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Selecting broiler chickens for ultimate pH of breast muscle: Analysis of divergent selection experiment and phenotypic consequences on meat quality, growth and body composition traits[1]

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ABSTRACT: Genetic parameters for ultimate pH of Pectoralis major (PM-pHu) and Sartorius (SART-pHu) muscles, color parameters L*, a*, b*, log of drip loss (LogDL) of PM muscle, breast meat yield (BMY), thigh yield (TY), abdominal fat percentage (AFP), and body weight at 6 weeks (BW6) were estimated in two lines of broiler chickens divergently selected for PM-pHu. Effects of selection on all the previous traits and on glycolytic potential (GP), pH measured 15 minutes post-mortem (PM-pH$_{15}$), curing-cooking yield (CCY), cooking loss (CL), and Warner-Bratzler shear force (WB-SF) of the PM muscle were also analyzed after five generations. Strong genetic determinism of PM-pHu was observed, with estimated h^2 of 0.57±0.02. There was a significant positive genetic correlation (r_g) between PM-pHu and SART-pHu (0.54±0.04), indicating that selection had a general rather than a specific effect on energy storage in skeletal muscles. The h^2 estimates of L*, a* and b* parameters were 0.58±0.02, 0.39±0.02 and 0.48±0.02, respectively. Heritability estimates for TY, BMY and AFP were 0.39±0.04, 0.52±0.01 and 0.71±0.02, respectively. Our results indicated different genetic control of LogDL and lightness of the meat between the two lines, these traits had a strong r_g with PM-pHu in the line selected for low (pHu-) pHu value (-0.80 and -0.71, respectively) which was not observed in the line selected for high (pHu+) pHu value (-0.04 and -0.29, respectively). A significant positive r_g (0.21±0.04) was observed between PM-pHu and BMY, but not between PM-pHu and BW6, AFP or TY. Significant phenotypic differences were observed after 5 generations of selection between the two lines. The mean differences ($P < 0.001$) in ultimate pH between the two lines were 0.42 and 0.21 pH units in the breast and thigh muscle, respectively. Breast meat in the pHu+ line exhibited lower lightness (-5 units, $P < 0.001$), redness (-0.22 units, $P < 0.001$), yellowness (-1.53 units, $P < 0.001$), and DL (-1.6 units, $P < 0.001$) than in the pHu- line. Breast meat of the pHu+ line was also characterized by greater CCY (+6.1 units, $P < 0.001$), lower CL (-1.66 units, $P < 0.01$), and lower WB-SF after cooking (-5.1 units, $P < 0.001$) compared to the pHu- line. This

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study highlighted that selection based on pHu can be effective in improving the processing ability of breast meat and reducing the incidence of meat quality defects without affecting chicken growth performance.

Key words: broiler, divergent selection, genetic parameters, meat quality, ultimate pH.

INTRODUCTION

Evolution in consumers' attitude towards foods and increased demand for further processed meat products has significantly increased the importance of meat sensory and technological qualities, and processing ability (Barbut et al., 2008; Petracci et al., 2009, 2013). Extreme meat quality conditions such as PSE-like or DFD meats are strongly associated with variations in meat pH (Fletcher, 2002). Normal broiler breast meat pHu values are approximately 5.8-5.9 (Qiao et al., 2001; Duclos et al., 2007). The more pHu deviates from this value the more defects occur. Meat with high pHu values (> 6.1) exhibits physicochemical properties resulting in DFD (Dark, Firm and Dry) syndrome, while meat with low pHu (< 5.7) is associated with acid meat, often referred to as PSE-like (Pale, Soft and Exudative) syndrome in broiler (Barbut, 1997; Woelfel et al., 2002). Breast meat pHu has also been shown to be negatively associated with cooking loss (Woelfel et al., 2001; Bianchi et al., 2005). Thus, low pHu values result in negative economic effects due to lower yield. The impact of meat pH on meat quality has been studied in broiler lines selected for different criteria such as growth rate (Nadaf et al., 2007) and body composition (Berri et al., 2001; Sibut et al., 2008). However, there has been no study of lines selected directly for muscle pHu or related meat quality traits. In 2009, two divergently selected broiler lines were created with the Pectoralis major pHu (PM-pHu) breeding value as selection criterion. These

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lines, specifically selected on breast meat pHu, should contribute significantly to understanding of genetic and physiological control of meat quality and the relationship with growth and body composition. This study investigated the genetic control of PM-pHu in relation to meat quality, body composition and growth and evaluated the impact of the divergent selection by phenotypic characterization of the two lines at the 5th generation.

MATERIALS AND METHODS

All animal care and experimental procedures needed for the selection of the two divergent lines for PM-pHu (Program N° 00880.02) were approved by the Ethics Committee for Animal Experimentation of Val de Loire (registered under N° 19 by the National Committee).

Birds and Housing

The base population used to initiate the divergent selection originated from 43 males and 127 females from a grand parental female broiler line selected for a balance between growth and reproduction traits. As the measurement of PM-pHu requires sacrificing the birds, selection was carried out on their sibs. Three batches were produced at each generation: one batch (462 to 674 birds) was kept for reproduction and the other two batches (725 to 917 birds) for measurements of muscle pH and other traits under study. On average, 28 males and 73 females in each line were used from each generation as parents of the next generation. The selection pressure applied on males and females was estimated at 19% and 50%, respectively.

In order to reduce the effects of the environment, birds from the two lines (pHu+, pHu-) were reared together in a standard closed broiler house equipped with a dynamic ventilation system, gas heaters, automatic pan feeders and nipple drinkers at PEAT (Pôle

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d'Expérimentation Avicole de Tours, Nouzilly, France). Classical rearing practices were followed for the two batches dedicated for measurements. The lighting program consisted of 24 h of light for the first week of age and of 20 h of light and 4 h of darkness for the remainder of the rearing period. A standard broiler starter feed (ME 12.50 MJ/kg, CP 21.7%) was used for the first three weeks, followed by a standard broiler finisher diet (ME 13 MJ/kg, CP 20%) from three to six weeks of age. Broilers were fed and watered ad libitum until 8h before slaughter.

Slaughter and Carcass Processing

At the age of six weeks, birds were weighed, transported to the experimental slaughter house of PEAT, slaughtered after being stunned in a water bath using an electric current (80 mA and 125 Hz) for 5 s/bird. After 24 h of storage at 2°C, carcasses were cut and the abdominal fat, right breast muscles (Pectoralis major and minor) and thigh + drumstick were removed from the carcass and weighed to calculate their respective yields relative to body weight at slaughter.

Meat quality measurements performed at each generation

The ultimate pH of the right PM muscle was measured one day after slaughter using a portable pH meter (Model 506, Crison Instruments SA, Alella, Bercelona, Spain) by direct insertion of the glass electrode into the thickest part of the muscle. A Miniscan Spectrocolorimeter (Hunterlab, Reston, VA) was used at the same time to measure the color of the ventral surface of the PM muscle according to the CIELAB trichromatic system as lightness (L*), redness (a*) and yellowness (b*).

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Additional meat quality measurements performed on the last (5th) generation

The glycolytic potential (**GP**) was measured on the left PM muscle that was sampled 15 minutes post-mortem and quickly frozen in liquid nitrogen (n = 6 per line and sex) according to the method of Dalrymple and Hamm (1973) and calculated according to Monin and Sellier (1985). The initial rate of pH decline in breast muscle was estimated through measurement of the PM muscle pH at 15 min postmortem (**PM-pH$_{15}$**, n = 40 per line and sex) as described in Berri et al. (2007). The ultimate pH of Sartorius (**SART-pHu**) muscle (thigh) was measured on all birds of the 5th generation (n = 756) one day after slaughter as described above for PM muscle. The PM muscles (n = 694) were then put into zip-lock plastic bags, hung from a hook and stored at 2°C for 5 days before estimating their drip loss (**DL**) expressed as a percentage of the initial sample weight. After DL measurement, PM muscle samples (n = 40 per line and sex) of 180 g ± 5g were weighed, then vacuum-packed and stored at +2°C overnight. Samples were then cooked in a water bath at 85°C for 13 min (core temperature of about 70°C), cooled in crushed ice for 10 min, wiped dry using absorbent paper and weighed again to estimate cooking loss (**CL**). CL was expressed as the percentage of the initial weight before vacuum-packing. Firmness of the cooked meat was then evaluated by measuring the Warner Bratzler shear force (**WB-SF,** N/cm^2) using an Instron universal testing instrument (Instron 5543, Guyancourt, France). For each sample (n = 40 per line and sex), measurement was performed on three adjacent strips (measuring 3 × 1 × 1 cm) and the average maximum WB-SF necessary to shear the meat was calculated for each sample. The curing-cooking yield (**CCY**) was measured on 60 g of PM muscle (n = 40 per line and sex) first lacerated with a scalpel, then mixed in a plastic bag (Stomacher 80 standard bags, Seward, UK) with 20% nitric salt solution (136 g/L of 0.6% sodium nitrite) for 30 seconds

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using a stomacher (BagMixer, Lab Blender, Interscience, France), and stored for 24 h at 4°C under agitation. The next day, the cured meat was cooked at 85°C for 10 min then cooled in crushed ice for 10 min, wiped dry using absorbent paper and weighed. The CCY was expressed as a percentage of the raw cured meat before processing.

Estimation of genetic parameters

The total population of 9,626 animals used for the genetic analyses consisted of 4,685 (48.7%) males and 4,941 (51.3%) females. This population was produced by 322 sires and 855 dams. Data inspection, tests for normality of trait distributions and detection of outliers were performed using PROC UNIVARIATE of SAS (SAS Inst. Inc., Cary, NC). Descriptive statistics of traits included in the genetic analysis were performed using PROC MEANS of SAS. Genetic parameters (i.e., heritabilities and genetic correlations) were estimated using the Restricted Maximum Likelihood (REML) method in a multivariate animal model. This model was fitted to the data using VCE 6.0.2 (Neumaier and Groeneveld, 1998). The equation of the model was as follows:

$$y_{ijkl} = \mu + H_i + S_j + c_k + a_l + e_{ijkl}$$

where y_{ijkl} is the performance of the *l*th individual (l = 1 to 9626), μ is the general mean, H_i is the fixed effect of the *i*th hatch (i =1 to 19), S_j is the fixed effect of the *j*th sex (j = 1 for males, 2 for females), c_k is the random environmental effect common to all the progeny of the *k*th dam (k = 1 to 855) which was included in the model only for BW6, a_l (or a_{kl} in case of BW6) the direct additive genetic effect of the *l*th individual. This model was first fitted to the complete data set to obtain estimates of genetic parameters for the total population (constituted from the two divergent lines). An intra-line analysis was then performed by fitting the same model to two data sets each containing the data of one of the two lines in

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order to reveal whether the genetic determinism of the traits studied was different between the two genetic lines. Estimated breeding values (**EBV**) were calculated by obtaining the solutions of the mixed model equations in the OUTPUT section of VCE (6.0.2) parameters file (Neumaier and Groeneveld, 1998) while genetic standard deviations (**GSTD**) were obtained by taking the square roots of estimated genetic variances. In order to improve the understanding of the relationship between the breeding values of PM-pHu (explanatory variable) and the other traits measured in the study (dependent variables), a series of simple linear regression models was fitted using the *lm* (Chambers and Hastie, 1992) function available in the R statistical environment (R Core Team, 2013). This series of fits allowed visualization of this relationship and made it possible to see how it differed in direction and strength between the two genetic lines.

Phenotypic analysis of growth, carcass composition, and meat quality traits

The effects of line, sex and their interactions were tested for all traits measured on the last generation using a two-way analysis of variance with interaction for unbalanced data (General Linear Model procedure of SAS). The Least Squares Means (LSMeans) and Tukey's test for multiple comparisons between levels of each factor and their different combinations (interactions) were obtained using the same procedure. The accepted type I error was set at 5%.

RESULTS

Genetic parameters in the whole population

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Descriptive statistics of traits included in the genetic analyses are summarized in Table 1. As an initial inspection of the data revealed a non-normal distribution of DL, it was decided to apply a logarithmic transformation to this trait prior to genetic analyses but not prior to the phenotypic characterization of the two divergent lines where raw values of DL (i.e. percentages) were used. All other traits had a normal or a very close to normal distribution as indicated by their skewness and kurtosis coefficients. As shown in Table 2, a strong genetic control of PM-pHu was observed with an estimated heritability (h^2) of 0.57. Except for BW6, the h^2 of which was moderate, all the other traits had high h^2 of between 0.39 and 0.71. Estimated genetic correlations (r_g) between PM-pHu and other traits measured in the present study varied greatly (Table 2). Strongly significant positive r_g was found between PM-pHu and SART-pHu. Strong and moderate negative r_g were observed with L* and b* while it was very close to zero with a*. A significant negative r_g was also found with LogDL. Correlations between PM-pHu and growth and body composition varied according to the traits. They were moderately positive with BMY, but close to zero with TY, AFP, and BW6.

Genetic parameters within each line

Similar estimates of h^2 for PM-pHu were obtained within each line and in the whole population (Table 2). In contrast, differences in h^2 between the two lines were observed for other traits. The h^2 estimate of LogDL for the pHu+ line was around than twice as high as that for the pHu- line ($P < 0.05$). The h^2 estimate for BMY was 27% higher (*P < 0.05*) in the pHu- line. The intra-line analyses indicated that PM-pHu did not always correlate in the same way with meat quality and carcass composition traits in the two selected lines. Indeed, PM-pHu was negatively correlated with L* in both lines, moderately in the pHu+ line but strongly in the pHu- line. Similarly, a very strong negative correlation was found in the pHu- line

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between PM-pHu and LogDL while in the pHu+ line the correlation was close to zero. The correlation with a* was close to zero in the pHu+ line whereas it was moderate and positive in the pHu- line.

To highlight the inter-line differences, especially in r_g between PM-pHu and LogDL and between PM-pHu and L*, estimated breeding values (EBV) of LogDL and L* were fitted against those of PM-pHu in a simple regression model separately for both lines (On-line supplement 1, Figures A to D). In the pHu+ line, only 4% of the variability of LogDL was accounted for by the PM-pHu. On the other hand, PM-pHu accounted for 85% of the total LogDL variability in the pHu- line. Similarly, for the relationship between breast meat L* and PM-pHu EBV, R^2 values from the regression fit were quite different between the two lines and reached 81% and 32% in the pHu- and pHu+ lines, respectively.

Genetic evolution of traits during selection

As shown in Figure 1, the two subpopulations (pHu- and pHu+) started to diverge for PM-pHu after only one generation of selection where the between-lines difference was estimated at 1.15 genetic standard deviation (GSTD). The difference increased by an average of 0.67 GSTD per generation to reach 3.84 GSTD after five generations of selection (in G5). Similar patterns of evolution but of lower magnitude were observed for L* and b* color parameters with the between-lines difference reaching 2.21 and 1.66 GSTD at G5 for these two traits, respectively. The genetic evolution of a* was less symmetric and of lower magnitude than the other color parameters, with a difference of 0.63 GSTD at G5. Carcass composition traits did not diverge to the same extent as meat quality traits. The two subpopulations started to diverge for BMY (0.36 GSTD) in the second generation, and the inter-line difference increased on average by 0.22 GSTD per generation, reaching 0.87 GSTD

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at G5. Non-symmetrical patterns of evolution were observed for AFP and BW6. Their respective between-lines differences were 0.06 and 0.21 GSTD at G5.

Phenotypic characterization of the pHu- and pHu+ lines

For PM-pHu (Figure 2), the between-lines difference at G1 was 0.13 units of pH (p<0.05). This difference increased by an average increment of 0.07 units/generation and reached 0.42 units at G5 (p<0.05). Based on the cut-off values published by Zhang and Barbut (2005), after five generations of selection 62.2% of the breast meat in the pHu- line could be classified as acid or PSE-like (PM-pHu $\leq$ 5.7) and 43.5% of breast meat in the pHu+ line as DFD (PM-pHu $\geq$ 6.1).

The marginal effects of line and sex on muscle, meat quality and body traits were estimated after five generations of selection (Table 3). There was no significant interaction between either factor except for BMY. As shown in Figure 3, BMY was significantly higher in the pHu+ line than in the pHu- line whatever the sex. The difference between sexes was significant only in the pHu+ line in which females exhibited higher BMY than males (P = 0.0002). As shown in Table 3, males were heavier and exhibited lower AFP but greater TY than females. A significant sex effect was also observed on some of the meat quality traits. The PM-pH$_{15}$ was slightly higher ($P < 0.001$) while the SART-pHu and CCY were lower ($P < 0.001$) in females than in males (Table 3). The line effect on all traits studied was highly significant except for BW6 and AFP (Table 3). The pHu+ line exhibited slightly higher ($P < 0.001$) TY. As for the PM-pHu, the SART-pHu was higher ($P < 0.001$) in the pHu+ line, with a difference of 0.21 pH units between the two lines. The PM muscle of the pHu- line was characterized by a higher GP (+17%, $P < 0.001$) and a slightly lower PM-pH$_{15}$ ($P < 0.001$) compared to the pHu+ line. Breast meat in the pHu+ line was less colored (lower a* and b*

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values, $P < 0.001$) but much darker (lower L*, $P < 0.001$) than in the pHu- line. Percentages of DL and CL were also reduced ($P < 0.01$) while that of CCY was considerably increased ($P < 0.001$) in the pHu+ line compared to the pHu- line. After cooking, the breast meat WB-SF was lower ($P < 0.001$) in the pHu+ than in the pHu- line.

DISCUSSION

It is well established in the literature that major meat quality defects such as PSE- and DFD-like meats are mainly associated with extreme variations in meat ultimate pH (Yang and Chen, 1993; Allen et al., 1997; Barbut, 1997; Fletcher et al., 2000; Swatland, 2008). PSE-like meat is characterized as being pale in color, with soft texture and lower water-holding capacity (WHC) while DFD-like meat is characterized by a dark color, firm texture and by being dry. Both types of defect contribute largely to variation in meat appearance, texture, juiciness, shelf-life and processing ability (Allen et al., 1997; Barbut, 1998; Fletcher, 2002; Bianchi et al., 2005; Zhuang and Savage, 2010). Thus, understanding the genetic and physiological control of meat pHu is essential in order to reduce the occurrence of extremely high or low values of this trait and consequently to reduce the incidence of these defects.

This is the first study to our knowledge in which genetic parameters of growth, carcass traits and meat quality were estimated in broiler lines divergently selected for breast meat pHu. Pedigree information (prior to the initiation of our experiment) of the female grand-parental broiler line from which birds in the present experiment were originated was not available so it was not included in the genetic analysis. Missing pedigree information has been shown to bias estimates of genetic parameters in case of non-random selection (Schenkel and Schaeffer, 2000). In our case, the original line was selected for growth and reproduction traits and as the relationship between growth and PM-pHu has been shown to be

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insignificantly different from zero (Gaya et al., 2011), there is no reason to think that the described lack of information could bias our estimated genetic parameters. On the other hand, to the best of our knowledge, papers on the relationship between muscle pHu and reproduction traits in poultry are not yet available. Strong genetic determinism (57%) of PM-pHu was observed. This was 10 to 15% higher ($P > 0.05$) than heritability obtained in broilers by Chabault et al. (2012) and Le Bihan-Duval et al. (1999, 2001) and around 40% higher ($P < 0.05$) than estimates obtained in the studies of Le Bihan-Duval et al. (2008) and Gaya et al. (2011). Differences in genetic background between the lines used in the selection process (male vs. female grandparent lines, commercial vs. experimental lines) could in part explain the differences in heritability observed between studies. In addition, the low standard error of h^2 we encountered compared to previous studies was probably due to the large number of pHu measurements (n= 4720) accumulated in our dataset over the 5 generations of selection. The strong genetic determinism of breast meat pHu resulted in a huge difference of 0.42 pH units, corresponding to more than two phenotypic STDs, between the pHu+ and pHu- lines after five generations of selection on this parameter. The breast muscle pHu in broilers is mainly determined by the glycolytic potential, which quantifies the amount of glycogen present in the muscle at slaughter (Berri et al., 2001, 2005). At the genetic level, the correlation between breast muscle GP and pHu is very close to -1 (Le Bihan-Duval et al., 2008). Therefore, selecting broilers for higher (or lower) breast meat pHu should result in lower (or higher) muscle glycolytic potential, and this was confirmed experimentally in our study with a significantly lower GP in the pHu+ line compared to the pHu- line. However, more research is needed to understand the mechanism by means of which the selection process induced this significant variation in GP. The heritability of the pHu of a thigh (Sartorius) muscle was estimated for the first time. In addition to being highly heritable, the SART-pHu had also a strong positive r_g with PM-pHu. From a physiological point of view, this indicates that

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selection on pHu in breast muscle affects the metabolism of other muscles, whose function and typology are quite different from the Pectoralis major muscle (Rémignon et al., 1994). This also means from an applied point of view that selection on breast meat pHu may have consequences on the sensorial and technological quality of thigh meat. The breast meat color parameters were found to be highly heritable. Our estimates were roughly in agreement with those reported for broilers in previous studies (Chabault et al., 2012; Le Bihan-Duval et al., 2001). The breast meat lightness (L*) and yellowness (b*) were negatively correlated with pHu, which was not the case for redness (a*). However, many studies have already reported negative genetic correlations between breast meat pHu and all color parameters including a* (Chabault et al., 2012; Gaya et al., 2011; Le Bihan-Duval et al., 1999, 2001, 2008). Redness of broiler breast meat is influenced by many factors such as genetic background, environmental conditions and pathogens (Debut et al., 2003; Fletcher, 2002). The complex determinism of breast muscle redness could explain why the relationship with pHu is not consistent between studies.

In addition to breast meat quality traits, our study provided original data on the genetic relationship between body composition and breast meat traits. The heritability estimates for BMY, AFP and TY were in agreement with those reported in the literature (Chabault et al., 2012; Le Bihan-Duval et al., 1999, 2001; Zerehdaran et al., 2004). There was a moderate positive correlation between BMY and pHu. Selecting broilers for higher breast meat pHu value would therefore lead to higher breast meat yield, which is one of the most important criteria of selection in commercial broiler lines. Such a positive relationship between breast muscle development and pHu has already been observed in several studies that analyzed the consequences of selecting body composition traits on breast meat quality (Berri et al., 2001, 2007; Sibut et al., 2008; Jlali et al., 2012). On the other hand, there was no significant genetic

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correlation between PM-pHu and BW6, TY or AFP, indicating that selection on breast meat pHu would not significantly affect these traits.

The high impact of selecting for breast muscle pHu on meat quality was confirmed at the phenotype level. Indeed, the pHu- and pHu+ lines diverged considerably for breast meat color and many traits related to water-holding capacity (**WHC**). An average variation of 0.42 units of pHu between the two lines led to differences of 5 and 1.5 points for L* and b*, and to 1.6% for DL during storage. The better WHC of meat from the pHu+ line was also observed during cooking (-1.6% less cooking loss) but especially during processing, with 6% more CCY than in the pHu- line. Our study also highlighted a very considerable difference in cooked meat texture between the two lines, with the pHu+ line producing a much more tender meat (-5 N/cm^2 for WB-SF) than the pHu- line. The determinism of breast meat texture is quite complex. Contrary to the significant correlation reported by Murphy and Marks (2000) between shear force and cook loss (r = 0.87) in ground and formed chicken breast meat, correlation between WB-SF and CL and between WB-SF and DL were not significant (r = 0.17, *P* = 0.13 and r = 0.21, *P* = 0.10, respectively) in the present study. Consequently, it was suggested that factors other than DL and CL contribute to the determinism of WB-SF in the population of the present study. According to a recent study (Wang et al., 2013), variations in post-mortem decline in pH in broiler breast muscle can affect the activity or release of several proteolytic enzymes involved in the post-mortem meat tenderization process. Whether or not selection on pHu affects the post-mortem proteolytic activity in breast muscle, and as a consequence meat texture, remains to be elucidated.

Within-line genetic analyses revealed some differences in the genetic control of meat quality parameters between the two divergent lines. The R^2 values obtained from the regression model fit between the EBV of PM-pHu and of LogDL and between the EBV of PM-pHu and of L* indicated that variation in PM-pHu would have a much greater impact on the variation

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of LogDL and L* in the pHu- line than in the pHu+ line. Indeed, increasing PM-pHu in the pHu+ line would result in a moderate decrease in the LogDL and the L* values, while in the pHu- line decreasing the PM-pHu would greatly increase the LogDL and L* values. Our data of the whole population indicate that, at the phenotypic level, the relationship between pHu and DL is not linear (data not shown). Such non-linear relationship has already been reported by Barbut (2002) and could explain why only a moderate increase in WHC was observed in the pHu+ line while a great decrease in WHC was observed in the pHu- line in the present study.

The greater water loss observed in the pHu- line could be explained by the post-mortem loss of functionality of muscle proteins, especially water-binding proteins, as they approach their isoelectric point (Barbut, 1998). When this point is reached (pH = 5.3 for myosin, Van Laak et al., 2000), muscle proteins lose their ability to attract, bind and retain water (Huff-Lonergan and Huff-Lonergan, 2005). Van Laak et al. (2000) have shown that contrary to what has been observed in pork, denaturation (i.e. irreversible loss of functionality) was unlikely the cause of PSE-like meat in chicken. According to these authors, myosin from white chicken muscles was more resistant to denaturation than that from red chicken muscles and from white and red pork muscles under different pH (5.4 vs. 6.5), temperatures (25 vs. 40 °C) and ATP concentrations (0.68 vs. 3.4 mM). Thus, the observed low WHC in the pHu- line would be a result of a reversible loss of functionality rather than an irreversible denaturation of muscle proteins.

Lightness (i.e. translucency or reflectance) of poultry meat is highly affected by pH-related light scattering effect giving low-pH meat a lighter color and high-pH meat a darker color (Swatland, 2008). According to this author, a decrease in pH reduces the negative electrostatic repulsion between myofilaments which then move closer together laterally and increase the refractive index of the meat in the lateral direction. In addition, at low pH values,

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free water (the proportion of water not contained within muscle cells) increases between muscle cells and on the surface of cut muscles and acts as a reflective surface, resulting in a lighter appearance of the meat, while at high pH values, more water is kept inside muscle cells giving the meat a less reflective surface and darker appearance (Braden, 2013).

Taking into consideration the estimates of the genetic parameters found in the current study and their phenotypic consequences, there is considerable impact of selection for PM-pHu on the determinism of meat quality traits. PM-pHu is therefore a major candidate for introduction into commercial breeding programs in order to reduce the incidence of PSE-like or DFD-like meat and the undesirable quality traits associated with these defects in broilers. The present study also showed that selection for PM-pHu had no significant impact on body weight and abdominal fat percentage which are traits of major economic importance for the industry. Thus, improvement of the qualitative aspects (i.e. reduction of PSE-like and DFD-line meat) does not seem to compromise the quantitative aspects of broiler chickens meat production.

LITERATURE CITED

Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. Poult. Sci. 76:1042-1046.

Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. Br. Poult. Sci. 38: 355-358.

Barbut, S. 1998. Estimating the magnitude of the PSE problem in poultry. J. Muscle Foods. 9:35-49.

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Barbut, S. 2002. Measuring sensory and functional properties. In: S. Barbut, editor, Poultry products processing: an industry guide. CRC Press, New York, USA. p. 467-511.

Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan, and E. W. Wilson. 2008. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Sci. 79: 46-63.

Berri, C., N. Wacrenier, N. Millet, and E. Le Bihan-Duval. 2001. Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poult. Sci. 80:833-838.

Berri, C., M. Debut, V. Santé-Lhoutelier, C. Arnould, B. Boutten, N. Sellier, E. Baéza, N. Jehl, Y. Jégo, and E. LeBihan-Duval . 2005. Variations in chicken breast meat quality: Implications of struggle and muscle glycogen content at death. Br. Poult. Sci. 46:572-579.

Berri, C., E. LeBihan-Duval, M. Debut, V. Santé-Lhoutelier, E. Baéza, V. Gigaud, Y. Jégo, and M. J. Duclos. 2007. Consequence of muscle hypertrophy on characteristics of pectoralis major muscle and breast meat quality of broiler chickens. J. Anim. Sci. 85:2005-2011.

Bianchi, M., D. L. Flether, and D. P. Smith. 2005. Physical and functional properties of intact and ground pale broiler breast meat. Poult. Sci. 84:803-808.

Braden, K. 2013. Converting muscle to meat: The physiology of rigor. In: C. R. Kerth, editor, The science of meat quality. Wiley-Blackwell, Iowa, USA. p. 79-94.

Chabault, M., E. Baéza, V. Gigaud, P. Chartrin, H. Chapuis, M. Boulay, C. Arnould, F. D'Abbadie, C. Berri, and E. Le Bihan-Duval. 2012. Analysis of a slow-growing line reveals wide genetic variability of carcass and meat quality-related traits. BMC Genet. 13:90.

18

Chambers, J. M., and T. J. Hastie. 1992. Linear models. In: J. M. Chambers, editor, Statistical models in S. Wadsworth & Brooks/Cole Advanced Books & Software, Pacific Grove, California 93950, USA. p. 96-138.

Dalrymple, R. H., and R. Hamm. 1973. A method for extraction of glycogen and metabolites from a single muscle sample. J. Food Technol. 8:439-444.

Debut, M., C. Berri, E. Baéza, N. Sellier, C. Arnould, D. Guemene, N. Jehl, B. Bouten, Y. Jégo, C. Beaumont, and E. Le Bihan-Duval. 2003. Variation of chicken technological meat quality in relation with genotype and stress pre-slaughter conditions. Poult. Sci. 82:1829-1838.

Duclos, M. J., C. Berri, and E. L. Bihan-Duval. 2007. Muscle growth and meat quality. J. Appl. Poult. Res. 16:107-112.

Fletcher, D. L., M. Qiao, and D. P. Smith. 2000. The relationship of raw broiler breast meat color and pH to cooked meat color and pH. Poult. Sci. 79:784-788.

Fletcher, D. L. 2002. Poultry meat quality. World's Poult. Sci. J. 58:131-145.

Gaya, L. G., G. B. Mourao, J. B. Sterman Ferraz, E. C. Mattos, A. M. M. A. Da Costa, T. M. Filho, A. F. Rosa, A. M. Felicio, and J. P. Eler. 2011. Estimates of heritability and genetic correlations for meat quality traits in broilers. Sci. Agric. (Piracicaba, Braz.). 68:620-625.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194-204.

Jlali, M., V. Gigaud, S. Métayer-Coustard, N. Sellier, S. Tesseraud, E. Le Bihan-Duval, and C. Berri. 2012. Modulation of glycogen and breast meat processing ability by nutrition in chickens: Effect of crude protein level in 2 chicken genotypes. J. Anim. Sci. 90:447-455.

19

Le Bihan-Duval, E., N. Millet, and H. Remignon. 1999. Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. Poult. Sci. 78:822-826.

Le Bihan-Duval, E., C. Berri, E. Baeza, N. Millet, and C. Beaumont. 2001. Estimation of the genetic parameters of meat characteristics and of their genetic correlations with growth and body composition in an experimental broiler line. Poult. Sci. 80:839-843.

Le Bihan-Duval, E., M. Debut, C. Berri, N. Sellier, V. Santé-Lhoutellier, Y. Jégo, and C. Beaumont. 2008. Chicken meat quality: Genetic variability and relationship with growth and muscle characteristics. BMC Genet. 9:53.

Monin, G., and P. Sellier. 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate postmortem period: The case of Hampshire breed. Meat Sci. 13:49-63.

Murphy, R. Y., and B. P. Marks. 2000. Effects of meat temperature on proteins, texture, and cook loss for ground chicken breast patties. Poult. Sci. 79:99-104.

Nadaf, J., H. Gilbert, F. Pitel, C. M. Berri, K. Feve, C. Beaumont, M. J. Duclos, A. Vignal, T. E. Porter, J. Simon, S. E. Aggrey, L. A. Cogburn, and E. Le Bihan-Duval. 2007. Identification of QTL controlling meat quality traits in an F2 cross between two chicken lines selected for either low or high growth rate. BMC Genomics 8:155.

Neumaier, A., and E. Groeneveld. 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genet. Sel. Evol. 30:3-26.

Petracci, M., M. Bianchi, and C. Cavani. 2009. The European perspective on pale, soft, exudative conditions in poultry. Poult. Sci. 88:1518-1523.

Petracci, M., M. Bianchi, S. Mudalal, and C. Cavani. 2013. Functional ingredients for poultry meat products. Trends in Food Sci. and Tech. 33:27-39.

Qiao, M., D. L. Fletcher, D. P. Smith, and J. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. Poult. Sci. 80:676-680.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Rémignon, H., L. Lefaucheur, J. C. Blum, and F. H. Ricard. 1994. Effect of divergent selection for body weight on three skeletal muscles characteristics in the chicken. Bri. Poult. Sci. 35:65-76.

SAS Institute Inc. 2009. Base SAS® 9.2 Procedures Guide. Cary, NC: SAS Institute Inc.

Schenkel, F. S., and L. R. Schaeffer. 2000. Effects of nonrandom parental selection on estimation of variance components. J. Anim. Breed. Genet. 117:225-239.

Sibut, V., E. Le Bihan-Duval, S. Tesseraud, E. Godet, T. Bordeau, E. Cailleau-Audouin, P. Chartrin, M. J. Duclos, and C. Berri. 2008. Adenosine monophosphate-activated protein kinase involved in variations of muscle glycogen and breast meat quality between lean and fat chickens. J. Anim. Sci. 86:2888-2896.

Swatland, H. J. 2008. How pH causes paleness of darkness in chicken breast meat. Meat Sci. 80:396-400.

Van Laak, R. L. J. M., C. H. Liu, and J. L. Lane. 2000. Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. Poult. Sci. 79:1057-1061.

Wang, S., C. Li, X. Xu, and G. Zhou. 2013. Effect of fasting on energy metabolism and tenderizing enzymes in chicken breast muscle early postmortem. Meat Sci. 93:865-872.

Woelfel, R. L., A. R. Sams. 2001. Marination performance of pale broiler breast meat. Poult. Sci. 80:1519-1522.

Woelfel, R. L., C. M. Owens, E. M. Hirschler, R.Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poult. Sci. 81:579-584.

Yang, C. C., and T. C. Chen. 1993. Effects of refrigerated storage, pH adjustment, and marination on color of raw and microwave cooked chicken meat. Poult. Sci. 72:355-362.

Zerehdaran, S., A. L. Vereijken, J. A. M. V. Arendonk, and E. H. V. D. Waaij. 2004. Estimation of genetic parameters for fat deposition and carcass traits in broilers. Poult. Sci. 83:521-525.

Zhang, L., and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Br. Poult. Sci. 46:687-693.

Zhuang, H., and E. M. Savage. 2010. Comparisons of sensory descriptive flavor and texture profiles of cooked broiler breast fillets categorized by raw meat color lightness values. Poult. Sci. 89:1049-1055.

Figure 1. Means ± standard deviations of the estimated breeding values (expressed in genetic standard deviation - GSTD) of ultimate pH of Pectoralis major muscle (PM-pHu).

Figure 2. Means ± standard deviations of the observed values of ultimate pH of Pectoralis major muscle (PM-pHu).

Figure 3. Impact of line-by-sex interaction on breast meat yield (BMY) expressed as least squares means ± standard error of the mean.

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Table 1. Descriptive statistics of traits included in the genetic analysis

Trait[1]	N	Mean	STD	Min	Max	Skewness	Kurtosis	CV
PM-pHu	4,720	5.90	0.20	5.38	6.58	0.27	-0.32	3.40
SART-pHu	756	6.53	0.20	5.94	7.00	-0.15	-0.32	3.00
L*	3,387	48.91	3.82	35.14	59.77	-0.04	-0.24	7.80
a*	3,247	-0.06	0.65	-1.85	1.82	0.09	-0.39	-
b*	3,390	10.47	1.47	5.76	15.56	0.08	-0.06	14.00
DL, %	691	2.99	1.53	0.44	7.86	0.72	0.17	51.29
BMY[2], %	4,700	20.18	1.41	14.98	25.76	-0.04	0.06	6.70
TY[2], %	764	22.60	1.20	18.78	26.97	0.16	0.51	5.30
AFP[2], %	4,698	1.96	0.44	0.69	4.02	0.44	0.32	22.30
BW6, g	5,062	2,737	395.8	1,527	4,408	0.43	0.37	14.40

[1]PM-pHu = pH of Pectoralis major muscle measured 24h postmortem; SART-pHu = pH of thigh (Sartorius muscle) measured 24h postmortem; L* = Pectoralis major lightness; a* = Pectoralis major redness; b* = Pectoralis major yellowness; DL = drip loss after 5 days of storage; BMY = breast muscle yield; TY = thigh + drumstick yield; AFP = abdominal fat percentage; BW6 = body weight at 6 weeks of age.

[2]Expressed in relation to body weight at 6 weeks of age.

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Table 2. Estimates and standard error of heritability (h^2) and genetic correlation (r_g) with ultimate pH of Pectoralis major muscle for body and meat quality traits in the whole population and in the two selected (pHu+ and pHu-) lines

Trait[1]	Total population h^2 (se)	Total population r_g (se)	pHu+ h^2 (se)	pHu+ r_g (se)	pHu- h^2 (se)	pHu- r_g (se)
PM-pHu	0.57 (0.02)	-	0.62 (0.04)	-	0.57 (0.03)	-
SART- pHu	0.41 (0.03)[b]	0.54 (0.04)	0.39 (0.04)[ab]	0.68 (0.07)	0.55 (0.06)[a]	0.64 (0.08)
L*	0.58 (0.02)	-0.47 (0.03)[b]	0.59 (0.03)	-0.29 (0.04)[c]	0.54 (0.03)	-0.71 (0.03)[a]
a*	0.39 (0.02)	-0.02 (0.03)[y]	0.43 (0.04)	-0.05 (0.03)[y]	0.33 (0.03)	0.19 (0.04)[x]
b*	0.48 (0.02)	-0.24 (0.03)	0.46 (0.04)	-0.22 (0.05)	0.49 (0.03)	-0.21 (0.04)
LogDL	0.52 (0.02)[b]	-0.34 (0.04)[y]	0.69 (0.05)[a]	-0.04 (0.08)[z]	0.37 (0.06)[c]	-0.80 (0.05)[x]
BMY, %	0.52 (0.01)[ab]	0.21 (0.03)	0.45 (0.03)[b]	0.15 (0.04)	0.57 (0.03)[a]	0.20 (0.04)
TY, %	0.39 (0.04)	0.06 (0.04)	0.44 (0.06)	-0.17 (0.10)	0.47 (0.04)	0.11 (0.09)
AFP, %	0.71 (0.02)	0.06 (0.03)	0.71 (0.03)	0.07 (0.04)	0.66 (0.02)	0.06 (0.04)
BW6, g	0.21 (0.02)	-0.05 (0.03)	0.29 (0.04)	-0.14 (0.04)	0.21 (0.04)	-0.06 (0.05)

[a-c] Different superscripts in the same row indicate significantly different h^2 estimates ($P \leq 0.05$).

[x-z] Different superscripts in the same row indicate significantly different r_g estimates ($P \leq 0.05$).

[1]PM-pHu = pH of Pectoralis major muscle measured 24h postmortem; SART-pHu = pH of thigh (Sartorius muscle) measured 24h postmortem; L* = Pectoralis major lightness; a* = Pectoralis major redness; b* = Pectoralis major

yellowness; LogDL = logarithm of drip loss after 5 days of storage; BMY = breast muscle yield; TY = thigh + drumstick

yield; AFP = abdominal fat percentage; BW6 = body weight at 6 weeks of age.

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Table 3. Effects of line and sex on muscle, meat quality and body traits[1]

Trait	Line effect		Sex effect		P-value	
	pH+	pH-	Males	Female	Line	Sex
PM-pH$_{15}$	6.78 ± 0.01	6.71 ± 0.01	6.72 ± 0.01	6.78 ± 0.01	***	***
PM-pHu	6.09 ± 0.01	5.67 ± 0.01	5.89 ± 0.01	5.87 ± 0.01	***	NS
SART-pHu	6.63 ± 0.01	6.42 ± 0.01	6.56 ± 0.01	6.49 ± 0.01	***	***
L*	47.50 ± 0.20	52.50 ± 0.20	50.00 ± 0.22	50.00 ± 0.19	***	NS
a*	-0.17 ± 0.04	0.05 ± 0.04	-0.009 ± 0.04	-0.11 ± 0.04	***	NS
b*	9.49 ± 0.09	11.02 ± 0.09	10.26 ± 0.1	10.25 ± 0.08	***	NS
DL, %	2.20 ± 0.06	3.80 ± 0.06	2.10 ± 0.07	2.90 ± 0.06	***	NS
CCY, %	86.6 ± 0.5	80.5 ± 0.5	85.1 ± 0.5	82.1 ± 0.5	***	***
CL, %	10.2 ± 0.36	11.9 ± 0.35	10.9 ± 0.34	11.2 ± 0.37	**	NS
WB-SF, N/cm^2	10.9 ± 0.4	16.0 ± 0.4	13.0 ± 0.4	13.9 ± 0.37	***	NS
GP^2, µM/g	102.6 ± 2.8	119.9 ± 2.8	110.3 ± 2.8	112.2 ± 2.8	***	NS
TY^3, %	22.8 ± 0.05	22.4 ± 0.1	23.0 ± 0.1	22.2 ± 0.1	***	***
AFP^3, %	1.78 ± 0.01	1.80 ± 0.01	1.61 ± 0.02	1.98 ± 0.02	NS	***
BW6, g	$2,727 \pm 11$	$2,698 \pm 11$	$2,910 \pm 12$	$2,515 \pm 11$	NS	***

[1]expressed as least squares means ± standard errors

[2]PM-pH$_{15}$ = pH of Pectoralis major muscle measured 15 minutes post-mortem; PM-pHu = pH of Pectoralis major muscle measured 24h postmortem; SART-pHu = pH of thigh (Sartorius muscle) measured 24h

postmortem; L* = Pectoralis major lightness; a* = Pectoralis major redness; b* = Pectoralis major yellowness;

DL = drip loss after 5 days of storage; CCY = curing-cooking yield; CL = cooking loss; WB-SF = Warner-

Bratzler shear force of cooked meat; TY = thigh + drumstick yield; AFP = abdominal fat percentage; BW6 =

body weight at 6 weeks of age.

[3]Expressed in relation to body weight at six weeks of age.
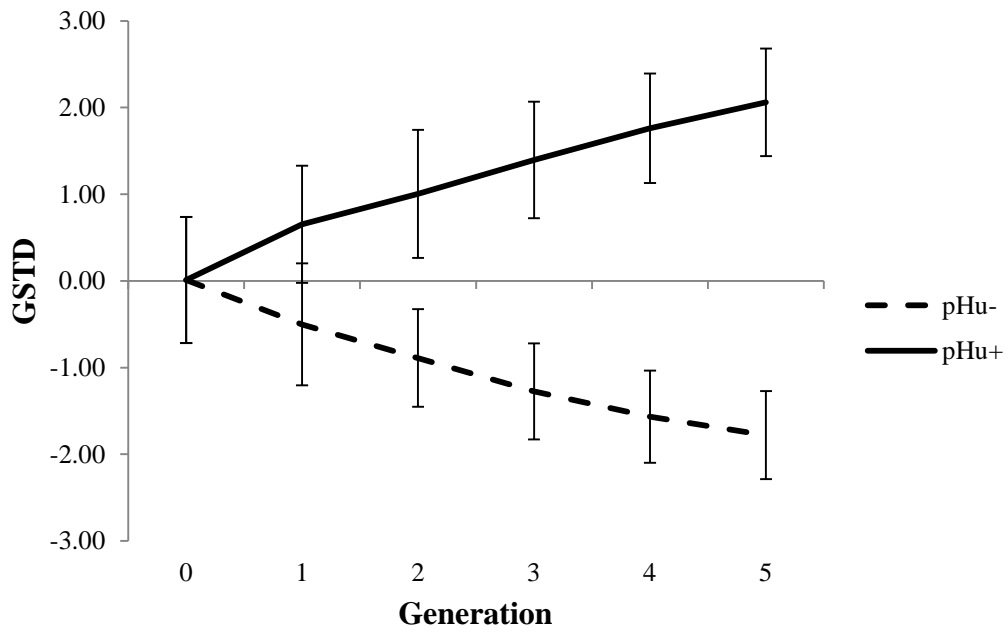
NS = not significant; **$P < 0.01$; ***$P < 0.001$.
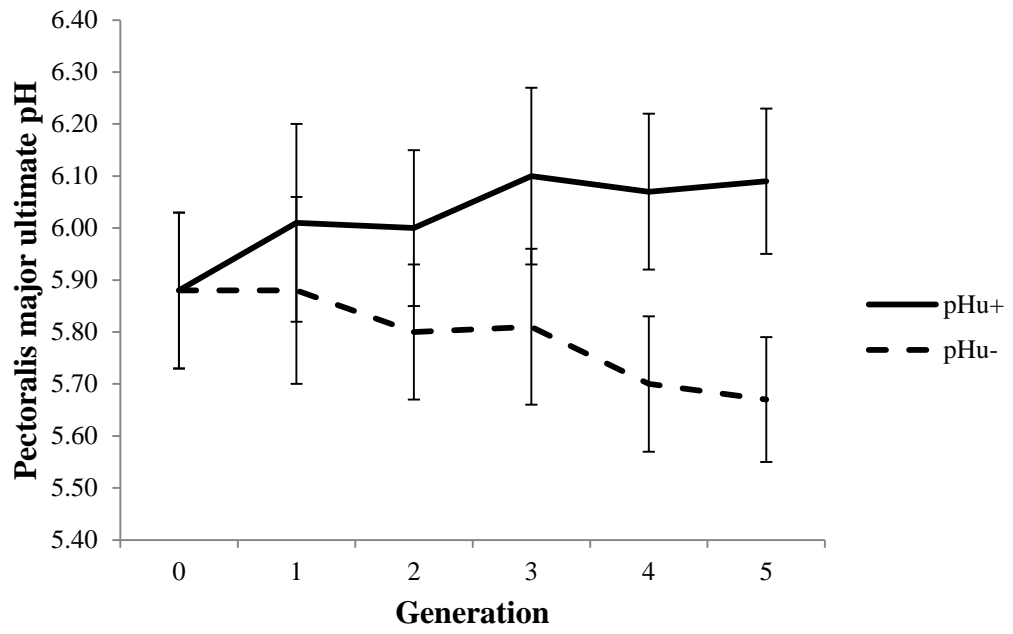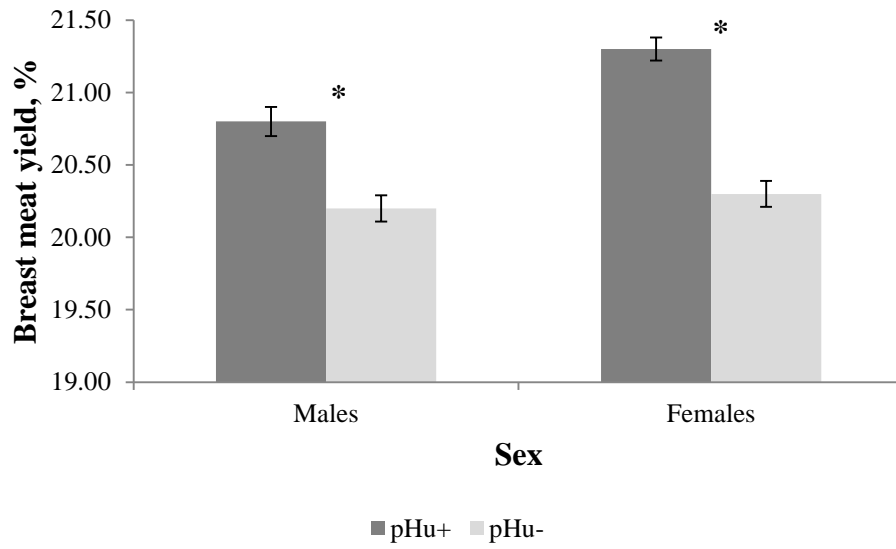
FIGURE 1

FIGURE 2

FIGURE 3

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Figure 1. Regression of estimated breeding values of L* on those of PM-pHu in the pHu+ line (A) and in the pHu- line (B), and of estimated breeding values of log (DL) on those of PM-pHu the pHu+ line (C) and in the pHu- line (D).