Meat quality characteristics of chickens as influenced by housing system, sex, and genetic line interactions

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ABSTRACT
This study evaluated the effects of housing system (cage versus pen), sex and line cross (EMY1 and EMY2) on meat quality in meat-type chickens. Chickens (n = 640) from each line cross (males: females = 1:1) were housed in batteries from d 1 to 28. Then, half of them were transferred to indoor floor pens, and the others were raised in single cages. Meat quality traits of breast fillets were measured at 91 d of age. Percent lipid and histidine were higher, whereas % total protein and myofibre density (MDS) were lower in caged than penned chickens. Cross EMY1 had higher MDS, but lower lipid % and myofibre diameters (MDM) than EMY2 (p < .05). Males had redder and brighter muscles and higher MDM and contents of glycine and proline than the females (p < .05). Penned females had smaller MDM and higher MDS than their caged counterparts (p < .05). Generally, housing systems alone, or interacting with sex and genetic line, affected yellowness, myofibre characteristics, % protein, % lipid, myofibre density, and % His of breast muscle.

Abbreviations: a*: redness; b*: yellowness; L*: lightness; pH: pH at 45 min post-mortem; IMP: inosine-5’-monophosphate; TP: total protein content; LC: lipid content; MC: moisture content; DM: dry matter; MDM: myofibre diameter; MDS: myofibre density; AA: amino acids; Thr: threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Lys: lysine; His: histidine; Asp: aspartic; Ser: serine; Glu: glutamic acid; Ala: alanine; Tyr: tyrosine; Arg: arginine; Gly: glycine; Pro: proline; FCR: feed conversion ratio

ARTICLE HISTORY
Received 23 March 2017
Revised 2 June 2017
Accepted 21 June 2017

KEYWORDS
Chicken; housing system; carcass; meat quality; correlation analysis

INTRODUCTION
The 21st century has seen a developing interest in the production of slower growing meat-type chickens. Although their relative share of the overall global market is small, they are an important component in niche markets. Comitant with this interest in slower growing chickens for meat is an emerging scientific literature on production systems (e.g. Fanatico et al. 2015; Comert et al. 2016; Stadeg et al. 2017) and meat quality (e.g. De Marchi et al. 2005; Fanatico et al. 2007) of these chickens.

Regardless of the sex, genetic stock, and production system, the main edible parts of a chicken are the skeletal muscles, especially the pectoralis major and minor. Important components of these muscles are their external features, size, nutritional profiles, and histological and chemical properties. Husbandry items that influence these meat quality components include ambient temperature (Zhang, et al. 2012), light intensity and photoperiod (Lien et al. 2007), access to pasture (Sales, 2014), and organic production practices (Castellini et al. 2002). Meat quality of chickens is also influenced by genetics (e.g. De Marchi et al. 2005; Sarsenbek et al. 2013; Harford et al. 2014) and sex (e.g. Brewer et al. 2012).

In a previous report (Zhao et al. 2015), we compared pen versus cage rearing, line crosses, and sexes in slower growing meat-type chickens. Observed were
significant effects of housing system and sex on carcase traits. Here, we further report their impact on quality and nutritional profiles on breast meat.

Materials and methods

All procedures for raising and slaughtering chickens were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University under permit number DKY-S20123118. The methods were carried out in accordance with the approved guidelines.

Animals

The EMY1 and EMY2 males have bright red feathers on their neck, back, and saddle, black tails, red combs, and light dark skin and shanks. The females of both crosses have dotted-yellow feathers and dark skin and shanks. Body weights of caged EMY1 and EMY2 males are approximately 2500 g on d 91, while those penned reared are about 2200 g. Body weights of females reared in cages are approximately 2000 g for both crosses, while the penned ones are about 1900 g (Zhao et al. 2015). Obviously, these days to market weight are quite different with commercial meat-type broilers (e.g. Rajkumar et al. 2016). Thus, they reflect what currently are slow-growing meat-type chickens.

Fertilised eggs of line crosses EMY1 and EMY2 (35 wk old) between Erlang Mountainous (EM) chickens with lines Y1 and Y2 of the Yao chickens were incubated in the same incubator from 28 June to 18 July 2012. At hatch, 320 males and 320 females from each line cross (a total of 1280 chicks) were randomly assigned within each subgroup to 16 batteries. There were four replicates for each combination of line cross by sex. The population density was 20 chicks/m². On d 28, half of the chicks were transferred to indoor floor pens, and the others were raised in single cages from d 29 to 91 (Zhao et al. 2015). The population density was 5 chicks/m². Cages were made from galvanised steel and their sizes were 500 mm × 400 mm × 370 mm, whereas the pens were framed with the concrete floor and the wood strips with the size 2000 mm × 5000 mm. Pens were covered with wood shavings as litter. There were three replicates of 50 individuals for each combination of line cross by sex for each housing system.

Management treatments

Diets

A corn-soy diet, in pellet form, was provided ad libitum. The diets consisted of 19% CP and 2897 Kcal of ME/kg to d 28, followed by 17% CP and 2998 kcal of ME/kg from d 29 to 90. Water was available throughout.

Photoperiod

The photoperiod during the first 3 d post-hatch was 24 h. It was then gradually decreased from 24 to 18 h by d 28. The light intensity was 20 Lux during the first 4 wks. After d 28, it remained 18 h photoperiod; however, the light intensity decreased to 5 Lux and maintained to d 91.

Ambient temperature

Heat was provided by chimney flue. During the first 3 days after hatch, the room temperature was maintained at 35 °C, and then decreased to 24 °C gradually by the end of 4 wk. The temperature was controlled by the subatmospheric pressure ventilation system.

Vaccination

Chicks were vaccinated with Marek’s disease, bird flu (H9 subtype), Newcastle, and bird flu (H5 subtype) on d 1, 8, 10, and 24, respectively.

Sample collections

On d 91, after 12 h feed withdrawal, each of 15 chickens per replicate (totally 120 birds) was slaughtered randomly. Chickens were stunned by electronic shock (70–90 Volt, 2–3 s), the blood then drained from the carotid artery (3–4 min), defeathered (70 °C water, lasting 40–60 s), and eviscerated. Breast muscles (pectoralis major and minor) were sampled from the chilled body (<10 °C). Sampling sites are shown in Figure 1.

Colour and pH

Meat colour and pH were measured in the internal section of left pectoral major muscle. Data were obtained within 45 min post-mortem with Chromameter (Minolta ChromaMeter CR-300, Minolta Inc., Osaka, Japan) and pH direct measuring apparatus (pH-STAR, R. Matthaus, Berlin, Germany) with three repeats for each sample, respectively. Reflective colours of surface in the CIELAB space were shown as values of lightness (L*), redness (a*), and yellowness (b*).

Moisture and lipid contents

Chemical analyses were conducted to determine the moisture and lipid contents of the left pectoralis major and minor. Each of 10 g fresh samples was finely
ground and weighed. Fresh muscle packed in filter paper was dried in a 105 °C oven to a constant weight (~4 h). The difference in weight between the fresh and dried samples divided by the fresh weight represents the moisture (%). Lipid content on the dry powder base was extracted with ether Soxhlet extraction method (Association of Official Analytical Chemists 1990). Lipid content percent was calculated as the difference in weight before and after extraction divided by pre-extracted weight.

Inosine-5′-monophosphate (IMP)

The IMP analysis was performed with an Agilent-1100 type high-performance liquid chromatograph (HPLC). The extraction of IMP from fresh right pectoralis major and minor was performed as follows: 5 g of fresh meat was homogenated by refiner and then mixed with 15 mL 3.5% chilled perchloric acid. The homogenate was centrifuged (4000 rpm, 5 min) and the supernatant extracted out. Added perchloric acid again and then the mix was centrifuged. The supernatants of two centrifugations were combined and immediately neutralised with 1 mol/L and 0.1 mol/L sodium hydroxides to pH 6.5, and the total volume diluted with ultrapure water to 100 mL. After filtered by 0.45 μm griddle, samples were analysed by high-performance liquid chromatography (Agilent 1100, Agilent Technologies, Palo Alto, CA) with chromatographic column HC C18 (4.6 mm × 150 mm, Agilent Technologies, Palo Alto, CA).

Total protein content and amino acid composition

Total protein was determined by the Kjeldahl method (Association of Official Analytical Chemists 1990), and a conversion factor of 6.25 was used to convert total nitrogen to protein. The amount of total protein in muscles was related to dry weight as a percent. Amino acid (AA) composition of the left pectoralis major and minor in dry powder form was determined by amino acid analyser (L-800, Hitachi High-Technologies Corporation, Tokyo, Japan) after acid hydrolysis according to the following procedure. 1.1 g powder was taken in the tube, 15 mL of HCl (6 mol/L) was added, and then the tube was sealed after filled with nitrogen and incubated at 110 °C for 24 h. After cooling to room temperature, the liquid to a volumetric flask was transferred, and the total volume to 100 mL was added with ultrapure water. 200 μL liquid to the Ampola was sucked and dried in a vacuum drier. 1 mL of 0.02 mol/L HCl was added, mixed, and uploaded to amino acid analyser. The AAs monitored were Met, Asp, Thr, Ser, Glu, Gly, Pro, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys, and Arg.

Histological properties of myofibres

Right pectoralis muscle sections were stained with haematoxylin and eosin (An et al. 2010). The images of the myofibre (10 × 40 enlarged) were captured by the digital microscope camera linked with a biological microscope (YS100, Nikon, Tokyo, Japan). An image analyser (Image Pro Plus 5.0, Media Cybernetics, Silver Spring, MD) was used to evaluate myofibre diameter and number of myoblast per square millimetre in the section (myofibre diameter and density, MDM and MDS). All fibres in each of 10 fields per slide were measured to obtain the mean of myofibre diameter for each bird. Fibre numbers per mm² were used to present the myofibre density.

Statistical analysis

All calculations were conducted by using procedures GLM of SAS 9.2 (SAS Institute Inc., Cary, NC) and graph was drawn by GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). The GLM model was as

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**Figure 1.** Sample collection sites on the breast fillet used to determine the meat quality traits. IMP: inosine-5′-monophosphate; TP: total protein; AA: amino acids; MDM: myofibre dimension; MDS: myofibre density; MC: moisture content; LC: lipid content.
Effects of housing system by sex on myofibre characteristics

There were interactions of housing system by sex for myofibre characteristics ($p < .05$). Penned males had larger myofibre diameters, but lower densities than their caged counterparts and penned females, respectively ($p < .05$, Table 2).

Effects of line cross by sex on content of His

There were interactions of line cross by sex on the contents of Leu, Tyr, and His of breast muscle ($p < .05$). The EMY2 females had higher His content than the other line cross and sex combinations ($p < .05$) (Figure 2). There were no differences among combinations for Leu and Tyr contents of breast muscle ($p > .05$).

Effects of housing system, line cross, and sex on meat quality traits

Housing systems affected % protein, % lipid content, myofibre density, and His content of breast muscle ($p < .05$). Caged chickens had higher intramuscular fat

**Table 1.** Means and standard errors for yellowness and Ser content of housing system by line cross and sex combinations*

| Sex  | Cross | Housing system | n | Yellowness (b*) | Ser, % |
|------|-------|----------------|---|----------------|-------|
| M    | EMY1  | cage          | 15 | 10.72 ± 0.74   | 3.12 ± 0.04 |
|      | EMY2  | cage          | 15 | 8.00± 0.74     | 3.05 ± 0.04 |
| F    | EMY1  | cage          | 14 | 10.27± 0.76    | 3.04 ± 0.04 |
|      | EMY2  | cage          | 15 | 11.04± 0.74    | 3.05 ± 0.04 |
| M    | EMY1  | Pen           | 14 | 7.18 ± 0.71    | 3.04 ± 0.03 |
|      | EMY2  | Pen           | 14 | 8.30± 0.74     | 3.09 ± 0.04 |
| F    | EMY1  | Pen           | 14 | 10.45± 0.76    | 3.14 ± 0.04 |
|      | EMY2  | Pen           | 14 | 10.81± 0.76    | 3.03 ± 0.04 |

*Multiple comparisons were conducted between the caged and penned chickens for the same sex and between sexes for the same housing system. $n$: 28 caged males; 30 penned males and caged females, respectively; 26 penned females.

**Table 2.** Means and standard errors for myofibre characteristics of housing system and sex combinations*

| Sex  | Caged | Penned |
|------|-------|--------|
|      | Myofibre diameter, μm | Myofibre density, n/mm² |
| M    | 40.47 ± 0.68 ns | 41.74 ± 0.71 * |
| F    | 40.58 ± 0.66 * | 37.49 ± 0.66 |

*Multiple comparisons were conducted between the caged and penned chickens for the same sex and between sexes for the same housing system. n: 28 caged males; 30 penned males and caged females, respectively; 26 penned females.

Figure 2. Means and standard errors for His content of line cross and sex combinations. Numbers above bars represent the sample sizes. Groups without the same letter (a, b, c) differ significantly ($p < .05$).
and His contents, and lower total protein and myofibre density than penned ones \((p < .05)\) (Table 3).

Although line cross affected protein and myofibre characteristics, there was no effect on the amino acid composition of breast muscle. Cross EMY1 had higher myofibre density, but lower protein and myofibre diameter than Cross EMY2 \((p < .05)\) (Table 4).

There was sexual dimorphism for lightness, redness, and yellowness of breast muscle. Males had redder and brighter breast muscles, larger myofibre diameters, and higher contents of Gly and Pro than females \((p < .05)\). However, females had more yellow breast muscle with higher lipid content, myofibre density, and His content than the males \((p < .05)\) (Table 5).

### Rank correlations between meat colour and pH45min, nutritional profiles, and histological properties

Spearman’s rank correlations between meat colour and \(\text{pH}_{45\text{min}}\), nutritional profiles (contents of moisture, lipid, total protein, IMP) and histological properties (myofibre diameter and density) were summarised in Table 6. Yellowness \((b^*)\) was positively correlated with \(\text{pH}_{45\text{min}}\) and lipid content \((p < .05)\), but negatively correlated with IMP \((p < .05)\). Lightness was negatively correlated with IMP \((p < .05)\), and redness was negatively correlated with myofibre diameter \((p < .05)\).

### Discussion

Chickens are an important component in the diets of consumers in both developed and developing countries. This is due, in part, to the convenience of portioned retail cuts at relatively lower prices than beef and pork (Jaturasitha et al. 2008). In addition, in some markets meat quality of chickens is given priority due to rising health concerns by consumers (Mehta et al. 2015). Slower growth stocks, e.g. the Label Rouge in Europe, have received increased attention for their meat quality. Studies have been conducted to analyse factors that affect their carcass performance and meat quality, included dietary enrichment with n-3 fatty acids (Baeza et al. 2013) and rearing systems (N’Dri et al. 2007). They show moderate significant interactions of genotypes by rearing systems for carcass traits of Label Rouge chickens. Also, the nutritional quality of raw and cured-cooked meat of Label Rouge chickens was improved (increased concentration of n-3 FA) by using of extruded linseed or linseed oil, whereas pH, juice loss after cold storage, susceptibility to oxidation, colour, processing yield, and shear force value were either not or slightly affected.

With increased attention to the exterior features, nutritional profiles, and histological properties of poultry meat, their colours became relevant because they can be correlated with physiological abnormalities (Russo et al. 2015). In broilers, selection for high and low \(L^*\) was an effective way to divergently select.

### Table 6. Spearman’s rank correlations between meat colour and nutritional profiles and histological properties of breast fillets.

| Coloura | Traitsb | Spearman \(r\) | Probability > |\(|p|) |
|---------|---------|---------------|--------------|-----------|
| Yellowness \((b^*)\) | \(\text{pH}_{45\text{min}}\) | 0.19 | 0.0384 |
| LC | 0.24 | 0.0096 |
| IMP | −0.28 | 0.0308 |
| Lightness \((L^*)\) | IMP | −0.21 | 0.0321 |
| Redness \((a^*)\) | MDM | −0.22 | 0.0175 |

*\(\text{pH}_{45\text{min}}\): \(\text{pH}\) within 45 min post-mortem; IMP: inosine-5’-monophosphate; LC: lipid content; MDM: myofibre diameter.

### Table 5. Means and standard errors of meat quality traits of males and females.

| Sex | Redness \((a^*)\) | Yellowness \((b^*)\) | Lightness \((L^*)\) | Glycine, % | Histidine, % | Proline, % | Lipid content, % | Myofibre characteristicsc |
|-----|----------------|----------------|----------------|-------------|-------------|-------------|----------------|--------------------------|
| M   | 2.14 ± 0.14   | 8.55 ± 0.36   | 54.85 ± 0.63   | 3.55 ± 0.02 | 3.08 ± 0.03 | 2.71 ± 0.02 | 2.55 ± 0.14 | 41.16 ± 0.48 |
|     | *             | *             | *             | *           | *           | *           | *              | *                        |
| F   | 1.45 ± 0.15   | 10.64 ± 0.38  | 52.27 ± 0.65   | 3.44 ± 0.02 | 3.24 ± 0.03 | 2.65 ± 0.02 | 3.14 ± 0.15 | 38.98 ± 0.47 |
|     | *             | *             | *             | *           | *           | *           | *              | *                        |

*aMDM and MDS: myofibre diameter and density.

*bM: male; F: female.

*c\(p < .05\).
the pale, soft, and exudative-like and dark, firm, and dry-like meat, respectively (Harford et al. 2014). They also reported that meat colour was influenced by breed, rearing environment, processing plant, chilling method, nutrition, and stunning method. Here, we found interactions of housing system by sex by line cross combinations influenced meat colour $b^*$. Line cross EMY2 females reared in pens had more yellow muscle than those from the other housing system by sex by line cross combinations. Also, there was sexual dimorphism of muscle colour, as males had brighter and redder, but lighter yellow breast muscle than females. In contrast, Ross females had brighter, paler, and more yellow breast muscle than males (Sirri et al. 2010). The differences between studies indicated that not only genetics (fast versus slower growing), housing system, and sex, but also the interactions between them, are the factors which affect the meat colours.

The values of lightness, redness, and yellowness reflect the degree of glycolysis post-mortem and the contents of lipid, moisture, and dry matter (including the total protein) in skeletal muscle (Lee et al. 2008). White striping in broiler breast fillets was accompanied with increased yellowness (Kuttappan et al. 2013). Our correlation analysis showed significant positive correlations of yellowness with pH45min post-mortem and lipid content, and a negative correlation between yellowness and IMP. Because IMP can enhance the flavour of poultry meat (Ma et al. 2015), selection on $b^*$ may be a way to improve muscle quality in pedigree lines.

Compared with other meat animals, such as pork and beef, chicken meat is lean because of its low content in intramuscular lipids (Mourot and Hermier 2001). Lipid content in the breast muscle was affected by the housing systems and sex in the current study. Here, caged chickens and females had higher lipid content in breast muscle than the penned ones and males. In a confined system with less area for activity, the oestrogen secreted by ovary could increase lipid deposition among myofibres (Choi et al. 2012; Oosthuysen and Bosch, 2012). This result was consistent with higher intramuscular fat in barrows than in gilts (Correa et al. 2006).

Conclusions
Our study evaluated the effects of housing system, sex and line cross on meat quality in slower growing meat-type chickens. Results showed that caged chickens had higher lipid and histidine %, whereas lower total protein content and myofibre density than those reared in floor pens. Cross EMY1 had higher myofibre density, but lower lipid content and myofibre diameter than Cross EMY2. Males had redder and brighter muscles, higher myofibre diameters, and contents of Gly and Pro than the females. Penned females had smaller myofibre diameter and higher myofibre density than their caged counterparts. Generally, housing systems interacting with sex and genetic line affected meat quality characteristics.

Acknowledgements
We thank Deyang Banghe Agricultural Science and Technology Company for providing the raising facilities and the chicks.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was financially supported by the China Agriculture Research System (CARS-41) and the National Natural Science Foundation of China (Grant No: 31402070).

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