Research Note: Divergent selection for breast muscle ultimate pH affects egg quality traits in broiler breeders

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ABSTRACT Two divergently selected broiler lines were created by selection for low (**pHu-**) or high (**pHu+**) *Pectoralis major* ultimate pH (**pHu**) in order to better understand the molecular mechanisms underlying meat quality traits in broilers and are also unique genetic resources reflecting low and high glycogen levels in chicken muscle. The present study aimed to reveal the correlated phenotypical changes of egg quality traits in broiler breeders from the 2 divergent lines at the 14th generation. Birds were reared on littered floor system until 18 wk of age and in individual cages up to 42 wk. Individual egg production was recorded daily from age at first egg to 42 wk. External (egg weight: **EW** and shape index: **SI**), internal (albumen height: **AH**, Haugh unit: **HU**, yolk index: **YI**, and yolk color: **YC**), and shell (shell percentage: **ESP**, thickness: **EST** and strength: **ESS**) characteristics of eggs in pHu- and pHu+ lines were measured in all eggs for 4 consecutive days at 26, 27, 28, 30, 31, 32, 41, and 42 wk of age. The pHu- line had significantly higher egg percentage than pHu+ (55.9 and 49.1%, respectively). The EW in pHu- line (57.2 g) was significantly lower than in pHu+ (59.0 g) and increased with age in both lines. The mean ESP, EST and ESS were lower in the pHu+ eggs compared to the pHu- line. ESP and EST decreased mainly from 26 to 27 wk of age and they had a stable trend with advancing age in the remaining weeks. AH and YI were lower in pHu- line eggs than in pHu+. YC was more intense and HU higher in pHu+ eggs than pHu- in pre-peak and peak laying period. In conclusion, these results showed that a divergent selection for muscle energy metabolism has led to correlated responses on internal and external egg quality traits and suggest that the production of good-quality eggs may be impaired in broiler breeders with low energy reserves.

Key words: broiler, egg quality, meat quality, metabolism, muscle glycogen

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INTRODUCTION

Selection for fast-growth and feed efficiency of broilers has been a major breeding objective for decades due to the high heritability of these traits and positive impact on the overall cost of meat production. It has been shown that advances in body weight, feed efficiency and breast meat yield are mainly achieved through genetic selection and resulted in a decrease in muscle energy reserves (glycolytic potential) resulting in a higher ultimate pH in the breast meat of broilers (Le Bihan-Duval et al., 2020). On the other hand, the increase in growth rate caused some reproductive dysfunctions in broiler breeders such as delayed sexual maturity age, defective egg syndrome, and decreased egg production (Decuypere et al., 2010).

The size, composition and quality of the egg depend on the physiological and maternal fitness of the females. The eggshell consists of ~98% calcium and is the primary source of calcium for bone development in chicken embryos. Albumen is less dense than egg yolk and contains mostly water (88−90%), while the remainder contains about 10% protein, 1% carbohydrates, and traces of lipids. Egg yolk lipids contribute about 90% of embryonic energy production used for maintenance, body growth, and development (van der Wagt et al., 2020).

Reproductive performance of broiler stocks has declined due to the continuous increase in growth rate, because the genetic merits of the 2 traits are negatively correlated. However, there are no studies investigating the direct effects of variation in muscle energy metabolism on egg production and quality in broiler breeders. Two divergent broiler lines (pHu+ and pHu-) were

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created by selection over 14 generations for low or high breast muscle pH (*Pectoralis major* pHu) in order to better understand the physiological and genetic mechanisms of meat quality traits in broiler chickens. These lines are also a unique genetic materials that reflects low and high muscle glycogen levels at early post-hatch (Métayer-Coustard et al., 2021) and 6 wk of age (Alnahhas et al., 2015). This study aimed to reveal the phenotypical changes of internal and external egg quality traits in their breeders.

MATERIALS AND METHODS

The present study was performed in agreement with the French National Regulation for human care and use of animals for research purposes. Animals were reared at the PEAT INRAE Poultry Experimental Facility (2018, https://doi.org/10.15454/1.5572326250887292E12) registered by the French Ministry of Agriculture under license number C-37-175-1 for animal experimentation (INRAE, Centre Val de Loire, Nouzilly, France).

Animals and Management Procedures

The pHu+ and pHu- lines have been divergently selected over 14 generations for breast meat ultimate pH (**pHu**) measured on 6 wk old broilers. The base population originated from a grand-parental female standard line selected for a balance between growth and reproduction traits. As the measurement of pHu requires sacrificing the birds, selection of breeders was performed on their siblings. On average, 34 male and 80 female breeders were selected as parents of the next generation. The present study was conducted on egg traits collected from 80 hens of the pHu- line and 77 hens of the pHu+ line, at the 14th generation of selection.

Similar procedures were applied for each line in terms of hatching, management, feeding, vaccination, and sanitary practices. To reduce the effects of the environment, birds from the 2 lines 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 INRAE Poultry Experimental Facility. The selection process has been quite efficient since after 14 generations of selection a difference of about 0.66 pH unit was observed between the 2 lines.

For breeders, chickens were reared on the littered floor system until 18 wk of age and transferred to the laying house with individual cages at 18 wk of age and they were kept in this house during later weeks. The first light stimulation was applied at 19 wk of age, so that they could start laying at around 23 to 24 wk of age. The birds freely accessed water at all ages. They were fed ad libitum for the first 3 wk and the same amount of restricted feed was given daily for both lines between 4 and 42 wk of age. From 4 to 34 wk of age, the amount of feed was gradually increased by 4 to 5 g/hen per week and decreased from the 35 wk onwards.

Data Collection

To characterize their reproductive traits, egg production was recorded daily and individually from the age at first egg to 42 weeks at 14th generation. Based on individual daily egg records, egg production (EGGPRO, number of eggs from first egg to 42 wk of age) and egg percentage (EGGPERC, % of eggs from first egg to 42 wk of age) were determined.

Internal and external quality traits of eggs were measured in all eggs for 4 consecutive days at 26, 27, 28, 30, 31, 32, 41, and 42 wk of age. Egg weight (**EW**, in g), eggshell strength (**ESS**, in N), albumen height (**AH**, in mm), Haugh unit (**HU**), and yolk color (**YC**) were measured with a digital egg tester (DET6000, Nabel Co., Ltd, Kyoto, Japan). Shape index (**SI**, in %) was defined as the ratio of the length on the width measured to the nearest 0.1 mm with electronic caliper. Egg shells were dried in an oven at 110°C for 2 h after the membranes were cleaned and then egg shell weight (**ESW**, in g) was determined. Egg shell thickness (**EST**, in mm) was measured at around the equatorial region of egg using a dial pipe gauge (Mutitoyo Corp., Kawasaki, Japan). Egg shell percentage (**ESP**, in %) was calculated as egg shell weight / egg weight × 100. HU was determined according to formula: $Haugh\ unit = 100 \times \log(AH + 7.57 - 1.7\, EW^{0.37})$. Yolk index (YI, in %) was calculated as yolk height divided by yolk diameter.

Statistical Analyses

Data analysis was carried out using XLSTAT (Version-2021.2.1, Addinsoft, Paris, France) statistical software. One-way analysis of variance was used to determine the effects of pHu selection (pHu- and pHu+) on egg percentage (%). The effects of pHu line (pHu- and pHu+), week (26−42 wk of age) and their interaction on egg quality traits (e.g., egg weight, shape index, shell traits, albumen height, Haugh unit, yolk index, and yolk color) were subjected to a 2-way analysis of variance and analyzed with the following ANOVA model:

$$Y_{ijk} = L_i + W_j + LW_{ij} + e_{ijk}$$

with Y_{ijk} being the dependent variable for mean values of each egg quality traits per hen and week, L_i the fixed effect of line (pHu- and pHu+), W_j the fixed effect of week (26−42 wk of age), LW_{ij} the interaction between line *i* and week *j*, and e_{ijk} the residual for hen k trait. Duncan's multiple range test was used when the difference between the means was significant at least at the 0.05 level. Each egg quality data were expressed by the least squares mean and standard error of the means (**SEM**). The values with a $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Weekly egg percentage (EGGPERC) of pHu lines between 23 and 42 wk of age is given in Figure 1A.

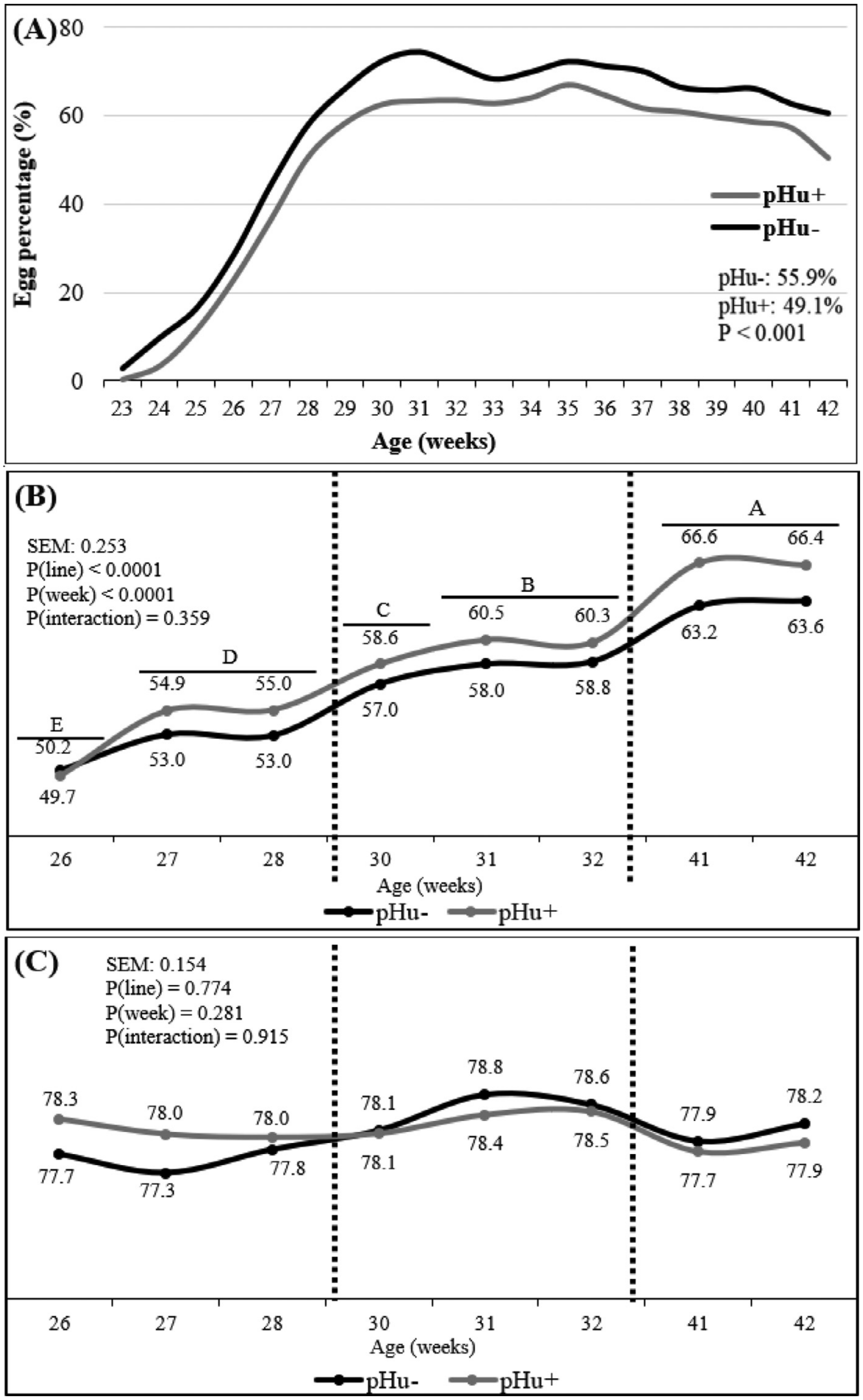

Figure 1. The change of weekly egg percentage (A), egg weight (B) and shape index (C) of pHu lines in 14th generation [1] SEM, Standard error of the means. [1] Data represent least square means for the given traits. Means with different letters indicate significant differences among weeks, $P < 0.05$. Vertical dotted lines indicate the different age periods (pre-peak [26−28 wk], peak [30−32 wk], and post-peak [41−42 wk]).

Average EGGPERC was significantly higher in pHu- (55.9%) than pHu+ (49.1%) line ($P < 0.001$). The pHu- line produced more eggs than pHu+ at all ages and the total EGGPRO was 78.3 and 68.8 eggs for pHu- and pHu+ line hens, respectively. The overall laying performance of the pHu lines are lower compared to commercial parent stocks with a 42-wk laying percentage of 74 to 77% (compared to 50−60% in the pHu lines) and a cumulative 91 to 92 eggs (Cobb-Vantress, 2020; Aviagen, 2021). However, unlike commercial parents, the pHu lines are pure-bred experimental lines that do not benefit from the heterosis effects arising from cross-breeding. Moreover, their selection focused on only breast meat pHu since 2009, without any weight put on the egg production in the selection index. As the growth characteristics of pHu broilers (over 2.7 kg BW and 20% breast meat yield at 42 d at the 14th generation) are close to those of commercial broilers, the divergent lines are, however, a valuable model to study the tradeoff between energy reserves and egg production in breeders of fast-growing lines.

The EW in pHu- line (57.2 g) was significantly lower than in pHu+ (59.0 g) and increased with age in both lines (Figure 1B). A wide range of changes occur in the internal and external egg quality traits with the advancing breeder age and the age-related increase trend in EW of pHu- and pHu+ hens was similar to the general literature (Nasri et al., 2020). These lower EGGPERC and heavier EW of the pHu+ hens may be related to reduced follicular maturation rate, in line with Johnston and Gous (2007). When EW and EGGPRO are evaluated simultaneously, egg mass is a good measure of productivity and reflects total nutrient output for a hen. The average weekly egg mass between 26 and 42 wk of age was 30.6 and 34.2 g/day for the pHu+ and pHu- line, respectively. In other words, the increase in EW of pHu+ hens with low egg number was insufficient to compensate for the total nutrient output. The fact that pHu+ line hens had less egg output than pHu- and it takes longer for egg formation clearly shows the inadequacy of the allocated resources in the pHu+ line for reproductive functions.

No significant effect of the line nor week was found for SI (Figure 1C), the average index value being 78.1. As for EW, the interaction effect on SI was found nonsignificant. Rajaravindra et al. (2015) also reported that the SI did not change with advancing age in broiler breeders. In our study, SI was similar between the lines for each week; that is, when the average egg length increased by 1 unit, the width increased by 0.78 units for both lines. Although the EW in pHu+ hens was higher than pHu-, this weight difference did not cause a change in the SI. It is obvious that meat quality traits and muscle energy metabolism that change over ultimate pH selection are not related to the SI of eggs, which suggests that these traits are controlled by different mechanisms.

ESP and EST were affected by line and week main effects (Figures 2A and 2B; $P < 0.0001$). The mean ESP was lower in the pHu+ line (8.9%) compared to the pHu-line (9.4%) between 26 and 42 wk and ESP decreased with advancing age. EST was higher in pHu- eggs (0.37 mm) than in pHu+ line (0.35 mm), and the highest values found at 26 wk of age for both lines ($P < 0.0001$). The pHu+ line eggs (34.3 N) had significantly less ESS values than pHu- eggs (36.4 N), irrespective of age. The interaction effect was nonsignificant for the ESP, EST, and ESS traits. Age-related worsening of ESP and EST in both lines were partially found to be compatible with the literature and as a result of aging physiology, the increase in shell weight occurred at a decreasing rate compared to the increase in EW, and the shell became thinner (Nasri et al., 2020). However, the age-related decrease in ESP and EST did not significantly affect the ESS, and although there were minor fluctuations, breaking strength was preserved in both pHu lines, whatever week effect.

While AH ($P < 0.0001$) and YI ($P < 0.0001$) were affected only by the line and week main effects, the interaction effect was significant for YC ($P = 0.008$) and HU ($P = 0.036$) traits. AH value was lower in pHu- line eggs (7.4 mm) than in pHu+ (7.8 mm). While AH did not change significantly between 26 and 32 wk of age, it decreased at 41 and 42 wk when it became similar between lines (Figure 2C). In pre-peak and peak laying periods, HU was higher in pHu+ line eggs compared to pHu- eggs. However, this situation changed at 41 and 42 wk since HU decreased with age to reach more similar values in both lines (Figure 2D). AH and HU are interrelated indicators that reflect albumen quality. Since the eggshell and yolk weight of the pHu lines are similar (Petit et al., 2022), the heavier egg weight of the pHu+ line is due to the higher albumen weight. The higher HU and AH in pHu+ eggs at 26 to 32 wk of age were probably due to the higher albumen viscosity, consistent with Zhang et al. (2020). AH and HU are also related to eggshell thickness, conductance, and albumen pH (Nasri et al., 2020). In our study, EST was lower in the pHu+ line, which is consistent with Petit et al. (2022). Although AH and HU were higher in pHu+ eggs in our study, the albumen pH of pHu lines was found similar in the study of Petit et al. (2022). This suggests the main role of viscosity and a higher concentration of protein and dry matter in the albumen of pHu+ eggs. In our study, while HU tended to decrease with advancing age, AH decreased only in the post-peak period. Age-related deterioration in albumen quality is mainly due to the increase in albumen pH and decrease in viscosity (Nasri et al., 2020). The larger deterioration of both AH and HU at 41 and 42 wk of age in pHu+ eggs compared to pHu- may be partially related to worsening of shell quality in pHu+ eggs. Although pHu+ eggs seem to be better until 41 wk of age in terms of the biological value of albumen and yolk traits, the same may not be the case in terms of embryonic development. Because more viscous albumen prevents gas diffusion and the use of nutrients in the very early stages of embryonic development. This situation seems likely to adversely affect chick yield and quality that need to be further investigated in the pHu lines.

YI values decreased significantly for both lines with age, and the mean YI in pHu- and pHu+ line eggs was determined as 0.43 and 0.45, respectively (Figure 2E). YI is related to vitelline membrane strength, an equally important component of the hatching eggs (Reijrink et al., 2008). In our study, the higher YI in pHu+ eggs at each week of age indicates that they may have a more resistant vitelline membrane. YC tended to increase with age, the highest values being obtained at 41 to 42 wk (Figure 2F). YC was more intense in pHu+ line than pHu- in pre-peak and peak laying period, but at 41 to 42 wk for which YC in the 2 lines reached similar values. The delay of ovulation intervals causes the same amount of yolk obtained from hepatic synthesis to accumulate in fewer follicles (Johnston and Gous, 2007). pHu+ line had ~9.5 eggs/hen less than pHu- line between 23 and 42 wk of age. In this case, it is speculated that the egg yolk synthesized in pHu+ hens accumulates in fewer follicles than pHu- line. Petit et al. (2022) reported that

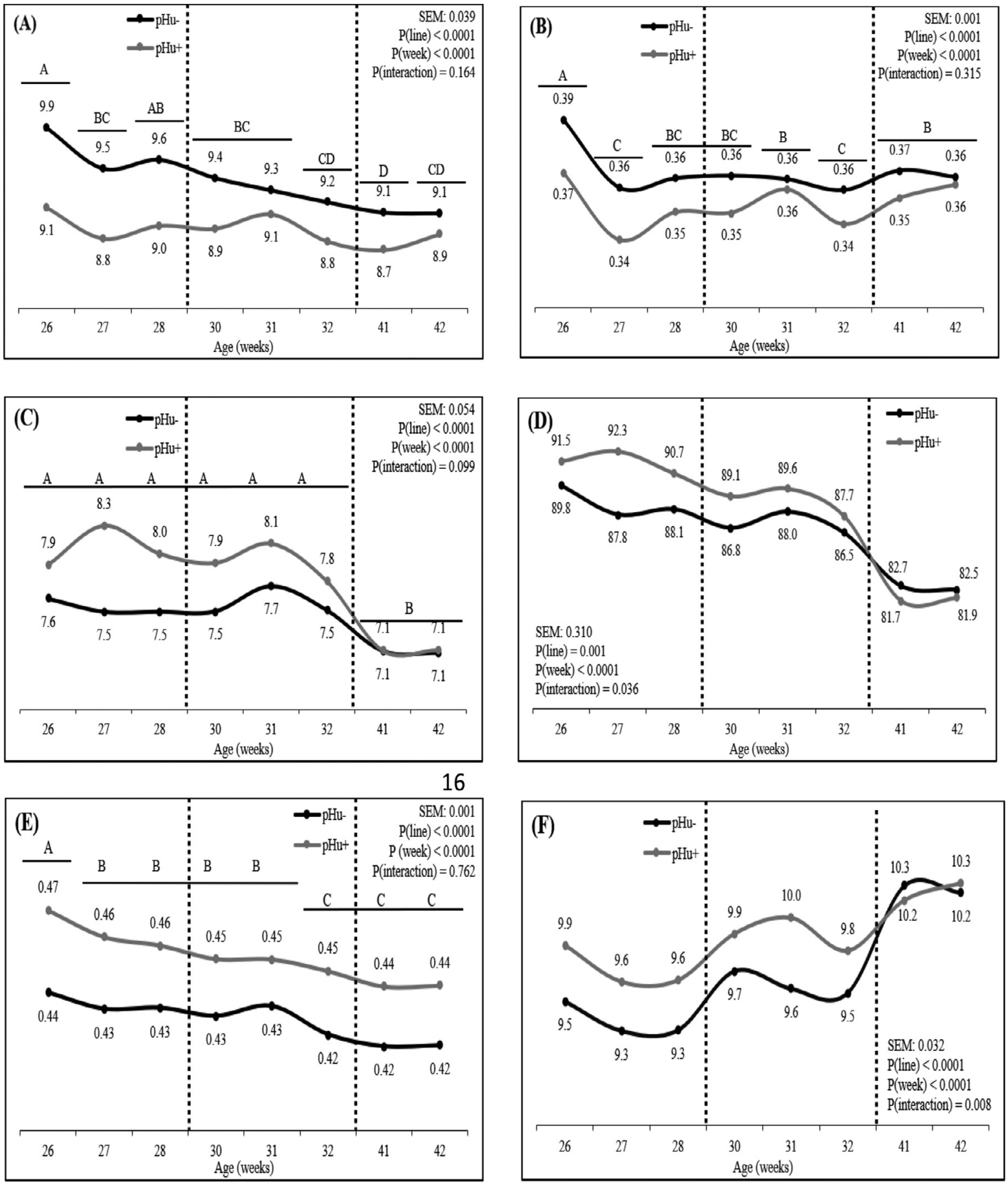

Figure 2. The change of egg shell percentage (A), egg shell thickness (B), albumen height (C), Haugh unit (D), yolk index (E), and yolk color (F) of pHu lines in 14th generation [1] SEM, Standard error of the means. [1] Data represent least square means for the given traits. Means with different letters indicate significant differences among weeks, $P < 0.05$. Vertical dotted lines indicate the different age periods (pre-peak [26−28 wk], peak [30−32 wk], and post-peak [41−42 wk]).

egg yolk of pHu+ hens have higher lipid percentage, largely explaining the more intense yolk color of pHu+ eggs compared to pHu- line. However, at 41 and 42 wk of age, the egg yolk color of pHu- hens increased more rapidly and reached a similar level with pHu+ line. The longer maturity duration of the egg yolk follicles at 41 and 42 wk may have resulted in greater darkening in the yolk color of both lines.

Our study results clearly showed that variation in energy metabolism resulting from the divergent selection on meat ultimate pH in broilers phenotypically affects internal and external egg quality traits in their breeders. We got strong indications that the production of eggs for broiler breeders with low muscle energy reserves (pHu+) has become more metabolically costly and that maternal influences on egg quality traits may contribute to some of the early phenotypic differences observed between the 2 lines. In our study on pHu fast-growing lines, we revealed that in addition to growth rate, the metabolic status of the broiler breeders is also an important factor to be considered in optimizing egg production and quality, and this is partly determined by genetics.

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DISCLOSURES

The authors state that they have no conflicts or competing interests regarding this work.

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