

Genetics and breeding of a black-bone and blue eggshell chicken line. 1. Body weight, skin color, and their combined selection

Huanhuan Wang,^{*} Avigdor Cahaner,[†] Lifeng Lou,^{*} Lei Zhang,^{*} Ying Ge,^{*} Qinghai Li,^{*} and Xuedong Zhang^{*,1}

^{*}Animal Husbandry Institute, Hangzhou Academy of Agricultural Sciences, Hangzhou 310024, China; and [†]Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

ABSTRACT An experimental population of chickens was developed from the cross between 2 indigenous Chinese breeds, Dongxiang blue eggshell and Jiangshan black-bone. This breeding was aimed at eventually combining dark heavy black-bone body and blue eggshell, into a single dual-purpose breed. BW was recorded and skin L*, a*, and b* color parameters were measured by a Chroma Meter at several ages (56, 105, 150, 200, 250, and 300 d). At 250 d, 3 independent observers classified skin darkness using a 3-level visual scale (1 = light, 2 = intermediate, 3 = dark). The 7-level average visual skin darkness, calculated for each chicken, was highly correlated (-0.658 and -0.612 in females and males, respectively) with skin L* (lightness), indicating that the accurately measured L* is reliable and useful reverse expression of visual skin darkness of black-bone chickens. Mean BW and skin L* of both sexes increased with age, to 2,063 and 1,522 g in males and females, respectively, at 300 d, and to 63 and 55 L* units in males and females,

respectively, at 250 d. The population's full-pedigree allowed estimating heritability and genetic correlations between traits. The heritability estimates of BW were similar in both sexes, increasing from around 0.25 at 56 d, to 0.53 to 0.60 at 150 d, and 0.57 to 0.62 at 300 d. Over the 5 ages, heritability estimates of skin L* were moderate to high, ranging from 0.45 to 0.58 in females, and from 0.31 to 0.65 in males, and the genetic correlations between BW and L* ranged mostly from 0.20 to 0.45. These low-to-moderate correlations between high BW and high L* (low darkness) are unfavorable; hence they were combined into an index, standardized BW minus standardized L*, allowing future selection for high BW with low L*. With high heritability of this index, 0.487 (females at 300 d) and 0.410 to 0.555 (males at 150 d or older), simultaneous improvements in BW and skin darkness appear to be feasible. The methodology used in this study can be useful in chicken populations experimentally bred for combination of high BW and other body characteristics.

Key words: black-bone chicken, BW, skin darkness, L*a*b* color scale, selection index

2021 Poultry Science 100:101035
<https://doi.org/10.1016/j.psj.2021.101035>

INTRODUCTION

In chickens with black skin, most other parts, organs, and tissues are also black, and traditionally they are called "black-bone chicken" (Wang and Li, 1987). Already Chinese ancient books, such as Compendium of Materia Medica (Li, 1596; translated by Luo, 2003), have indicated that black-bone chicken has unique effects on human health, and the darker the chicken the more profound its health effects. The black color is due to melanin hyperpigmentation, and studies have proved

that melanin is the main material responsible for the health-related effects of black-bone chicken such as anti-oxidation, anti-ultraviolet, and anti-aging properties (Xu et al., 1999; Wang et al., 2007; Tu et al., 2009). Therefore, black-bone chicken has been popular in oriental countries, especially China, holding an important share in poultry meat consumption (Zhang, 2002).

There are nearly 20 black-bone chicken breeds listed in China's records of animal genetic resources, including Silkies, Jiangshan, Muchuan, Yugan, and Yanjin (China National Commission of Animal Genetic Resources, 2010), and high levels of genetic diversity were revealed in 10 black-bone breeds by microsatellite markers (Li et al., 2006). Some of these breeds have been studied for the distribution and darkness of their black color. Liu et al. (1999) used a visual scale with 10 levels of darkness to classify 53 tissues and organs (including skin) in Taihe Silkies. Luo et al. (2000) used

© 2021 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received July 7, 2020.

Accepted January 23, 2021.

¹Corresponding author: bigzhengliang@hotmail.com

a 4-level visual scale of darkness to classify dozens of tissues and organs in Yanjin black-bone chicken. Recently, the presence and quantity of melanin pigmentation were microscopically measured in 33 organs (Nganvongpanit et al., 2020) and 34 skeletal muscles (Kriangwanich et al., 2021) of 10 Thai black-bone chickens. The muscles' darkness was also classified visually using 4 levels.

However, visual classification of the level of darkness is subjective and therefore multiple independent experienced judges are required for reliable assessment, making it complicated and costly. Because visual assessment seemed unpractical for actual breeding, objective methods to measure skin color have been developed. Clarys et al. (2000), who compared 3 skin reflectance instruments in vitro and in vivo, found the system of $L^*a^*b^*$ color parameters, measured by CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan), to be the most sensitive and reliable. The $L^*a^*b^*$ color parameters are recommended by the Commission International d'Eclairage, with lightness (L^*) ranging from 0 (maximum darkness) to 100 (maximum brightness), while a^* and b^* are negative or positive values on the green-red and blue-yellow axes, respectively (Nozaki and Makita, 1998). Huang et al. (2018) reported a strong correlation ($r = -0.847$) between the L^* value and melanin content of the skin, suggesting L^* as an objective reversed measurement of skin darkness.

Using classical genetics, it has been shown almost 100 yr ago that the autosomal dominant allele $*FM$ (fibromelanosis) leads to melanin hyperpigmentation (Bateson and Punnet, 1911; Dunn and Jull, 1927). Modern methods of molecular genetics revealed that the $*FM$ allele consists of inverse duplication of 2 close genomic regions that include the *EDN3* gene (Dorshorst et al., 2011; Tian et al., 2013). However, these studies considered melanin hyperpigmentation as a yes-or-no trait, ignoring the apparent variation among individual black-bone chickens in the amount of deposited melanin, and in visual darkness, even within genetically distinct and closed black-bone breeds.

Due to the association between human-health effects and the amount of melanin deposited in the chicken body, high level of skin and carcass darkness has been the most important trait in scientific studies with, and in the practical breeding of, black-bone chickens (Luo et al., 2000; Wei et al., 2017). Additionally, with the carcass being the economical product of black-bone chicken, high BW has been a desired breeding goal (Qian et al., 2018). To facilitate a successful breeding program for higher BW and darker skin, quantitative genetic parameters of these traits must be estimated in the base population. Studies in black-bone chicken populations yielded estimates of heritability of BW (Li et al., 1998; Wu et al., 2010) and of L^* , a^* , and b^* (Pan et al., 2018). However, there are no reports on the genetic parameters of BW and L^* at various ages, and on the genetic correlation between these 2 traits, and between L^* and visual skin darkness.

The present study is part of the ongoing development of the dual-purpose black-bone chicken line named BG, bred since 2015 for improved production of carcass

(higher BW, darker body) and eggs (higher laying rate of blue-shell eggs). This study determines and discusses the genetic parameters of BW and skin darkness at various ages, and their potential use in selecting females and males for these 2 body traits. The second study in this series will cover the genetic parameters of egg production and eggshell color, and the aggregate selection for body and egg traits, aiming at maximal dual-purpose production of black carcasses and blue-shell eggs.

MATERIALS AND METHODS

The BG Line

Breeding Process The BG line of chickens has been bred at the Hangzhou Academy of Agricultural Sciences, under the approval of the Academy's committee on animal ethics (Hangzhou, China). It originated from the segregating progeny of the cross between 2 indigenous Chinese breeds, Dongxiang blue eggshell chicken and Jiangshan black-bone chicken. The Dongxiang blue eggshell chickens are characterized by black feathers, blue eggs, and gray skin, except for a few with white skin. The Jiangshan black-bone chickens are characterized by white feathers, tint eggs, and black skin (darker than Dongxiang), and higher BW than the Dongxiang chickens (1,095 vs. 790 g and 1,000 vs. 640 g, in males and females, respectively, at 12 wk of age) (China National Commission of Animal Genetic Resources, 2010). The breeding program of the BG line has been aimed at combining black-bone body (and skin), black feathers, and blue eggshell.

In 2013, 10 Dongxiang blue eggshell males were mated with 60 Jiangshan black-bone females, producing F_1 generation chicks. In 2014, 5 F_1 males and 48 F_1 females were mated to produce the segregating population of F_2 generation. In 2015, PCR technology (Dorshorst et al., 2011; Wragg et al., 2013) was used to detect the F_2 individuals homozygous for black skin (Fibromelania gene, $*Fm$) and for blue eggshell (Oocyan gene, O). From these double-homozygous F_2 chickens, 8 males and 26 females were randomly mated to produce the base population of the BG line. In the next generation (2016), 22 males served as sires, and randomly mated with 77 females serving as dams. In the years 2017 and 2018, the BG line was further expanded by 2 cycles of random mating of 35 sires and 210 (2017) and 223 (2018) dams per generation. In 2018, all chicks were tagged by numbered wing bands, associating each chick to its sire.

In 2019, 35 random sires were mated, by artificial insemination, with 4 to 6 randomly assigned non-sib dams per sire. Full-pedigree progeny chicks were obtained in 3 consecutive hatches (May 1, 7, and 13, 2019), and they were marked by wing bands with pedigree-related identification numbers.

In each generation, the increment in inbreeding coefficient (ΔF) and the cumulative F were calculated from the effective number of breeders (N_e) per generation, derived from the corresponding numbers of sires and dams. ΔF and F calculated from N_e were found to be

similar to those determined from full-pedigree data (Nordskog and Cheng, 1988).

Animal Management After 56 d in stack-style brooding batteries of group cages, all birds were moved to stack-style growing group cages for an additional 50 d. The stocking density from 1 to 30 d, from 31 to 56 d, and from 57 to 105 d was 100, 50, and 25 birds per square meter, respectively. After the age of 105 d, females and males were housed in individual cages. All management procedures were carried out in accordance with the guidelines of Hy-Line International Breeding Co. Ltd. (<https://www.hyline.com/>), including temperature, water, feeding, lighting, beak trimming, disease control, etc. Major nutrients during the brooding, growing, and laying periods were 19.5% CP and 2,900 kcal/kg metabolizable energy (ME), 16.5% CP and 2,800 kcal/kg ME, 17.5% CP and 2,850 kcal/kg ME, respectively.

Family Structure At each age of measurements, data were obtained from all the existing chickens. However, due to some sporadic mortality, wing-band losing, and culling, the number of chickens slightly decreased with age, up to 200 d. Therefore, to ensure unbiased age effects, the data used to calculate means and genetic parameters at all ages were taken only from the chickens that were measured at 200 d. Additionally, in order to allow an unbiased estimation of the variance among full-sib individuals, dam families with a single progeny per sex (females=30, males=27) were excluded from all the calculations and analyses. Accordingly, 450 females and 339 males were included in all analyses at all ages. They were progeny of 35 sires, each mated at random to unrelated (non-sib) dams: 2 to 6 per sire, 124 in total. The average number of progeny per sire and dam, by sex, was 12.86 and 3.63 females, and 9.69 and 2.73 males.

BW and Skin Color Measurements

BW and skin color of all the males and females were measured at 6 ages: 56, 105, 150, 200, 250 (skin only), and 300 d (BW only). The color of the skin at the lateral thoracic region of each chicken was measured with a CR-400 Chroma Meter (Konica Minolta), yielding data of 3 parameters: L^* that measured lightness (100=maximum brightness, 0=maximum darkness), a^* that measured the balance of red and green, and b^* measuring the balance of yellow and blue (<https://www.konicaminolta.com.cn/>). Additionally, at 250 d, 3 independent observers scored the visual darkness of each chicken on 1 of 3 levels (1=light, 2=intermediate, 3=dark) of the skin at the lateral thoracic region. In addition to recording each observer's score (1, 2, or 3), the average score of the 3 observers was calculated for each chicken, yielding the average visual skin darkness (AVSD) with 7 levels: 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, and 3.00, as illustrated in Figure 1.

Statistical Analysis

Following confirmation of normal distribution using the Shapiro-Wilk test, data of BW and of each color

parameter (L^* , a^* , and b^*) from all ages were subjected to a two-way ANOVA, and Student's t tests were used to make comparisons between sexes within age, and between consecutive ages within sex. The association between the data of each color parameter (L^* , a^* , b^*) and the visual darkness score of each observer, and the average of the 3 observers (AVSD), were tested by their linear regression on the numerical values of the 3 levels or 7 levels, considering them to be continuous variables. All these analyses were carried out using JMP 13.0 software (<https://www.jmp.com/>, SAS Institute Inc., Cary, NC).

Estimates of heritability (h^2), genetic correlation (r_G), and phenotype correlation (r_P) of BW and each color parameter (L^* , a^* , b^*) were calculated at each age, separately for females and for males, by ASReml 4.1 software (<https://asreml.kb.vsnl.co.uk/>, VSN International Ltd., Hemel Hempstead, England UK), with sire and dam as random effects, and hatch as the fixed effect. The genetic parameters were estimated separately for each sex and age because females and males are typically selected at specific and different ages, and because the means and variances of BW and L^* differ significantly between males and females and between ages.

The ASReml software was used also to calculate the breeding value (BV) of each chicken. Heritability and BV were calculated, at each age and sex, also for an index that combines BW and L^* ($I_{BW\&L}$). To facilitate possible selection for higher BW and lower L^* values (darker skin), the index's value of each chicken was calculated by its standardized BW minus its standardized L^* , giving equal weight to both traits. Specifically, $I_{BW\&L}$ of the i th chicken at the j th age was calculated by the following equation, where SD denotes the standard deviation:

$$I_{BW\&L_{ij}} = BW_{ij}/SD_{BWj} - L_{ij}^*/SD_{L^*j}$$

RESULTS

BW and Skin Color Phenotypic and Genetic Values

As expected, mean BW of the males and females increased significantly with age, with males being heavier than females by 20% at 56 d to 36% at 300 d, when the BW of males and females averaged 2,063 and 1,522 g, respectively (Figure 2). Mean skin lightness (L^*) increased from about 50 at 56 d to 55 at 105 d, similarly in both sexes. After 105 d, mean L^* of females remained around 55, whereas in males it continued to increase with age, up to 63 at 250 d (Figure 2). Consequently, males had significantly lighter skin (higher L^*) than females by 2 to 4% up to 105 d, 6% at 150 d, 9% at 200 d, and 13% at 250 d. Differences in skin redness (a^*) were not related to age, and neither to sex, whereas skin yellowness (b^*) was significantly higher in females and at the age of 56 d (Figure 2).

The estimates of heritability of BW, L^* , a^* , and b^* , by sex and age, are presented in Figure 3 and Tables 1 and 2








	Chicken #1	Chicken #2	Chicken #3	Chicken #4	Chicken #5	Chicken #6	Chicken #7
Observer							
Ob.1	1	1	1	2	2	2	3
Ob.2	1	1	2	2	2	3	3
Ob.3	1	2	2	2	3	3	3
AVSD	1.00	1.33	1.67	2.00	2.33	2.67	3.00

Figure 1. Skin photos of 7 chickens demonstrate the difficulty of visual scoring due to the continuous variation in actual skin darkness. An observer can score each chicken to 1 of only 3 levels: low (=1), intermediate (=2), and high (=3) darkness, and different observers may differently score the same chickens. Here, scores given by 3 observers to the same 7 chickens are presented. Ob.1 tended to score low darkness, giving score 1 to chickens #1 to #3, score 2 to chickens #4 to #6, and score 3 only to chicken #7. Ob.3 tended to score high darkness, giving score 1 only to chicken #1, score 2 to chickens #2 to #4, and score 3 to chickens #5 to #7. Ob.2 was intermediate, giving score 1 to chickens #1 and #2, score 2 to chickens #3 to #5, and score 3 to chickens #6 and #7. The 7 values of AVSD, calculated from the 3 scores given to each chicken, are shown in the bottom row. Abbreviations: AVSD, average visual skin darkness; Ob., observer.

(BW and L^* only). At each age, the heritability of BW in females and males did not differ significantly, and they increased from around 0.25 at 56 d and 0.42 at 105 d, to 0.56 at 150 d; at 200 and 300 d, the heritability of BW was 0.47 and 0.57, respectively, in females and 0.62 in males (Tables 1 and 2). The heritability estimates of skin color parameters (L^* , a^* , b^*) did not exhibit a trend with age, and neither consistent differences between sexes (Figure 3). For L^* , the heritability estimates were moderate at 56 d, and low (0.2–0.3) at 150 d; they were moderate to high (0.45 at 105 d, 0.53 at 200 d, and 0.58 at 250 d) in females, and further higher (>0.62) in males at 105 and 250 d (Tables 1 and 2). The estimates of heritability of a^* and b^* were mostly moderate, between 0.2 and 0.6, with no consistent difference between sexes and no apparent age effect (Figure 3).

The main part of Table 1 lists correlation coefficients (phenotypic = r_P , genetic = r_G) between ages for BW and for L^* . It should be noted that all these coefficients differed significantly from zero; hence their SE are not presented, to avoid overloading of the table. For BW, in each type of correlation, the highest coefficients were obtained between adjacent ages, with r_P ranging from 0.693 to 0.842 in females, and from 0.738 to 0.915 in males (Table 1). The corresponding coefficients of r_G were further higher—from 0.875 to 0.934 in females, and from 0.875 to 0.962 in males. All the coefficients of r_P and r_G became gradually lower as the difference in age of measurement increased. With age of selection being an important factor in a breeding program, it should be noted that the final BW (300 d) was very highly genetically correlated with BW at 200 d (0.934 and 0.962, females and males, respectively), and gradually lower with earlier ages: 0.859 and 0.852 with BW at 150 d, 0.789 and 0.573 with BW at 105 d, and 0.643 and 0.351 with BW at 56 d (Table 1).

Also for skin lightness (L^*), the coefficients of r_G were larger than the corresponding coefficients of r_P in both sexes; yet the r_G coefficients hardly decreased as age differences increased. In females the lowest r_G was 0.831 (56 vs. 200 d), and most of them were >0.9 ; in males,

most r_G coefficients were >0.96 , even when L^* data at 56 d were correlated with L^* data at 250 d (Table 1). For the other skin color parameters, a^* and b^* (results not presented), r_G values between adjacent ages were very high (mostly >0.9), although as age differences increased, the coefficients of r_G were slightly lower (0.6–0.9); yet all were highly significant, indicating that the ranking of the chickens by these color parameters remained similar over the range of age, from 56 to 250 d.

Considering the practical objective of selecting for high BW and dark body (low L^*), Table 1 also shows the coefficients of r_P and r_G between BW and L^* at each age. The r_P were quite low (from 0.068–0.244) and mostly not significant, whereas the corresponding coefficients of r_G were somewhat higher and significant, ranging from 0.243 to 0.546 in females, and from 0.204 to 0.454 in males (Table 1).

Association Between Measured Skin Lightness (L^*) and Visually Classified Skin Darkness

For marketing, consumers prefer dark black-bone chickens, but their visual judgment may differ from the skin lightness (L^*) measured by the Chroma Meter. To check the association between L^* and visual skin darkness, 3 independent observers (Ob.1, Ob.2, and Ob.3) classified the visual darkness (1=light, 2=intermediate, 3=dark) of the skin at the lateral thoracic region of each chicken at 250 d, demonstrated schematically in Figure 1. The scoring was done in the entire flock, independent of sex, but the results are presented by sex, in Table 3.

In females, the 3 observers classified a similar percentage (31.6–33.8%) as dark, but more females were classified as light by Ob.1 (26.7%) than by Ob.2 and Ob.3 (16.5–17%). Inversely, Ob.1 classified only 41.5% females as intermediate, as compared to 49.7% (Ob.2) and 51.4% (Ob.3). The male scoring of Ob.2 and Ob.3 was also similar, with about 30, 56, and 14% classified as light 1), intermediate 2), and dark 3), respectively,

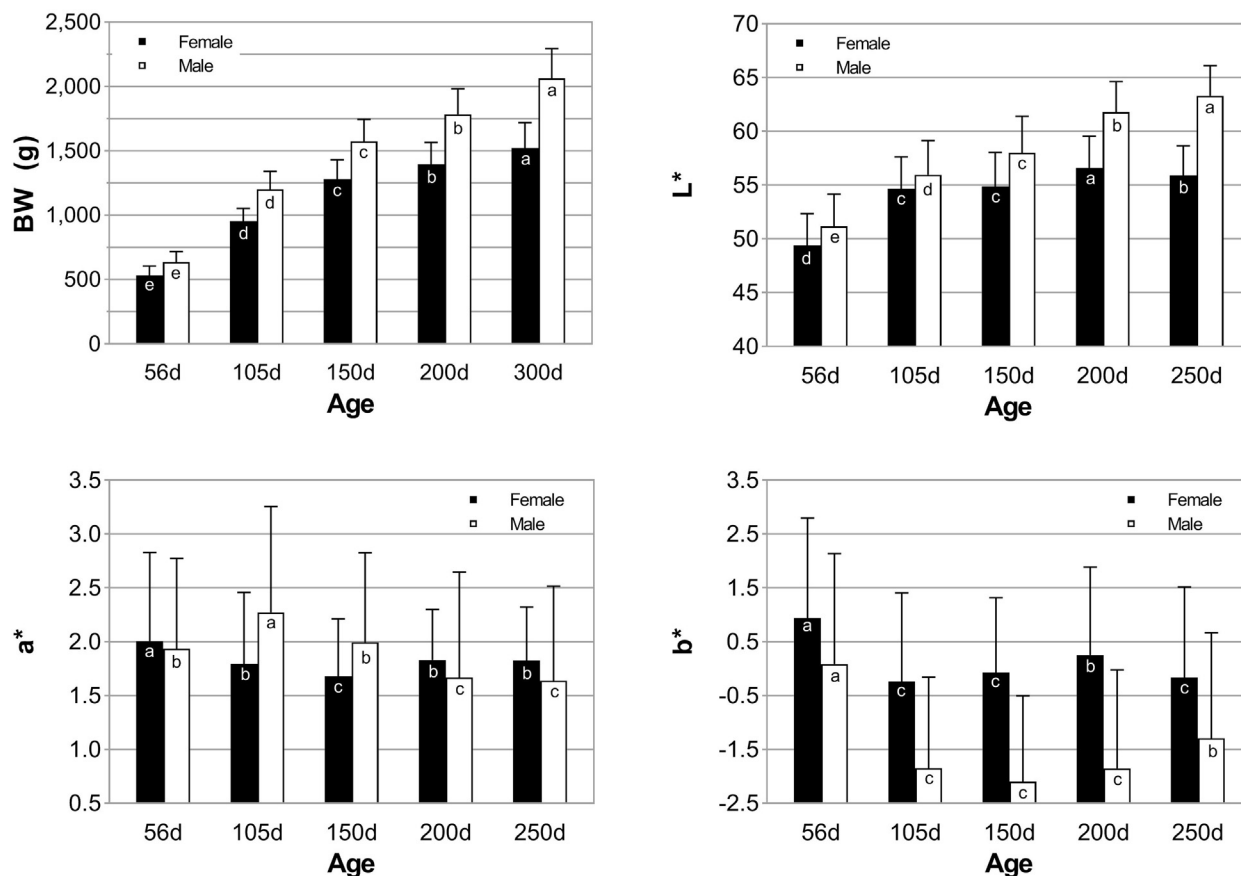


Figure 2. Means (and SD) by sex and age, of BW and the 3 skin color parameters: L* (lightness), a* (redness), and b* (yellowness). All the differences between females and males within age were significant at $P < 0.01$. ^{a,b,c,d,e}Age means within sex with no common superscript differ significantly at $P < 0.05$.

whereas the corresponding percentages of Ob.1 scoring were 35.9, 46, and 18.1% (Table 3).

To overcome differences between the individual observers in their subjective classification of visual skin darkness, their numerical scores (1, 2, or 3) of each chicken were used to calculate the AVSD with 7 levels: 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, and 3.00. Figure 1 illustrates that AVSD's 7 values are more accurately associated with the gradual continuous variation in actual skin darkness, compared to the 3 levels of each single observer.

In both sexes, the percentages of the 7 levels of AVSD were spread quite evenly between 1.00 and 3.00 (Table 3). Although females and males had similar percentages of level 2.00 (25 and 26%) and level 2.33 (12.5 and 12.4%), they differed significantly in the percentages of the lower and higher levels. The combined percentage of the lighter levels (1.00, 1.33, 1.67) was 30.3% in females vs. 48.6% in males, and inversely, the combined percentage of the darker levels (2.67, 3.00) was 32.2% in females vs. only 13% in males (Table 3). Reflecting these distributions, the numerical means of the AVSD were 2.12 and 1.84 in females and males, indicating that females had darker skin than males. These results are in agreement with the means of skin lightness (L*) at 250 d, 55.9 and 63.2 in females and males, respectively (Figure 2).

The association between L* and the visual scoring of the 3 single observers and of their means (AVSD) was tested—separately in females and males—by linear regression and correlation of the L* data on the numerical values of the 3 levels of each single observer, and the 7 levels of AVSD. All the correlation coefficients were highly significant and negative, indicating that higher visual darkness was negatively correlated, in a linear manner, with lower values of L*, a measure of skin lightness (Table 4). In females, the correlation coefficients with the 3 levels of each individual observer ranged between -0.557 and -0.616 , whereas a more close correlation (-0.658) was obtained from the regression on AVSD's 7 levels. Also in males, the correlation coefficients with the 3 levels of each individual observer (-0.543 , -0.486 , and -0.518) were considerably weaker than -0.612 , the correlation between L* and AVSD's 7 levels (Table 4). The R^2 values represent the proportion of the total variance (within sex) in L* that was explained by the linear association with the visual scoring. The R^2 values (the correlation coefficients raised to the power of 2) also indicate stronger association of L* data with the 7 levels of ASVD than the 3 levels of a single observer. The regression equations of females' and males' L* data of AVSD are presented in Figure 4, showing clearly the linear nature of the association between visual skin darkness (AVSD) and the measured

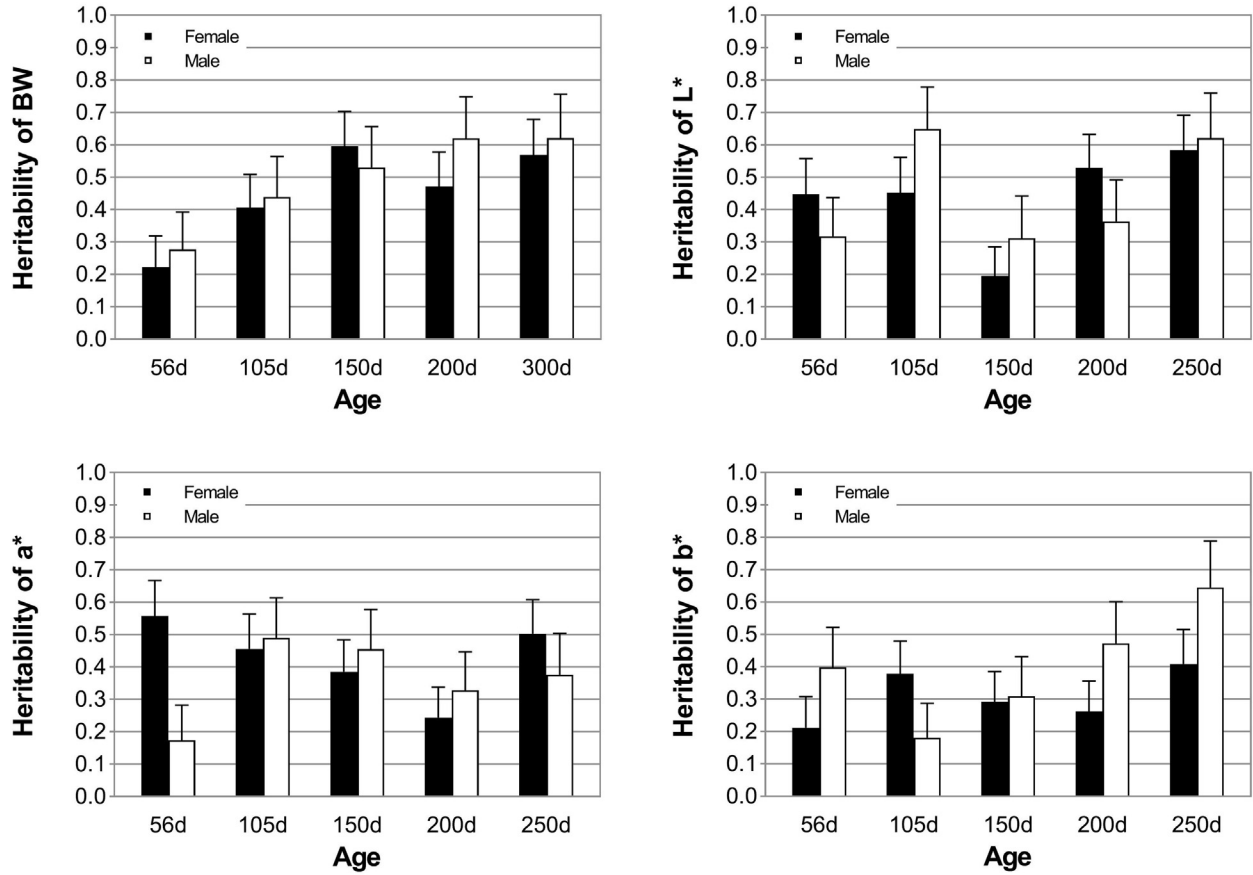


Figure 3. Estimates of heritability (and their SE) by sex and age, of BW and the 3 skin color parameters: L* (lightness), a* (redness), and b* (yellowness).

skin lightness (L*). No such associations were observed between AVSD and the data of a* and b* color parameters (Figure 4).

Considering AVSD, with its 7 levels, to be an accurate expression of the underlying continuous variation in

visual skin darkness, its heritability (h^2) was calculated. The obtained estimates were 0.520 in females and 0.375 in males. These significant estimates indicate that AVSD is heritable, yet higher estimates of h^2 were obtained for L* at 250 d (0.584 in females and 0.621 in

Table 1. Genetic parameters of BW and skin lightness (L*).

Trait	Sex	Age (day)	56 d	105 d	150 d	200 d	250/300 d	r_p with BW ¹	r_G with BW ¹
BW	Female	56	0.223	0.722	0.454	0.437	0.364		
		105	0.919	0.406	0.693	0.651	0.609		
		150	0.616	0.875	0.596	0.747	0.696		
		200	0.651	0.807	0.898	0.471	0.842		
		300	0.643	0.789	0.859	0.934	0.569		
	Male	56	0.277	0.738	0.552	0.460	0.367		
		105	0.880	0.439	0.781	0.672	0.583		
		150	0.691	0.875	0.530	0.904	0.806		
		200	0.483	0.712	0.957	0.620	0.915		
		300	0.351	0.573	0.852	0.962	0.621		
L*	Female	56	0.448	0.557	0.520	0.585	0.585	0.220	0.314
		105	0.958	0.452	0.550	0.618	0.613	0.169	0.392
		150	0.962	0.934	0.195	0.640	0.634	0.128	0.546
		200	0.831	0.854	0.957	0.529	0.764	0.101	0.370
		250	0.877	0.925	0.966	0.929	0.584	0.109	0.243
	Male	56	0.318	0.571	0.488	0.530	0.585	0.170	0.440
		105	0.880	0.649	0.583	0.632	0.618	0.224	0.454
		150	0.952	0.975	0.313	0.658	0.660	0.074	0.204
		200	0.986	0.968	0.986	0.364	0.777	0.068	0.234
		250	0.985	0.923	0.978	0.963	0.621	0.244	0.273

For each sex and trait, a 5×5 matrix (of 5 ages) shows heritability at each age (in the diagonal, bold), and correlation between the trait values at different ages: phenotypic (r_p) above the diagonal and genetic (r_G) below the diagonal.

¹The r_p and r_G correlations between BW and L* at each age are shown in the last 2 columns; L* at 250 d was correlated with BW at 300 d.

Table 2. Heritability (h^2) estimates (\pm SE) of BW, skin lightness (L^*), and the index combining the 2 traits ($I_{BW\&L}$ = standardized BW minus standardized L^* , calculated for each chicken) at the ages of 56, 105, 150, 200, and 250/300 (L^*/BW) days.

Sex	Age (day)	h^2 ($r_{Ph,BV}$)		
		BW	L^*	$I_{BW\&L}$
Females	56	0.223 \pm 0.096 (0.751)	0.448 \pm 0.110 (0.897)	0.314 \pm 0.098 (0.841)
	105	0.406 \pm 0.103 (0.881)	0.452 \pm 0.109 (0.899)	0.309 \pm 0.100 (0.882)
	150	0.596 \pm 0.107 (0.952)	0.195 \pm 0.090 (0.674)	0.264 \pm 0.096 (0.810)
	200	0.471 \pm 0.107 (0.914)	0.529 \pm 0.103 (0.926)	0.357 \pm 0.100 (0.922)
	250/300	0.569 \pm 0.110 (0.950)	0.584 \pm 0.107 (0.957)	0.487 \pm 0.106 (0.953)
Males	56	0.277 \pm 0.116 (0.785)	0.318 \pm 0.119 (0.857)	0.201 \pm 0.110 (0.827)
	105	0.439 \pm 0.126 (0.902)	0.649 \pm 0.130 (0.969)	0.378 \pm 0.122 (0.930)
	150	0.530 \pm 0.126 (0.932)	0.312 \pm 0.130 (0.757)	0.410 \pm 0.123 (0.833)
	200	0.620 \pm 0.129 (0.956)	0.364 \pm 0.128 (0.888)	0.555 \pm 0.126 (0.929)
	250/300	0.621 \pm 0.135 (0.961)	0.621 \pm 0.139 (0.949)	0.493 \pm 0.135 (0.953)

Values within the parentheses are $r_{Ph,BV}$, the correlation coefficients between the phenotype values of BW, L^* , and $I_{BW\&L}$, and their corresponding calculated BV.
Abbreviation: BV, breeding value.

males), and very high genetic correlations were found between ASVD and L^* (-0.862 in females and -0.894 in males), suggesting L^* to be a better selection criterion for skin darkness.

DISCUSSION

Excluding Possible Effect of Inbreeding

The cumulative inbreeding coefficient (F) in the studied population was very low ($F = 0.0355$), similar to that found in a randomly mated control line by Sewalem et al. (1999). Recently, Dou et al. (2020) studied the effect of F on BW of layers, and found higher inbreeding to be associated with higher BW. However, they studied a unique F_2 population; hence their inbreeding-related findings are not comparable with those of this study. In turkeys, F was found to slightly reduce BW, but only at levels higher than 0.1 (Cahaner et al., 1980). Moreover, Sewalem et al. (1999) found F levels of <0.1 to depress only fertility and hatchability in layer chickens. Therefore, it can be safely assumed that inbreeding had no significant effect on BW and skin color of the chickens in this study, and it neither biased the estimated genetic parameters.

Skin Color Measurement and Genetics

Skin darkness, a major objective in the breeding of black-bone chickens, is determined by the amount of hyperpigmentation due to melanin deposition, but consumers judge the darkness by visual appearance. Several researchers tried to classify black-bone chicken darkness by using different levels of visual darkness. Liu et al. (1999) found differences in darkness between tissues, ages, and sexes in Taihe Silkies by using 10 levels. Luo et al. (2000) used 4 visual levels (black, dark gray, gray, light gray) to show that in Yanjin black-bone chicken, the darkness of skin and claws was strongly associated with that of the tongue, and that the skin became less dark after 90 d of age. Also (Kriangwanich et al., 2021) used a 4-level darkness scale in Thai black-bone chickens and showed differences in darkness between specified skeletal muscles, but not between sexes.

In the present study, a visual scale with only 3 levels was used, due to the difficulty to subjectively distinguish between 4 or more visual levels of darkness. The visual classification of skin darkness was carried out by 3 independent observers, each scoring the level of darkness of every single chicken as 1 (light) or 2 (intermediate) or 3 (dark). The scoring of the 3 observers, presented as percentages of each level (Table 3), were quite similar

Table 3. Percentages of the 3 levels of visual skin darkness (1=light, 2=intermediate, 3=dark) of 250 d females and males, as judged by each of 3 observers (Ob.1, Ob.2, Ob.3), and the percentages of the 7 levels of the AVSD, calculated by averaging (for each chicken) the scores of the 3 observers.

Sex	Observer	Levels of visual skin darkness							Total
		1.00	1.33	1.67	2.00	2.33	2.67	3.00	
Females	Ob.1	26.7	–	–	41.5	–	–	31.8	100
	Ob.2	16.5	–	–	49.7	–	–	33.8	100
	Ob.3	17.0	–	–	51.4	–	–	31.6	100
	AVSD	9.9	9.0	11.4	25.0	12.5	13.1	19.1	100
Males	Ob.1	35.9	–	–	46.0	–	–	18.1	100
	Ob.2	30.5	–	–	55.9	–	–	13.6	100
	Ob.3	29.4	–	–	55.9	–	–	14.7	100
	AVSD	15.0	15.5	18.1	26.0	12.4	6.8	6.2	100

Abbreviations: AVSD, average visual skin darkness; Ob., observer.

Table 4. Significance of the association between the 3-level and 7-level skin visual darkness, and the continuous skin lightness (L^*), is expressed by correlation coefficients and R^2 of the linear regression of L^* on the numerical values of the 3 darkness levels (1, 2, or 3) of each observer, and of the 7 levels of AVSD (1.00, 1.33, 1.67, 2, 2.33, 2.67, 3.00).

Sex	Observer	Levels	Correlation	R^2 of regression
Females	Ob.1	3	-0.616	0.379
	Ob.2	3	-0.557	0.311
	Ob.3	3	-0.581	0.337
	AVSD	7	-0.658	0.434
Males	Ob.1	3	-0.543	0.295
	Ob.2	3	-0.486	0.236
	Ob.3	3	-0.518	0.268
	AVSD	7	-0.612	0.374

Abbreviations: AVSD, average visual skin darkness; Ob., observer.

within sex and similarly different between sexes, supporting the use of a scale with 3 levels only. However, having 3 independent observers, and averaging of their scores (AVSD), had the advantages of reducing the subjectivity of a single observer, and generating a 7-level gradual scale that better represented the continuous variation among chickens in their visual darkness (Figure 1, Table 3). Yet, visual scoring by multiple observers is time consuming and costly, is not truly continuous, and depends on the subjective judgment of the specific observers and on their working conditions (e.g., ambient light in the chicken house).

The L^* , a^* , and b^* , 3 continuous color parameters measured easily, objectively, and accurately by the Chroma Meter, were determined for each chicken in this study at 5 ages (Figure 2). At the age of 250 d, the association between each color parameter (L^* , a^* , b^*) and AVSD, the expression of visual skin darkness, was tested. Significant negative linear association between AVSD and L^* was observed in females and in males, whereas no such associations were observed between AVSD and a^* or b^* .

Further, when using the joint regression analysis to compare the slope (of L^* on AVSD) in females (-2.87) vs. males (-3.06), the interaction of AVSD with sex was not significant, indicating that the 2 slopes do not differ significantly. Therefore, the slope calculated from the joint regression analysis (-2.96) is more accurate, and its SE is 0.12, as compared to 0.15 and 0.21 of the separate slopes of the females and males, respectively. Based on these results, it is concluded that skin L^* can be used as an accurate measurement of visual skin lightness (the exact opposite of darkness) in studies of black-bone chickens, whereas a^* and b^* are useless for this purpose.

Successful selection for skin darkness in a population of black-bone chickens requires the selected trait to be reasonably heritable, indicating the presence of additive genetic variance. In this study, the heritability of AVSD, the semi-continuous expression of visual skin darkness in the BG line, was quite high in females (0.520) and moderate in males (0.375). The estimates of heritability of L^* at 250 d (when visual scoring of skin darkness was done) were higher than those of AVSD in females (0.584 vs. 0.520) and much higher in males (0.621 vs. 0.375), reflecting the accuracy and continuous nature of the L^* measurement. Heritability estimates of L^* in the other ages were moderate to high in females (0.45 at 56 and 105 d, 0.53 at 200 d), and higher in males at 105 d (0.649) (Figure 3, Table 2). These estimates of heritability, along with very high correlations between ages (Table 1), indicate that L^* may serve as an age-independent effective selection criterion in a breeding program aimed at increasing skin darkness of black-bone chickens.

Combined Selection for High BW and Low Skin Lightness (L^*)

In black-bone chicken production, income is generated by selling dressed bodies (carcasses) and therefore

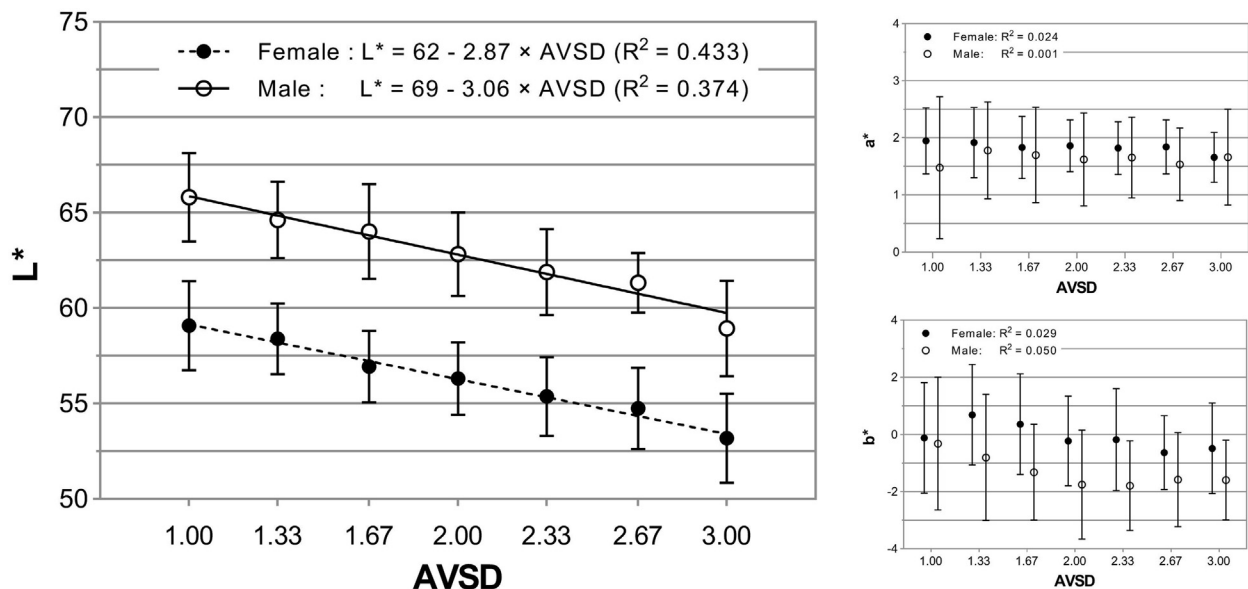


Figure 4. Significant linear regression of the skin lightness (L^*) data on the 7 levels of AVSD (1.00, 1.33, 1.67, 2, 2.33, 2.67, 3.00), in females and in males. There were no similar regressions of skin redness (a^*) and skin yellowness (b^*) on AVSD. Abbreviation: AVSD, average visual skin darkness.

genetic elevation of BW at marketing ages is the second breeding objective, along with body darkness. The means of both BW and L^* increased with age, but differently in each sex. In males, the means linearly increased with age, up to 300 d (BW) and 250 d (L^*), whereas in females, as age increased it had a declining effect on further BW gain and elevated L^* (i.e., reduced darkness) (Figure 2). These differences do not interfere with the selection for BW and L^* , because separate breeding schemes should be applied in females and in males, for additional reasons to be discussed below.

Selection for BW has been known to be effective in chicken populations with moderate-to-high heritability for this trait. In the Yugan black-bone female chickens, Li et al. (1998) reported BW heritability of 0.346 and 0.135 at age of first egg (around 157 d) and at 300 d, respectively, with high (0.776) genetic correlation (r_G) between BW at these 2 ages. Wu et al. (2010) found the heritability of BW in Saibei black-bone chicken to be 0.331 at 300 d of age. In the present study, heritability of BW in the BG line was estimated for both sexes at 5 ages. As expected, the estimates of heritability were low (around 0.25) at 56 d, when maternal effects are still substantial; they were moderate (around 0.42) at 105 d, high (0.53–0.60) at 150 d, and mostly further higher (≥ 0.6) at 200 and 300 d (Figure 3, Table 2). With high genetic correlations (mostly > 0.85) between BW at the ages of 150, 200, and 300 d (Table 1), significant response to selection on BW at this range of ages can be expected.

The genetic correlations between the 2 selected traits (BW and L^*) within sex and age (in the potential selection ages of 150 to 300 d, as discussed below) were low to moderate (0.243–0.546) in females, and lower (0.204–0.273) in males (Table 1). These positive genetic correlations are contrary to the selection objective; yet their low values should allow for combined simultaneous selection for high BW and low L^* values (i.e., higher darkness). To achieve this goal, a simple index was used, combining + BW and $-L^*$. To account for the different units and scales of BW and L^* , the actual index was calculated by summing standardized values, that is original BW and L^* values of each chicken were divided by the corresponding SD of BW and L^* (within sex at each age). Thus, the index of the i th chicken at the j th age was calculated by the following equation:

$$I_{BW\&L_{ij}} = BW_{ij}/SD_{BW_{ij}} - L_{ij}^* / SD_{L_{ij}^*}$$

With no known difference in the economic importance of BW vs. skin darkness, this index gives equal weights to these 2 traits, but differential weights can be easily introduced into this equation. Estimates of heritability were calculated for the $I_{BW\&L}$ values of the individual chickens at each age. These estimates are presented in Table 2, along with the corresponding estimates of BW and L^* . At each age and sex, the heritability of $I_{BW\&L}$ was lower than the mean heritability of each trait separately, due to the negative genetic correlation between + BW and $-L^*$. Yet, because these correlations

were only low to moderate, the heritability estimates of $I_{BW\&L}$ ranged from 0.264 to 0.487 in females, and from 0.201 to 0.555 in males (Table 2), suggesting a significant genetic response to selection on this index.

Heritability determines the response to selection on the phenotypic values of the individual candidates. Higher response is theoretically expected if individuals are selected by their BV. The BV was calculated for all 450 females and 339 males by the ASReml software, and they were found to be highly correlated to the corresponding phenotypic values (r values from ~ 0.8 at the early ages to ~ 0.95 at the later ages, Table 2), apparently due to high heritability and short pedigree (1 generation only). Thus, the responses to selection on BV vs. phenotypic values are expected to be similar in the current generation of the BG line. The actual response to these 2 options of selection will be compared empirically in the next generation of the BG line.

Age of selection is an important factor in a breeding program, according to 4 criteria: 1) earlier selection saves the costs of keeping extra candidates for a longer time; 2) all relevant performance data must be obtained for each selection candidate; 3) good heritability and favorable genetic correlations; 4) near the age of marketing, to assure selection on commercially relevant records. In the case of the BG line, bred also for egg production, selection of females should take place not before 250 d of age, to allow sufficient data on egg production from the onset of lay (at about 150 d). At the suggested selection ages, 250 to 300 d, the heritability estimates of the selection index as well as BW and L^* were the highest, indicating that also by the third criterion, females should be selected at these ages. These are also the earliest ages for selling black-bone female layers for meat consumption.

The selection of males, if based only on BW and L^* , can be conducted as early as 105 d, when these traits (and $I_{BW\&L}$) were sufficiently heritable. However, if males are to be selected after their sexual maturity is confirmed, the earliest age would be around 150 to 200 d. The heritability of the selection index (and BW and L^*) in males was highest at 200 d, but sufficiently high at 105 and 150 d. Indigenous black-bone males are marketed at BW ranging from 1,500 to 1,750 g, typically when they are around 150 d old, favoring selection around this age. Thus, it can be concluded that BG males can be selected as early as 105 or 150 d, whereas selection at 200 d would be expected to result in a somewhat higher improvement in BW and skin darkness, as well as male fertility. It should be noted that because skin L^* (lightness) of males increases with age (Figure 2), the younger they are, the darker their skin is. Thus, because market requirements are by BW (rather than age), selection for higher BW (i.e., higher growth rate) will gradually reduce the males' age-at-marketing and consequently lead to age-related darker skin, in addition to the expected genetic response to the selection for darker skin.

In summary, through estimating the heritability of BW, skin visual darkness and skin L^* , a^* , and b^* color parameters, and the genetic correlations among them, at several ages covering the main growth and production

period, this study revealed that skin L* represents (inversely) visual skin darkness, and allowed the integration of BW and skin L* into a combined index. The methodologies presented in this study can also be useful for the breeding of a combination of BW and other body characteristics such as yellow skin, breast meat yield, shank length, etc., in experimental chicken populations like the BG line.

ACKNOWLEDGMENTS

This work was supported by the Agricultural and Social Development Research Project of Hangzhou (grant number 20162012A04, 2016).

DISCLOSURES

The authors declare that they have no conflicts of interest to this manuscript entitled “Genetics and breeding of a black-bone and blue eggshell chicken line. 1. Body weight, skin color, and their combined selection.”

REFERENCES

- Bateson, W., and R. C. Punnett. 1911. On gametic series involving reduplication of certain terms. *J. Genet.* 1:293–302.
- Cahaner, A., H. Abplanalp, and F. T. Shultz. 1980. Effects of inbreeding on production traits in Turkeys. *Poult. Sci.* 59:1353–1362.
- China National Commission of Animal Genetic Resources. 2010. *Animal Genetic Resources in China (Poultry)*. China Agriculture Press, Beijing, China (in Chinese).
- Clarys, P., K. Alewaeters, R. Lambrecht, and A. O. Barel. 2000. Skin color measurements: comparison between three instruments: the Chromameter, the DermaSpectrometer and the Mexameter. *Skin Res. Technol.* 6:230–238.
- Dorshorst, B., A. M. Molin, C. J. Rubin, A. M. Johansson, L. Stromstedt, M. H. Pham, C. F. Chen, F. Hallbook, C. Ashwell, and L. Andersson. 2011. A complex genomic rearrangement involving the Endothelin 3 locus causes dermal hyperpigmentation in the chicken. *PLoS Genet.* 7:e1002412.
- Dou, T. C., J. Guo, L. Qu, X. G. Wang, Y. P. Hu, and K. H. Wang. 2020. The effect of inbreeding on the body weights of layers. *J. Fujian Agric. For. Univ.* 49:212–216 (in Chinese with English abstract).
- Dunn, L. C., and M. A. Jull. 1927. On the inheritance of some characters of the silky fowl. *J. Genet.* 19:27–63.
- Huang, W. S., Y. W. Wang, K. C. Hung, P. S. Hsieh, K. Y. Fu, L. G. Dai, N. H. Liou, K. H. Ma, J. C. Liu, and N. T. Dai. 2018. High correlation between skin color based on CIELAB color space, epidermal melanocyte ratio, and melanocyte melanin content. *PeerJ* 6:e4815.
- Kriangwanich, W., P. Piboon, W. Sakorn, K. Buddhachat, V. Kochagul, K. Pringproa, S. Mekchay, and K. Nganvongpanit. 2021. Consistency of Dark Skeletal Muscles in Thai Native Black-Bone Chickens (*Gallus gallus Domesticus*). *PeerJ* 9:e10728.
- Li, S. Z. (author), X. W. Luo (translator). 1596 (2003). *Compendium of Materia Medica (Bencao Gangmu)*. Beijing, China: Foreign Languages Press.
- Li, X. Y., J. Q. Lu, S. S. Dong, G. Q. Wu, and N. Yang. 2006. Genetic variation in Chinese native black-bone chicken populations as revealed by microsatellite markers. *Sciencepaper Online* 3, 70 (in Chinese with English abstract).
- Li, F. Q., J. F. Xie, and D. D. Jiang. 1998. Estimated for genetic parameters of Yugan black feather and black bone chicken's major economic traits. *China Poult.* 20:6–8 (in Chinese with English abstract).
- Liu, X. P., J. H. Lu, S. C. Wang, G. H. Chen, J. Y. Wang, K. W. Chen, X. Y. Zhang, K. H. Wang, and D. W. Zhao. 1999. Studies on the melanin deposit of tissues and organs in Taihe silkies. *Anim. Sci. Vet. Med.* 16:12–14 (in Chinese with English abstract).
- Luo, Z. X., C. W. Gao, J. Li, and S. J. Wu. 2000. The darkness characteristics and breeding of Yanjin black-boned chicken. *China Poult.* 22:34–36 (in Chinese).
- Nganvongpanit, K., P. Kaewkumpai, V. Kochagul, K. Pringproa, V. Punyapornwithaya, and S. Mekchay. 2020. Distribution of melanin pigmentation in 33 organs of Thai black-bone chickens (*Gallus gallus domesticus*). *Animals* 10:777.
- Nordskog, A. W., and S. Cheng. 1988. Inbreeding effects on fertility and hatchability associated with the formation of sublines. *Poult. Sci.* 67:859–864.
- Nozaki, A., and T. Makita. 1998. The surface color measurement of major tissues of Silky fowls and white Leghorns. *J. Vet. Med. Sci.* 60:489–493.
- Pan, Z. S., K. Zhang, L. Y. Bian, S. H. Xu, J. Zhang, K. N. Zhang, M. L. Yu, Y. L. Lu, W. X. Du, and D. F. Li. 2018. Study on skin color and FM copy number variation of specialized Silkie chicken. *China Poult.* 40:10–14 (in Chinese with English abstract).
- Sewalem, A., K. Johansson, M. Welhelson, and K. Lillpers. 1999. Inbreeding and inbreeding depression on reproduction and production traits of White Leghorn lines selected for egg production traits. *Br. Poult. Sci.* 40:203–208.
- Qian, F. K., J. Liao, X. X. Chen, G. Wang, H. Liu, J. Chen, P. Yan, and S. G. Yu. 2018. The correlation analysis of body weight and skin color in Muchuan black-bone chicken. *Heilongjiang Anim. Sci. Vet. Med.* 61:195–197 (in Chinese).
- Tian, M., Y. Q. Wang, X. R. Gu, C. G. Feng, S. Y. Fang, X. X. Hu, and N. Li. 2013. Copy number variants in locally raised Chinese chicken genomes determined using array comparative genomic hybridization. *BMC Genomics* 14:262–272.
- Tu, Y. G., Y. Z. Sun, Y. G. Tian, M. Y. Xie, and J. Chen. 2009. Physicochemical characterisation and antioxidant activity of melanin from the muscles of Taihe black-bone silky fowl (*Gallus gallus domesticus Brrison*). *Food Chem.* 114:1345–1350.
- Wang, M. N., and S. B. Li. 1987. Black-boned chicken and its medicinal history. *Agric. Archaeol.* 7:392–395 (in Chinese).
- Wang, Z. P., X. M. Deng, and A. R. Wang. 2007. Photoprotective effect of melanin derived from white Silky fowl on *Drosophila melanogaster* against ultraviolet radiation. *J. China Agri. Univ.* 12:17–21 (in Chinese with English abstract).
- Wei, X. F., C. Y. Li, X. F. Pan, and S. G. Yu. 2017. The measurement of skin color and extraction of melanin from skin and muscle in Muchuan black-bone chicken. *Contemp. Anim. Husb.* 35:29–31 (in Chinese with English abstract).
- Wragg, D., J. M. Mwacharo, J. A. Alcalde, C. Wang, J. L. Han, J. Gongora, D. Gourichon, M. T. Boichard, and O. Hanotte. 2013. Endogenous retrovirus EAV-HP linked to blue egg phenotype in Mapuche fowl. *PLoS One* 8:e71393.
- Wu, Z. F., H. B. Liu, and X. X. Wang. 2010. Preliminary estimation of heritability of major economic traits in Saibei black-bone chicken. *Heilongjiang Anim. Sci. Vet. Med.* 53:150–151 (in Chinese).
- Xu, X. L., S. Zhuang, and B. X. Chen. 1999. The effect of melanin from white Silky fowl on antiageing in *Drosophila melanogaster*. *J. Nanjing Agri. Univ.* 22:105–108 (in Chinese with English abstract).
- Zhang, X. Y. 2002. The way to develop the production of black-bone chickens. *Gu. Chin. Poult.* 13:37–39 (in Chinese).