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GENOME-WIDE ASSOCIATION STUDIES OF CHICKEN (*Gallus gallus* L.) BREAST MEAT COLOR CHARACTERISTICS

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Abstract

One of the most important parameters of meat quality is its color characteristics, which largely determines consumer demand for these products. Special color scales are used to assess the quality of meat based on its color spectrum. The $L^*a^*b^*$ scale is common the effectiveness of which has been shown in meat livestock farming. A number of studies have established the genetic determination of meat color characteristics for farm animals and poultry. SNPs and candidate genes that determine the expression of this trait have been identified (J. Sun et al., 2022; X. Guo et al., 2023). Here, we submit data on genome-wide association studies of the spectrum of color parameters of breast meat of F_2 chickens of the resource population based on genome-wide genotyping data. The aim of research was to search for SNPs and identify genes associated with meat color in chickens. For the research, an F_2 model resource chickens population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry, 2021-2023) was obtained by crossing two chicken breeds contrasting in meat quality, the Russian White (slow growth) and Cornish (fast growth). The poultry of F_2 resource population was genotyped using high-density Illumina Chicken iSelect BeadChip 60k (Illumina, Inc., USA). At the age of 9 weeks, birds were slaughtered. The spectra of breast meat were measured according to the $L^*a^*b^*$ color scale using a portable spectrophotometer CM-700d (Konica Minolta, Japan). Based on the genotype and phenotype data, genome-wide association studies were carried out using PLINK 1.9 software with accepted restrictions (geno 0.1, mind 0.1, maf 0.03). The threshold significance criterion was set to $p < 0.000001$. The chickens of F_2 resource population was characterized by a high coefficient of variability in the green (a^*) and blue (b^*) spectrum of meat color, from 19.99 % to 97.23 %. According to the L parameter, chickens showed relatively low variability not exceeding 9.75 %. Based on the GWAS analysis, 60 significant SNPs were identified, including those associated with the color spectrum L^* (28 SNPs), a^* (48 SNPs), and b^* (4 SNPs). These SNPs were located on chromosomes GGA1 (10 SNPs), GGA2 (3 SNPs), GGA3 (18 SNPs), GGA7 (2 SNPs), GGA8 (4 SNPs), GGA10 (2 SNPs), GGA12 (7 SNPs), GGA13 (9 SNPs), GGA17 (4 SNPs), and GGA18 (1 SNP). We identified 270 candidate genes associated with the studied traits, including 30 genes that contain the identified SNPs. The results of the study can be helpful in further genomic selection of chickens for improving meat quality.

Keywords: *Gallus gallus*, chicken, SNP, GWAS, candidate genes, meat quality, meat color, $L^*a^*b^*$ color scale

Progress in poultry farming is associated with high demand for the poultry products. Every year the requirements for the quality of poultry meat and its marketable yield are increasing [1, 2]. According to FAO, total production and consumption of this product is expected to increase annually by 1.8% until 2050, which is significantly higher than the expected growth in pork production and

consumption of 0.8% annually [3]. Chicken meat is a source of protein with high biological value, especially compared to plant proteins, in particular, in terms of the content of iron, phosphorus, vitamin A, thiamine, nicotinic acid [4]. In addition, the low energy value makes chicken meat a healthy food with a reduced fat content and a higher content of polyunsaturated fatty acids (PUFAs) compared to other types of meat [5].

Currently, poultry production emphasizes on improving quality for various characteristics of the final product, including appearance, texture and firmness, water holding capacity, color, pH, shelf life, collagen content, protein solubility, fat binding capacity [6]. Many of these parameters are significantly by poultry feeding, housing conditions [7], sex, age and breed [7, 8].

Color is an important indicator of meat quality and largely determines consumer demand [9]. Products with the desired color and without defects in appearance ensure better sales and final price [9, 10]. Pale, soft and exudative (PSE) meat is a color defect. PSE is becoming a growing problem in the meat industry. In the PSE condition, the water holding capacity (WHC) of the meat decreases and its texture becomes softer [11]. In broilers, meat PSE is influenced by various pre-slaughter factors, stunning methods, and cooling regimes [3, 12].

Poultry is the only animal species that has dark and light meat depending on the type of muscle. The breast meat is pale pink, the thighs and legs are dark red [13]. Direct correlations have been established between the meat color and pH. Dark meat, as a rule, has a higher pH, and very light meat has a lower pH value [14]. In the meat industry, pH also influences the PSE (pale, soft, exudative) and DFD (dark, firm, dry) appearance [15]. Fresh poultry meat is often classified as PSE based solely on high L^* (lightness) color parameter and low pH, which also reduces WHC [16]. A number of studies have reported the genetic basis of meat color of farm animals and poultry, including loci of quantitative traits [17, 18], SNPs [19, 20] and candidate genes [19–22] for color parameters.

This paper results from a genome-wide association study of the color spectrum indicators of breast meat in F_2 chicken resource population. We identified novel SNPs and candidate genes that are highly significantly ($p < 0.00001$) associated with meat color parameters in chickens. In the future we plan to assess the discovered SNPs as genetic markers in breeding for chicken meat quality.

The goal of the work is to search for SNPs and identify genes associated with meat color in chickens.

Materials and methods. Experiments were carried out in 2021–2023 at vivarium of the Ernst Federal Center for Livestock Husbandry — VIZh (Moscow Province) on an F_2 chicken (*Gallus gallus* L.) model resource population ($n = 260$) derived from crossing Cornish meat breed and Russian White egg breed. Chicks up to 3 weeks of age were raised in brooders with a gradual decrease in temperature from 34 °C (in the first hours after hatching) to 23 °C, and then was floor-housed. The keeping conditions met the birds' age requirements and provided free access to complete feed, fresh water and normal lighting, good ventilation ensured the absence of dampness, drafts and gas pollution. The birds aged 9 weeks were slaughtered after starvation period of 8–10 h in accordance with the Russian Federation national standard the GOST R 52837–2007 “Agricultural poultry for slaughter. Technical conditions”. After slaughter, the carcasses were scalded, the plumage was removed and the carcasses were deboned.

The color parameters of breast meat were measured using a portable spectrophotometer CM-700d (Konica Minolta, Japan) based on the $L^*a^*b^*$ system which is a three-dimensional space where negative values of a and b correspond to cold colors, positive values correspond to warm colors. The color index L characterizes the light reflection from meat surface [23]. Color spectra were recorded

24 h after slaughter at five points of the breast fillet sample and the average value was determined for each scale of the spectrum.

DNA was isolated from feather pulp using the commercial kit DNA Ekstran-2 (NPF Syntol LLC, Russia) according to the manufacturer's recommendations. The quality and integrity of the isolated DNA was assessed by a 1% agarose gel horizontal electrophoresis, the DNA purity spectrophotometrically (a NanoPhotometer® N60 spectrophotometer, Thermo Fisher Scientific, USA), samples with an $OD_{260/280} > 1.8$ were used. DNA concentration was assessed fluorometrically (a Qubit® 2.0 fluorometer, Invitrogen/Life technologies, USA) with the Qubit™ dsDNA BR Assay kit for 2-1000 ng DNA quantification (Invitrogen/Life technologies, USA).

Whole-genome genotyping was performed with the Illumina Chicken iSelect BeadChip DNA chip (Illumina, Inc., USA) for 60 thousand SNPs. Quality control and data filtering for each sample and each SNP were performed using PLINK 1.9 software in R (<http://zzz.bwh.harvard.edu/plink/>). The filters were --mind 0.10, --geno 0.10, --maf 0.05, --hwe 1e-3. Regression analysis commands --assoc, --adjust, --qt-means were used to perform genome-wide association studies (GWAS) and identify SNPs associated with muscle color indices. To confirm the influence of SNPs and identify significant regions in the chicken genome, a Bonferroni test for null hypotheses was used. Data were visualized in the qqman package (<https://github.com/qqman>). The candidate genes in the regions of identified SNPs were searched with the Genome Data Viewer in the NCBI Gallus gallus (chicken) database (<https://www.ncbi.nlm.nih.gov/datasets/genome/>). For functional annotations, the GeneCards database (<http://www.genecards.org/>) and the DAVID program (<https://david.ncifcrf.gov/>) were used.

Mean values (M), standard errors (\pm SEM), minimum (min), maximum (max), coefficient of variation (C_v , %) were calculated using the Microsoft Office 365 package.

Results. Table 1 shows the color parameters of breast meat in F2 chickens of the resource population studied.

1. Breast meat color parameters of chickens (*Gallus gallus* L.) from the F2 resource population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry – VIZh, 2021-2023)

| Parameter | M | \pm SEM | min | max | C_v , % |
|-----------|-------|-----------|-------|-------|-----------|
| L | 38.88 | 1.98 | 42.08 | 62.28 | 7.62 |
| a | 2.12 | 0.19 | -0.31 | 14.07 | 97.23 |
| b | 10.07 | 0.18 | 5.73 | 16.81 | 19.99 |

Note. a – spectrum from green (–128) to purple (127); b - spectrum from blue (–128) to yellow (127).

According to Table 1, the values of the a* and b* spectra of breast fillet highly fluctuated coefficient of variation, C_v of 97.23 and 19.99%, respectively. This indicates the influence of crossbreeding on the variability of these traits, when birds' feeding, housing and clinical health are controlled and stress during slaughter is minimized.

After data filtering, 16,630 SNPs were involved in genome-wide association studies. Figure 1 shows the distribution of identified SNPs across chromosomes.

Based on all the studied color indicators of meat in chickens from the F2 resource population, we eventually identified 60 significant SNPs ($p < 0.00001$) on chromosomes GGA1, GGA2, GGA3, GGA7, GGA8, GGA10, GGA12, GGA13, GGA17, GGA18 (Fig. 2).

Table 2 shows significantly significant SNPs ($p < 0.00001$) associated with the color characteristics of breast meat in chickens from the F2 resource population. It was found that 28 SNPs are associated with the color parameter L^* .

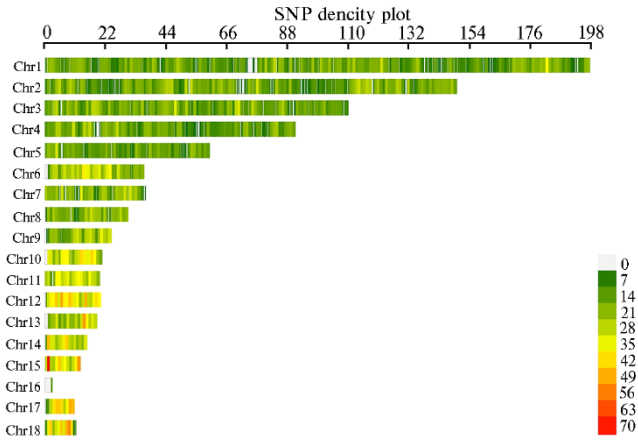


Fig. 1. Distribution density of SNPs for meat color parameters across chromosomes of chickens (*Gallus gallus* L.) from the F2 resource population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2021-2023).

These SNPs are located at GGA1 (4 SNPs), GGA2 (5 SNPs), GGA3 (8 SNPs), GGA8 (1 SNP), GGA12 (4 SNPs), GGA13 (3 SNPs), GGA17 (2 SNPs) and GGA18 (1 SNP). In total, we identified 48 significant SNPs for the a^* parameter on chromosomes GGA1, GGA2, GGA3, GGA7, GGA8, GGA10, GGA12, GGA13, GGA17 and GGA18. The largest number of SNPs was found on GGA3 (12 SNPs), the smallest on GGA18 (1 SNP). For breast meat color criterion b^* , only 4 SNPs were identified on GGA3.

terion b^* , only 4 SNPs were identified on GGA3.

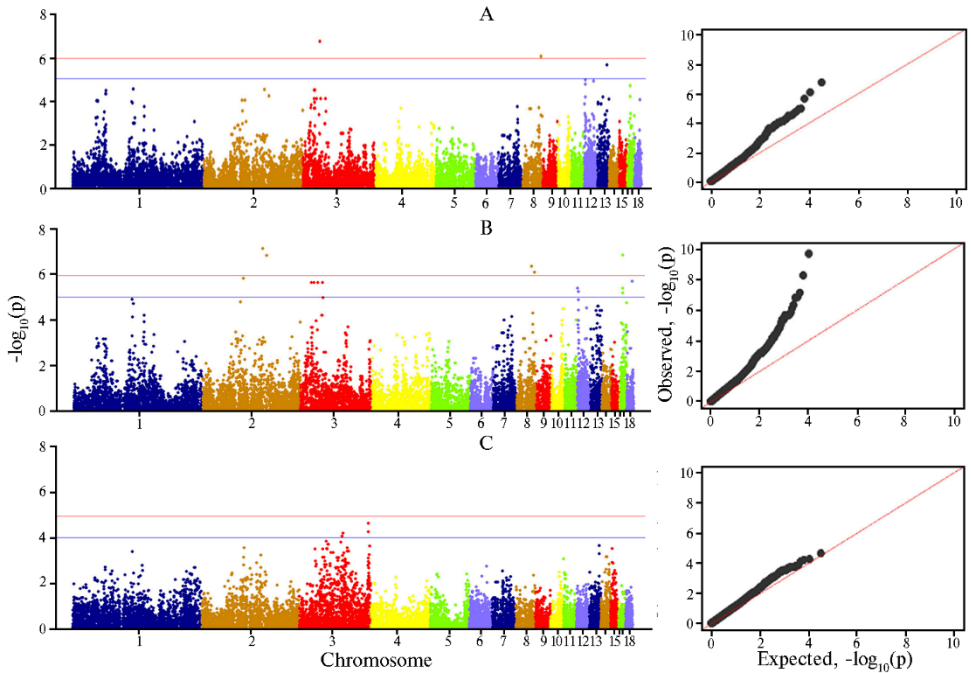


Fig. 2. Genetic structure of breast meat color parameters in chickens (*Gallus gallus* L.) from the F2 resource population: on the left — genome-wide plots, on the right — Q-Q (probability) graphs; A — spectrum of color L^* , B — spectrum of color a^* , — spectrum of color b^* ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2021-2023).

2. Significant ($p < 0.00001$) SNP associations with breast meat color parameters in chickens (*Gallus gallus* L.) from the F2 resource population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2021-2023)

| Chromosome | GGA | SNP | Position | p | Trait |
|------------|-----|---------------------|---------------------|----------|-------|
| 1 | | GGaluga017028 | 49518104...50473515 | 4.87E-05 | L |
| | | Gga_rs13865002 | | 4.87E-05 | |
| | | GGaluga017292 | | 3.33E-05 | |
| | | Gga_rs13899455 | | 1.27E-05 | |
| | | Gga_rs14856616 | | 7.36E-05 | |
| | | 88864063...90363842 | 1.27E-05 | a | |

| | | | | |
|----|----------------|-----------------------|----------------------------------|------|
| | GGaluGA031490 | 91050938...91450938 | 2.87E-05 1.95E-05 | L, a |
| | Gga_rs13917314 | 107440599...107940361 | 6.13E-05 | a |
| 2 | Gga_rs13917480 | | 6.13E-05 | |
| | Gga_rs14187600 | 57548810...58000666 | 9.71E-05 | L, a |
| | Gga_rs14187774 | | 1.62E-05 9.71E-05 1.62E-05 | |
| | GGaluGA150095 | 62037051...62437051 | 9.72E-05 | L, a |
| | Gga_rs14219701 | 91397215...91797215 | 1.47E-06 2.99E-05 | L, a |
| | Gga_rs15133231 | 97797354...98197354 | 7.47E-08 5.91E-05 | L, a |
| 3 | Gga_rs16228851 | 16704934...17317837 | 1.49E-07 7.91E-05 | L, a |
| | GGaluGA210154 | | 2.24E-06 3.22E-05 | |
| | Gga_rs14321392 | 18109888...18509888 | 3.22E-05 | L |
| | Gga_rs14323710 | 20423383...20823383 | 7.91E-05 | L, a |
| | Gga_rs16239991 | 25213895...25613895 | 2.24E-06 1.85E-07 | L, a |
| | Gga_rs15303835 | 26562059...26962059 | 2.39E-11 7.91E-05 | L, a |
| | GGaluGA215531 | 32726056...34925170 | 2.24E-06 6.15E-05 | L, a |
| | Gga_rs16250047 | | 6.15E-05 | |
| | Gga_rs14337156 | | 6.15E-05 | |
| | Gga_rs16250652 | | 6.15E-05 | |
| | Gga_rs14337823 | | 6.15E-05 | |
| | GGaluGA215952 | | 7.91E-05 | |
| | Gga_rs16251735 | | 2.24E-06 7.91E-05 2.24E-06 | |
| | GGaluGA216144 | | 1.04E-05 | |
| | Gga_rs15375140 | 65279421...65679421 | 8.35E-05 | b |
| | GGaluGA226948 | 66888191...67288191 | 6.24E-05 | |
| | GGaluGA238450 | 105376493...105875531 | 5.50E-05 | |
| | Gga_rs16338886 | | 2.25E-05 | |
| 7 | GGaluGA313956 | 16384962...16784962 | 9.96E-05 | a |
| | Gga_rs13598324 | 28338012...28738012 | 6.98E-05 | |
| 8 | GGaluGA330168 | 22596941...22996941 | 4.46E-07 | a |
| | Gga_rs14653321 | 24397555...24797555 | 4.96E-05 | a |
| | GGaluGA332278 | 27324733...27840907 | 8.92E-07 | L, a |
| | Gga_rs15937915 | | 2.06E-10 7.96E-07 | |
| 10 | Gga_rs14953406 | 18446784...19677731 | 3.25E-05 | a |
| | GGaluGA072861 | | 3.25E-05 | |
| 12 | GGaluGA080532 | 366564...1003693 | 4.08E-06 | a |
| | GGaluGA080537 | | | |
| | Gga_rs15628463 | | | |
| | Gga_rs15630811 | 1703306...2418508 | 6.82E-05 | L, a |
| | GGaluGA081274 | | 1.33E-05 1.09E-05 | |
| | Gga_rs14031390 | | 5.67E-06 1.71E-05 | |
| | GGaluGA087110 | 14173406...14573406 | 3.72E-05 1.21E-05 | L, a |
| 13 | GGaluGA093806 | 9428125...9828125 | 3.01E-05 6.88E-05 | L, a |
| | Gga_rs15698305 | 11860386...13195035 | 3.86E-05 5.92E-05 | a |
| | Gga_rs14060024 | | 2.46E-05 | |
| | GGaluGA095191 | | 2.46E-05 | |
| | GGaluGA001139 | | 2.46E-05 | |
| | Gga_rs14063043 | 15435179...16143937 | 6.59E-05 | L, a |
| | Gga_rs14063186 | | 2.26E-06 5.55E-09 | |
| | GGaluGA097116 | | 3.85E-05 | |
| | Gga_rs14065976 | 18469260...18869260 | 8.32E-05 | L, a |
| 17 | GGaluGA114289 | 4212179...5004176 | 3.78E-05 6.34E-05 | L, a |
| | | | 4.00E-06 | |

| | | | | |
|----|----------------|---------------------|----------|------|
| | GGaluGA114391 | | 2.04E-05 | |
| | | | 1.44E-07 | |
| | GGaluGA114420 | | 6.48E-06 | |
| | Gga_rs15788572 | 10517209...10917209 | 1.78E-05 | a |
| 18 | Gga_rs14114367 | 8362502...8762502 | 9.17E-05 | L, a |
| | | | 2.02E-06 | |

We identified candidate genes containing or linked to the identified SNPs (± 0.2 Mb). Structural annotation revealed 270 genes that, according to a preliminary assessment, are responsible for the color spectra of breast sirloin, including 30 genes in the positions of identified SNPs (Table 3).

3. Structural annotation of candidate genes in the region of identified SNPs associated with breast meat color parameters in chickens (*Gallus gallus* L.) from the F₂ resource population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry – VIZh, 2021-2023)

| Chromosome GGA | Candidate gene | | | Trait |
|-------------------|---|--|---|---------|
| | SNP location | | linked with SNP ($\pm 0,2$ Mb) | |
| | gene ID | positions | | |
| 1 | <i>TEF</i> <i>MKL1</i> <i>TNRC6B</i> <i>EPHA6</i> <i>ERG</i> | 49701507..49717640 50003390..50101264 50146094..50287628 90900356..91399201 107658703..107811187 | <i>CD200</i> , <i>CD200L</i> , <i>CRYBG3</i> , <i>KCNJ15</i> , <i>MEI1</i> , <i>EP300</i> , <i>BTLA</i> , <i>CGGBP1</i> , <i>ARL6</i> , <i>KCNJ6</i> , <i>ETS2</i> | L, a |
| 2 | <i>VAPA</i> | 97965836..97997739 | <i>ACO2</i> , <i>RANGAP1</i> , <i>C1H3ORF52</i> , <i>EPHA3</i> , <i>CCDC134</i> , <i>L3MBTL2</i> , <i>ARL13B</i> , <i>PROS1</i> , <i>CSDC2</i> , <i>RBX1</i> , <i>NSUN3</i> , <i>STX19</i> , <i>XRCC6</i> , <i>XPNPEP3</i> , <i>POLR3H</i> , <i>ST13P5</i> , <i>TOB2</i> , <i>SGSM3</i> , <i>SREBF2</i> , <i>ADSL</i> , <i>SHISA8</i> , <i>GRAP2</i> , <i>PHF5A</i> , <i>FAM83F</i> , <i>DES11</i> , <i>SNU13</i> , <i>DCDC2</i> , <i>NRSN1</i> , <i>MRS2</i> , <i>GPLD1</i> , <i>PHACTR1</i> , <i>EDN1</i> , <i>CNDP1</i> , <i>CNDP2</i> , <i>MOG</i> , <i>ALDH5A1</i> , <i>HIVEP1</i> , <i>CYB5A</i> , <i>FAM69C</i> , <i>APCDD1</i> , <i>PPP4R1</i> , <i>FBXO15</i> , <i>TIMM21</i> , <i>RAB31</i> , <i>RALBP1</i> , <i>TWSG1</i> | L, a |
| 3 | <i>TMEM63A</i> <i>KCNK2</i> <i>TTC7A</i> <i>KIF28P</i> <i>AHCTF1</i> <i>KIF26B</i> <i>AKT3</i> <i>LAMA4</i> <i>AFG1L</i> <i>FZD3</i> | 16879683..16908662 20526712..20653121 26628446..26790506 33508057..33530730 33454877..33507931 34003714..34297816 34651696..34796287 65400812..65497195 67086571..67151321 105634168..105686155 | <i>PARP1</i> , <i>CNIH3</i> , <i>HLX</i> , <i>SHKBP1</i> , <i>CAMKMT</i> , <i>SIX3</i> , <i>MCFD2</i> , <i>SCCPDH</i> , <i>PRKD3</i> , <i>SPAST</i> , <i>SRD5A2</i> , <i>QPCT</i> , <i>CEBPZ</i> , <i>ELP3</i> , <i>LIN9</i> , <i>MIXL1</i> , <i>DNAH14</i> , <i>MTARCI</i> , <i>CENPF</i> , <i>PTPN14</i> , <i>SIX2</i> , <i>CALM2</i> , <i>PPP1CB</i> , <i>NDUFAF7</i> , <i>SULT6B1</i> , <i>DPY30</i> , <i>MEMO1</i> , <i>SMYD3</i> , <i>CNST</i> , <i>TFB2M</i> , <i>ADSS2</i> , <i>COX20</i> , <i>DES12</i> , <i>TUBE1</i> , <i>ARMC2</i> , <i>FBXO16</i> , <i>EXTL3</i> | L, a, b |
| 7 | – | – | <i>ACBD3</i> , <i>SDE2</i> , <i>WDR26</i> , <i>PFN3</i> , <i>EFCAB2</i> , <i>HNRNPU</i> , <i>SDCCAG8</i> , <i>WISP3</i> , <i>FOXO3</i> , <i>INTS9</i> , <i>LEFTY1</i> , <i>ENAH</i> , <i>DEGS1</i> , <i>MARK1</i> , <i>FYN</i> , <i>SNX3</i> , <i>NR2E1</i> , <i>OSTM1</i> , <i>SEC63</i> , <i>CHN1</i> , <i>INSIG2</i> , <i>WIPF1</i> , <i>CCDC93</i> , <i>CHRNA1</i> , <i>DDX18</i> | L, a |
| 8 | <i>BEND5</i> <i>GLIS1</i> <i>EFCAB7</i> | 22380811..23220947 24591950..24764561 27494881..27547348 | <i>GPR155</i> , <i>CIR1</i> , <i>SCRN3</i> , <i>SP9</i> , <i>OLA1</i> , <i>SP3</i> , <i>FOXD3</i> , <i>ALG6</i> , <i>LRP8</i> , <i>ITGB3BP</i> , <i>PGM1</i> , <i>ROR1</i> , <i>DMRTB1</i> , <i>YIPF1</i> , <i>NDC1</i> | L, a |
| 10 | – | – | <i>DIO1</i> , <i>MEGF11</i> , <i>ANP32A</i> , <i>DIS3L</i> , <i>NOX5</i> , <i>MAP2K1</i> , <i>GLCE</i> , <i>TIPIN</i> , <i>KIF23</i> , <i>ZWILCH</i> , <i>PAQR5</i> , <i>LCTL</i> , <i>TLE3</i> , <i>RPL4</i> , <i>UACA</i> , <i>SNAPC5</i> , <i>SMAD6</i> , <i>SMAD3</i> | L, a |
| 12 | <i>PBRM1</i> <i>HEMK1</i> <i>DOCK3</i> <i>MAG11</i> | 771096..833446 1847096..1930905 2034647..2317912 14181633..14474643 | <i>TNNC1</i> , <i>RPL29</i> , <i>MAPKAPK3</i> , <i>CISH</i> , <i>NISCH</i> , <i>STAB1</i> , <i>NT5DC2</i> , <i>SMIM4</i> , <i>GNL3</i> , <i>SPCS1</i> , <i>GLT8D1</i> , <i>NEK4</i> , <i>ITIH3</i> , <i>MUSTN1</i> , <i>SFMBT1</i> | L, a |
| 13 | <i>GLRA1</i> <i>FLT4</i> <i>VDAC1</i> <i>FSTL4</i> | 12434258..12486014 12951742..12998047 15589702..15650724 15845754..16032315 | <i>SFXN1</i> , <i>DRD1</i> , <i>MFAP3</i> , <i>GRIAI</i> , <i>UBE2B</i> , <i>SKP1</i> , <i>BRD8</i> , <i>KIF20A</i> , <i>NMUR2</i> , <i>G3BP1</i> , <i>SPARC</i> , <i>ATOX1</i> , <i>PPP2CA</i> , <i>TCF7</i> , <i>RAPGEF6</i> , <i>GRK6</i> , <i>LMAN2</i> , <i>FAT2</i> , <i>CCDC69</i> , <i>GM2A</i> , <i>ANXA6</i> , <i>RGS14</i> , <i>ARL2</i> , <i>LACAAAT2L</i> , <i>TNIP1</i> , <i>GPX3</i> , <i>DCTN4</i> , <i>PRR7</i> , <i>PDLIM7</i> , <i>NDST1</i> , <i>RPS14</i> , <i>CD74</i> , <i>TCOF1</i> , <i>B4GALT7</i> , <i>ADRA2BL2</i> , <i>SMIM3</i> , <i>RBM22</i> , <i>MYOZ3</i> , | L, a |

| | | | | |
|----|---------------|--------------------|--|------|
| | | | <i>SYNPO, DOK3, DBN1, ARSI, CAMK2A, PDGFRB, CDX1, DDX41, RAB24, HMGXB3, CSF1R, TRIM105, NPY7R, PRELID1, NSD1, TBC1D9B, MGAT4B, SQSTM1, MAMLI, CANX, HNRNP1, DCK2</i> | |
| 17 | <i>BRINP1</i> | 4664527..4744154 | <i>TLR4</i> | L, a |
| 18 | <i>LMX1B</i> | 10665426..10802338 | <i>GNA13, MVB12B, RGS9, ARSG, ALC, WPI1, PRKARIA, ANGPTL2, ABCA8, MAP2K6, RALGPS1, ABCA5</i> | L, a |
| | <i>ABCA9</i> | 8559320..8581766 | | |

Note. Dashes in the table mean that the found SNP position was not localized within the gene

Of the 270 identified genes associated with the color characteristics of breast meat, 39 significant candidate genes for biological functions were selected, including 3 genes in which the identified SNPs were located (Table 4).

4. Functional annotation of genes associated with breast meat color parameters in chickens (*Gallus gallus* L.) from the F₂ resource population ($n = 260$, vivarium of the Ernst Federal Research Center for Animal Husbandry – VIZh, 2021–2023)

| Gene | Position | Biological functions |
|---|----------------------|---|
| In the SNP position: | | |
| <i>BRINP1</i> | 4664527..4744154 | Cell cycle, cell death, behavior |
| <i>FSTL4</i> | 15845754..16032315 | Development of multicellular organisms, development of the nervous system |
| <i>TTC7A</i> | 26628446..26790506 | Cellular homeostasis of iron ions |
| Linked to the SNP position (± 0.2 Mb): | | |
| <i>ARL13B</i> | 89891671..89940839 | Looping the heart, forming a neural tube pattern |
| <i>ABCA5</i> | 8582298..8608484 | Lipid transport |
| <i>ABCA8</i> | 8536924..8557588 | Lipid transport |
| <i>CD200L</i> | 88913076..88921182 | Regulation of the immune response |
| <i>DMRTB1</i> | 24557324..24563156 | Development of germ cells, sex differentiation |
| <i>FBXO15</i> | 91609665..91633896 | Protein binding |
| <i>FBXO16</i> | 105598226..105634014 | Protein binding |
| <i>FYN</i> | 65580445..65711082 | Cardiac process, forebrain development, innate immune response |
| <i>GNA13</i> | 8386712..8414335 | Aging of a multicellular organism |
| <i>G3BP1</i> | 12485905..12506960 | Innate immune response, a protective response to the virus |
| <i>GM2A</i> | 12614487..12617622 | Lipid transport |
| <i>LRP8</i> | 24349072..24499027 | Regulation of the innate immune response |
| <i>NDST1</i> | 12716758..12736200 | Polysaccharide chain biosynthesis process |
| <i>PDLIM7</i> | 9768860..9788764 | Heart development |
| <i>PRELID1</i> | 9812879..9814674 | Lipid transport |
| <i>SIX3</i> | 25520822..25523953 | Eye development, maturation of epithelial cells |
| <i>SMAD3</i> | 18814590..18878841 | Response to hypoxia, development of the immune system |
| <i>SP3</i> | 16704259..16735630 | Liver development |
| <i>B4GALT7</i> | 9710664..9712643 | Carbohydrate metabolism, glycosaminoglycan biosynthesis |
| <i>CCDC134</i> | 49550539..49554893 | Angiogenesis, embryonic hematopoiesis, embryonic liver development |
| <i>DIO1</i> | 24792883..24797740 | Biosynthesis of hormones |
| <i>DEGS1</i> | 17295979..17300997 | Biosynthesis of fatty acids |
| <i>EDN1</i> | 62406640..62411539 | Cellular homeostasis of calcium ions, contraction of smooth muscle veins |
| <i>GPX3</i> | 12661488..12663434 | Response to oxidative stress |
| <i>INSIG2</i> | 28449946..28462775 | Cholesterol biosynthesis |
| <i>LCTL</i> | 18547541..18551236 | Carbohydrate metabolism |
| <i>LEFTY1</i> | 16864275..16879586 | Spleen development |
| <i>LACAAT2L</i> | 12628444..12633004 | Amino acid transport |
| <i>MAP2K1</i> | 18496305..18527713 | Heart development, thyroid development |
| <i>NRSN1</i> | 57666592..57673732 | Nervous system development |
| <i>PGM1</i> | 27548636..27567569 | Carbohydrate metabolism, glucose metabolism process |
| <i>PPP1CB</i> | 26901055..26932174 | Glycogen metabolism |
| <i>SCCPDH</i> | 33530313..33540359 | Biosynthesis of glycolipids |
| <i>SFXN1</i> | 9634295..9669465 | Ion transport, amino acid transport |
| <i>STX19</i> | 89905548..89921452 | Intracellular protein transport |
| <i>TNNC1</i> | 643058..648527 | Contraction of skeletal muscles, regulation of muscle contraction |

Color is an important quality indicator closely related to glycolysis and intramuscular fat metabolism. The functions of a number of identified candidate genes (transport of lipids, amino acids, proteins, cholesterol, biosynthesis of fatty acids, development of the spleen, liver, heart, protein binding) directly or indirectly affect chicken meat color. The identified genes are responsible for the development of a multicellular organism and its organs, fatty acid homeostasis, and

biochemical processes. As is known, stress greatly affects the organoleptic characteristics of meat, therefore the function of nervous system is important meat color spectra. For the 7 candidate genes we identified, there are many reports about their connection with valuable traits in chickens. In particular, highly reliable associations of the *AKT3* gene with feather pigmentation in chickens [24] and the development of muscle fibers [25] have been established. The influence of the *FSTL4* and *VDAC1* genes on the moisture content in the egg yolk has been shown [26], and the *TTC7A* gene has been shown to influence the accumulation of glycogen in the muscles of chickens [27]. Association of the *FSTL4* gene with rapid muscle growth in broilers has also been reported [28]. The *MAG11* and *VDAC1* genes influence immunity [29-31], and the *FZD3* and *EPHA6* genes influence the function and development of the nervous system [32]. For other candidate genes we identified, a number of studies have found a connection with indicators of meat productivity and quality in other species of farm animals. E.g., the *TTC7A* and *AFGIL* genes are associated with back fat thickness in pigs [33, 34], and the *BRINP1* gene is associated with growth indices in pigs [35] and linear measurements in goats [36].

Thus, we identified 60 significant SNPs associated with meat color in chickens, 28 SNPs for the L* color spectrum, 48 SNPs for the a* spectrum, and 4 SNPs for the b* spectrum. The SNPs we discovered are located in the chromosomes GGA1 (10 SNPs), GGA2 (3 SNPs), GGA3 (18 SNPs), GGA7 (2 SNPs), GGA8 (4 SNPs), GGA10 (2 SNPs), GGA12 (7 SNPs), GGA13 (9 SNPs), GGA17 (4 SNPs), and GGA18 (1 SNP). We also identified 270 candidate genes associated with the studied traits, of which 30 genes harbored SNPs. These results can promote genomic selection of chickens for meat quality.

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