
Ash (%)	2.04
CP (%)	12.76
Lipid (%)	1.62
Fiber (%)	4.42
NDF (%)	13.42
ADF (%)	4.51
ADL (%)	0.72

Note: ADL = acid detergent lignin; NDF = neutral detergent fibre; ADF = acid detergent fibre.

The amount of total collagen was determined based on the content of hydroxyproline (conversion factor 7.52), accordingly to PN-ISO3496:2000. Soluble collagen was determined using the method described by Palka (1999). The collagen solubility was calculated as the percentage of soluble collagen in the total collagen.

Histological Evaluation

From the carcasses (8 males and 8 females for each group, randomly chosen) approximately 1 cm³ muscle sample of each PM (*m. pectoralis major*) was removed and immediately frozen in liquid nitrogen (-196°C). Each specimen was cut in a cryostat (Cryostat Microm HM 525, GmbH, Germany, Thermo SCIENTIFIC, Series No. 52827, Runcorn, UK) into sections of 10 µm thick, which were then used for histochemical staining based on the hematoxylin and eosin method (Dubovitz et al. 1973). The microscopic images of the specimens (at the magnification of 100x) were taken using Opta-Tech microscope equipped with an Opta-View™ camera (Opta-Tech microscope, Warsaw, Poland), Model: MN-800, Series No. 04783. Histomorphometric analysis, including the calculation of the shortest diameters of 300 muscle fibres in each individual according to Brooke (1970), was conducted by means of Multiscan 18.03 software for computer analysis of microscopic pictures (MultiScanBase v.18.03 – Computer Scanning Systems II Ltd., Warsaw, Poland).

Statistical analyses

Data were analysed in a 2 × 2 factorial design (ANOVA), including the effects of rearing system and sex and their interaction using SPSS package (SPSS Inc. 2010. PC + Statistics. 18.0. SPSS Inc., Chicago, IL). None of the interactions were significant and are not reported in the results. For the meat quality characteristics, BW at slaughter was included as a covariant. Each bird was considered as the experimental unit.

Results

Live body weight and slaughter performance

Live body weight and slaughter performance of broiler chickens are reported in Table 3. Compared with the IS group, the SIS

Table 3. Effects of rearing system and sex on live body weight and slaughter performance of chickens.

Item	Rearing system (RS)		Sex (S)		SEM	RS	S
	IS	SIS	Male	Female			
n	30	30	30	30			
Final live BW (g)	2618.9	2713.8	2857.9	2479.6	30.1	NS	**
Daily weight gain (g)	61.28	47.66	58.70	50.52	0.68	**	**
Carcass weight (CW, g)	1921.6	2033.1	2100.5	1857.5	25.4	*	**
Carcass yield (%)	73.34	74.93	73.44	74.78	0.26	*	*
Pectoral muscle (g)	513.3	452.56	506.04	461.61	10.3	**	*
Pectoral muscle (% CW)	26.64	22.20	24.09	24.81	0.28	**	NS

Note: IS = Intensive; SIS = Semi-intensive; Significance: NS = P > 0.05; *P < 0.05; **P < 0.01.

group had slightly higher final BW (+3.5%). These differences were not significant (P > 0.05) and were mainly due to the different slaughter age.

As expected, IS group birds showed higher (P < 0.01) daily weight gain (DWG) than those of SIS group (P < 0.01). Chickens from SIS group showed higher (P < 0.05) carcass weight and carcass yield compared with IS group. Differently, IS birds had higher (P < 0.01) weight and yield (+4.44 percentage points) of PM compared to SIS ones. Males had a higher (P < 0.01) DWG than females and, therefore, a higher (P < 0.01) BW. The carcass weight was higher (P < 0.01) in males, while carcass yield was higher (P < 0.05) in females. Moreover, males had higher PM weight (P < 0.05) and lower PM yield (P < 0.05) than females.

Physicochemical properties and fibre diameter

Physicochemical characteristics and fibre diameter of PM are reported in Table 4. The pH values of PM measured at 15 min and 24 h post mortem were higher (P < 0.01) in IS birds compared with SIS. Sex did not affect (P > 0.05) the PM pH.

Chickens from IS group had darker meat (lower L* and b*, P < 0.01) than SIS ones. Sex did not affect the colour of PM. The housing system and sex did not affect (P > 0.05) the WHC value.

Meat from SIS chickens had lower (P < 0.01) shear force value than that of IS birds, indicating that meat from SIS birds is more tender. This fibre diameter was smaller (P < 0.01) in SIS chickens in comparison with the IS group.

The largest diameters of muscle fibres, observed in broiler chickens from IS group, coincided with heaviest pectoral muscle (Tables 3 and 4). The shear force and the fibre diameter were parameters independent of the bird sex (P > 0.05).

The chemical composition of PM (Table 4) was affected by rearing system (moisture and protein percentages; P < 0.01) and was unaffected by sex. The PM of broiler chickens reared conventionally showed a lower content of water (74.22%) compared with that of SIS group (75.85%). In addition, protein content was higher in birds of IS group (+1.17 percentage points) than in those of SIS. Compared with IS broilers, those of SIS groups had slightly lower (P = 0.10) fat content.

Table 4. Effects of rearing system and sex on physicochemical traits and fibre diameter of pectoral muscle.

Item	Rearing system (RS)		Sex (S)		SEM	RS	S
	IS	SIS	Male	Female			
n	30	30	30	30			
pH ₁₅	6.32	6.10	6.16	6.26	0.03	**	NS
pH ₂₄	5.76	5.68	5.73	5.71	0.01	**	NS
Color 24h							
L*	56.88	58.91	57.72	58.03	0.28	**	NS
a*	10.53	10.56	10.66	10.44	0.17	NS	NS
b*	3.79	6.30	4.80	5.24	0.17	**	NS
WHC (%)	67.31	65.94	66.78	66.50	0.44	NS	NS
Shear force (N/cm)	26.36	22.75	24.68	24.51	0.43	**	NS
Fibre diameter (µm)	50.80	41.40	45.43	46.44	0.91	**	NS
Dry matter (%)	25.78	24.15	24.93	25.02	0.08	**	NS
Protein (%)	23.76	22.59	23.22	23.14	0.11	**	NS
Fat (%)	1.60	1.44	1.50	1.54	0.10	NS	NS
Total collagen (%)	0.54	0.56	0.56	0.53	0.02	NS	NS
Soluble collagen (%)	0.19	0.20	0.21	0.18	0.01	NS	NS

Note: IS = Intensive; SIS = Semi-intensive; WHC = water holding capacity; Significance: NS = P > 0.05; ** P < 0.01; For the trait fibre diameter n = 16.

Total intramuscular collagen and soluble collagen were not influenced ($P > 0.05$) by rearing system or sex.

Discussion

Live body weight and slaughter performance

Rearing system is one of the key factors affecting the final body weight of animals. Various studies reported that free-range birds have lower live body weight compared to the animals kept indoors (Castellini et al. 2002; Połtowicz and Doktor 2011; Kuźniacka et al. 2014) this due to the fact that birds exposed to fluctuating temperatures and to an increased exercise in yards, have a higher energy requirement, and consequently a higher feed conversion rate (Tong et al. 2014). Chickens from SIS group showed higher ($P < 0.05$) carcass weight and carcass yield compared with IS group. Differently, IS birds had higher ($P < 0.01$) weight and yield of PM compared to SIS ones. Many authors provide conflicting information concerning the effect of rearing system on dressing percentage and carcass cutting yields. Kuźniacka et al. (2014) did not indicate any effect of rearing system (intensive vs. semi-intensive) on dressing percentage in Hubbard-Flex broilers. Instead, Skomorucha et al. (2008) reported a higher carcass yield in 42-d-old Cobb broilers housed without outdoor access compared to birds grown with outdoor access. In accord with the results obtained in the present study, Skomorucha et al. (2008), Połtowicz and Doktor (2011) and Funaro et al. (2014) observed a higher pectoral muscle yield in chickens kept indoors compared to birds grown with outdoor access. On the contrary, the results by Castellini et al. (2002) showed that in the carcasses of free-range birds the pectoral and thigh muscle yields were higher than in those of birds reared indoor. These results are not confirmed by Fanatico et al. (2005) and Bogosavljević-Bošković et al. (2011), who found that the housing system had no effect on the proportions of primal cuts in dressed broiler carcasses.

As expected, males had a higher ($P < 0.01$) DWG than females and, therefore, a higher ($P < 0.01$) BW. The carcass weight was higher ($P < 0.01$) in males, while carcass yield was higher ($P < 0.05$) in females. Moreover, males had higher PM weight ($P < 0.05$). Maiorano et al. (2017) reported an important sex effect in the final BW, carcass weight, carcass yield and pectoral muscle weight of Ross chickens slaughtered 42 d of age, higher in males than in females, but not in the carcass yield. Similar results were obtained by Kuźniacka et al. (2014) in Hubbard-Flex chickens. Differently, Lopez et al. (2011) found that male broilers had a higher final BW, carcass weight, and PM weight and lower carcass and breast meat yields when compared with females. A sex effect on cut-up yield was also reported by Young et al. (2001), who found that females yielded larger proportions of forequarters, breasts and fillets, but smaller proportions of drumsticks than males, under commercial-like conditions.

Physicochemical properties and fibre diameter

The pH values of PM measured at 15 min and 24 h post mortem were higher ($P < 0.01$) in IS birds compared with SIS. Sex did not affect ($P > 0.05$) the PM pH. These findings are consistent with

Castellini et al. (2002) and Fanatico et al. (2007) who found a lower pH in birds reared with outdoor access. In contrast, Alvarado et al. (2005) found that outdoor access resulted in higher pH. Nevertheless, literature often reported that meat from poultry (Ponte et al. 2008) and pigs (Maiorano et al. 2013) reared on outdoor access is characterized by lower pH indicating more glycogen in the muscle at slaughter, which then results in more lactate in the post-mortem process (Bonneau and Lebret 2010). However, the pH_u values (ranging from 5.63–5.76) observed in this study varied within the pH range accepted for commercial meats. Meat quality is closely related to the decrease in muscle pH post-mortem. The pH drop rate is related to the glycolytic enzymes activity immediately after death, and the ultimate pH is shaped by the initial muscle glycogen levels (Bendall 1973). It is well known that the ultimate pH is of importance when considering meat preservation and stability; in fact, high muscle pH affects warehousing and sensorial quality by its undesirable effect on bacterial growth and meat moistness (Allen et al. 1997), while a low pH is associated with poor water-holding capacity and meat colour, as well as meat texture (Woelfel et al. 2002; Husak et al. 2008).

Meat colour is an important quality attribute and it is also relevant to consumers when they buy meat products (Fletcher 2002). However, differences in lightness (L^*) and redness (a^*) are of little practical importance for poultry meat (Funaro et al. 2014). In the present study, chickens from IS group had darker meat (lower L^* , $P < 0.01$) than SIS ones. Similar results were observed by several authors (Fanatico et al. 2007; Mikulski et al. 2011), indicating that outdoor access had a positive effect on lightness of meat. Redness (a^*) did not differ significantly between groups. It is important to note that yellowness (b^*) was higher in SIS ($P < 0.01$) compared to IS. As suggested by other works (Fanatico et al. 2007; Funaro et al. 2014), both diet (corn) and organic free-range systems can lead to yellower meat products. In particular, the consumption of vegetation present in the outdoor space could contribute to increased meat yellowness because plant material contains abundant carotenoid pigments. Sex did not affect the colour of PM. Generally, meat from females exhibits a higher yellowness value than males (Fanatico et al. 2005; Lopez et al. 2011). However, Fanatico et al. (2005) did not find any differences in b^* values between fast-growing genotype reared indoor or outdoor. The housing system and sex did not affect ($P > 0.05$) WHC value. WHC is an important attribute of meat quality, and if it is poor whole meat and further processed products will lack juiciness.

Appearance and tenderness are two characteristics of utmost significance for the quality of poultry meat (Fletcher 2002). Specifically, meat tenderness is the one decisive sensory characteristic on which the final quality result depends (Fletcher 2002), and it is an important attribute for consumers. Numerous aspects can influence tenderness, including breed, sex, age, fibre resistance, sarcomere length, pH and collagen morphology (Lepetit 2007; Elminowska-Wenda et al. 2014). In the present study, meat from SIS chickens had lower ($P < 0.01$) shear force value than that of IS birds, indicating that meat from SIS birds is more tender. This could be due to the fibre diameter that was smaller ($P < 0.01$) in SIS chickens in comparison with that of the IS group. In agreement with

our findings, the research by Elminowska-Wenda et al. (1997) on White Italian geese (slaughtered at the same age) fed under intensive and semi-intensive rearing system, reported larger diameters of muscle fibres of both types (red and white) in pectoral muscles of ganders fed intensively. The largest diameters of muscle fibres, observed in broiler chickens from IS group, coincided with heaviest pectoral muscle. Thinner fibres are advantageous for meat quality, therefore, this characteristic can serve as an indicator of meat fibrillarity and its tender structure (Maiorano et al. 2017). The shear force and the fibres diameter were parameters independent of the bird sex ($P > 0.05$).

The chemical composition of PM was affected by rearing system (moisture and protein percentages; $P < 0.01$) and was unaffected by sex. The PM of broiler chickens reared conventionally showed a significantly lower content of water compared with that of SIS group. In addition, protein content was significantly higher in birds of IS group than in those of SIS. Compared with IS broilers, those of SIS groups had slightly lower fat content. Mikulski et al. (2011) and Funaro et al. (2014) observed that the breast meat of chickens with outdoor access contained higher content of protein and dry matter, and lower intramuscular fat than that of chickens reared indoor. Differently, Fanatico et al. (2005) reported that the nutrient composition (water, protein, and fat) of chicken muscle was not significantly affected by outdoor access. Similar results were also found by Wang et al. (2010), except to abdominal fat that was lower in chickens reared outdoor. Free range rearing can promote higher energy consumption, stronger lipogenesis and more intense physical exercise resulting in reduction in abdominal fat (Castellini et al. 2002).

Total intramuscular collagen and soluble collagen were not influenced ($P > 0.05$) by rearing system or sex. Similar findings were found by Funaro et al. (2014) in pectoral muscle from conventional older and heavier (average carcass weight = 2.45 kg) birds and free range older and heavier birds. To our knowledge, no information is available from current literature on the effect of sex on intramuscular collagen properties in chickens. In adult (20 weeks old) quails collagen maturity appeared to vary depending on bird's sex (Tavaniello et al. 2014). Furthermore, different collagen content and maturity, associated with the animal sex and being a consequence of hormonal activity, were also observed in pork, beef, lamb, and deer (cited by Maiorano et al. 2013). According to a general opinion, connective tissue is a factor influencing the initial toughness of meat (Maiorano et al. 2012), giving a sensation of chewiness (Duizer et al. 1996) and, in addition, contributing to the technological yield (Boutten et al. 2000).

Conclusion

Fast-growing chickens are more suitable for indoor system in terms of productive performance. As expected, a sex effect was observed in chickens reared in both intensive and semi-intensive system. Compared to SIS birds, IS birds exhibited: (i) a greater fibre muscle diameter; (ii) a darker and less yellow meat with higher pH and shear force; (iii) a meat with higher dry matter and protein content and a slightly higher fat content.

A better understanding of meat quality of fast-growing genotypes raised in different production systems and provided

different diets, will help producers in making informed decisions about their production systems. However, other factors than improved live body weight and meat quality, such as those related to animal welfare, might be responsible for consumers' preferences for chicken meat from broilers reared under non-industrial systems.

Disclosure statement

No potential conflict of interest was reported by the authors.

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