104 (2025) 105324

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Genome-wide association study reveals the genetic mechanism of wing bone strength in Cornish White

Longyu Zhuang [a,b,c], Yuanhang Shi [a,b,c], Xuefeng Shi [a,b,c], Caiyun Jiang [a,b,c],
Lin Xuan [a,b,c], Bingxin Luo [a,b,c], Honglei Jin [a,b,c], Runzhe Wang [a,b,c],
Jiahui Lai [a,b,c], Guangqi Li [d], Yiyuan Yan [d], Guiqin Wu [d], Guiyun Xu [a,b,c], Jiangxia Zheng [a,b,c,*]

[a] *National Engineering Laboratory for Animal Breeding and MOA Key Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China*
[b] *State Key Laboratory of Animal Biotech Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China*
[c] *Key Laboratory of Animal Genetics, Breeding and Reproduction (livestock), Ministry of Agriculture and Rural Affairs, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China*
[d] *Beijing Huadu Yukou Poultry Industry Co. Ltd., Beijing, 101206, China*

ARTICLE INFO

Keywords:
Cornish White chicken
Genome-wide association studies
Wing bone
Bone strength

ABSTRACT

Broiler products mainly refer to breast and leg meat, and also include wings. The price of wings is relatively high, about twice that of breast and leg meat. However, in this study, the incidence of wing bone deformity reaches more than 10 %. Such a high fracture rate reduces the quality and benefits of wing products. It is necessary to study the wing bones, find the relevant gene loci and enhance the strength of wing bones. A total of 436 Cornish White chickens were used as experimental birds. The weight, length, width, strength and toughness of ulna, radius, humerus and tibia, as well as growth and breast muscle traits, were measured, and correlation analysis was performed. A genome-wide association study (GWAS) was performed and a linear mixed model was used to analyze 39,000 SNPs from a self-developed broiler microarray. The genetic parameters of the traits were calculated. The results showed that the coefficient of variation of the toughness of the wing bone was greater than 20 %, and that of the ulna was 34.45 %. Wing bone traits were negatively correlated with feed conversion ratio and positively correlated with tibia traits and growth traits, reaching 0.8, and weakly correlated with breast muscle traits ($P > 0.05$). The heritability of wing bone traits was low, and the heritability of humerus strength was the highest (0.27). There were 27 significant SNP in the GWAS results, with the lowest P value being 2.263×10^{-7}. Eight bone-related genes, represented by nucleotide binding oligomerization domain containing 1, were annotated and enriched in the guanine nucleotide exchange factor activity pathway, which may affect bone strength by regulating bone homeostasis through FERM, ARH/RhoGEF and pleckstrin domain proteins. The research lays the foundation for further improving broiler product quality and animal welfare.

Introduction

In recent years, with the adjustment of the broiler industry structure, the total output and proportion of broilers have continued to increase. According to data from the United States Department of Agriculture, global broiler production in 2023 was 102,389,000 tons, an increase of 549,000 tons from 2022, of which white-feathered broilers accounted for about 70 %(USDA, 2025). The demand for high-yield broilers has driven the advancement of breeding technology, resulting in an increase in the weight of white-feathered broilers at market price. The greater weight during growth has put a test on their bone support capacity. Cornish White chickens weighing about 2.5 kg are mostly used for meat production. The strength and toughness of bones affect the bone fracture rate, thereby affecting economic benefits. In previous studies, the abnormal rate of leg bone of white feather broilers was up to 13 % (Zheng et al., 2023). The factors that affect the bone strength of broilers are relatively complex, including nutritional factors, genetic factors and pathological factors. Regarding broiler bone traits, the effects of 25-hydroxyvitamin D3 on bone weight, length and composition have been explored, and an improving effect was found (He, et al., 2023). In

---

* Corresponding author at: China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District, Beijing, China.
E-mail address: jxzheng@cau.edu.cn (J. Zheng).

https://doi.org/10.1016/j.psj.2025.105324
Received 11 March 2025; Accepted 19 May 2025

addition, leg diseases, such as viral arthritis and tibial achondroplasia, can also cause lameness and affect bone strength (Liu, et al., 2023). However, there is less discussion about the genetic factors that determine wing skeletal traits.

The world's first genome-wide association analysis (**GWAS**) was the identification of human myocardial infarction susceptibility genes in 2007 (Ikegawa, 2012), and now GWAS has become a powerful tool for mining effective SNP sites. In broilers, a study using GWAS found that the SRY-Box Transcription Factor 6 gene affected breast muscle production and breast muscle disease (Tan, et al., 2024). Tang et al. revealed the genetic mechanism of broiler leg disease through GWAS technology, and screened out candidate genes such as Sirtuin 1 (Tang, et al., 2024). Studies showed that the heritability of broilers' leg disease was about 0.16 (Zheng, et al., 2023), which was a medium-to-low heritability trait. Therefore, it is of certain significance to study the genetic selection and breeding of broiler bones. However, systematic studies on the genetic aspects of wing bone strength are lacking.

The necessity of studying broiler wing bones is to reveal the growth, development and genetic rules of broiler bones in terms of bone weight, size and mechanical properties, etc., and then carry out targeted breeding. The significance of GWAS analysis of wing bone strength lies in analyzing the relationship between wing bone development and SNP and gene function, providing a theoretical basis for improving the production efficiency of Cornish White chicken. In this study, chip sequencing was used to obtain the genome data of 42-day-old Cornish White chickens after data quality control. The weight, size, strength and toughness of ulna, radius, humerus and tibia, as well as growth traits and breast muscle traits were measured. SNP related to bone traits were discovered through GWAS, and a series of genes were obtained. This will lay the foundation for improving broiler bone strength, reducing the chance of wing breakage, increasing economic benefit and improving animal welfare.

Materials and methods

Ethics statement

All experimental protocols associated with the broilers used in this study were conducted with the guidelines established by the Ministry of Science and Technology (Beijing, China). Ethical approval was granted by the Animal Welfare Committee of China Agricultural University (AW71802202-1-2) and performed in accordance with the procedures outlined in the "Guide for Care and Use of Laboratory Animals" (China Agricultural University).

Market survey on chicken wings, legs and breasts in East Asia

For chicken wings, chicken drumsticks and chicken breast (all refrigerated), three online stores in China (Dingdong Maicai (https://www.100.me/home/index), Jingdong (https://www.jd.com/) and Meituan (https://bi-mall.meituan.com/#/)), and one online store in Japan and South Korea (Amazon (https://www.amazon.co.jp/ref=nav_logo) and Coupang (https://www.coupang.com/)) were selected. Three items from each part of each store were selected, and the mean value and the standard deviation of their prices were calculated.

Birds and phenotypic determination

Growth Traits. In this study, 436 Cornish White pure line roosters from Beijing Huadu Yukou Poultry Industry Co., LTD. were used. Their weight was measured at 3 weeks and 6 weeks of age, respectively, and the feed consumption from 3 weeks to 6 weeks of age was measured. The feed conversion ratio (**FCR**) from 3 weeks to 6 weeks of age was calculated. Residual feed intake (**RFI**) was calculated according to the formula in Prakash's study (Prakash, et al., 2021).

Bone Traits and Breast Muscle Traits. Blood from the wing vein was collected at 42 days of age and stored at −80°C for sequencing analysis, and then the broilers were slaughtered. The ulna, radius, and humerus of the left wing were observed, and any fractures or deformities was recorded. The whole wing and calf of the broiler were removed with scissors, the muscle and fascia attached to the ulna, radius, humerus and tibia were stripped away, the wing tips and chicken feet were discarded, and 4 bones were left for subsequent analysis in each chicken. The length of bones was measured with a vernier caliper, accurate to 0.01 cm; the width of the bone was measured with a spiral micrometer, accurate to 0.001 cm; the weight of the bone was measured with a balance with an accuracy of 0.1 g. The strength (g) and toughness (g·sec) of all bones were determined by three-point measurement using the fracture test function of the TA-XT plus texture analyzer from Stable Micro Systems, UK., see Fig. 1.

The chicken breast, including pectoralis major and pectoralis minor muscles, was separated with a scalpel and surgical scissors, and its weight was measured with a balance. Breast muscle rate was calculated according to the formula in Tan et al. (Tan, et al., 2022).

DNA extraction, genotyping and quality control

The blood samples were transferred to Oyi Biomedical Technology Co., Ltd. for DNA extraction and genotyping. Genotyping was performed using the self-developed chip. A chip with high efficiency and low cost was designed and developed for Cornish chicken. The SNP density was 50 K. After evaluation, the SNP loci in the chip were evenly distributed on the chromosome. The proportion of loci with a minimum allele frequency (**MAF**) higher than 0.1 was 96.47 %. The homogeneity reached 98.96 %, the detection rate reached 99.41 %, and the consistency rate of repeated samples reached more than 99.91 %. It has been verified that the indicators of the chip have reached a better level, which can meet the detection needs of large groups. The chip sequencing data can be obtained in the genomic variation map of National Genomics Data Center, with the accession number is GVM001034.

The obtained sequencing files were cleaned using PLINK 1.9. Firstly, SNP with a deletion rate of more than 2 % were deleted. Then, SNP with sub allele frequencies below 2 % were deleted. Finally, the Hardy-Weinberg equilibrium results of all sites were calculated, and the sites that did not conform to the Hardy-Weinberg equilibrium were deleted. After the above three operations, the number of SNP decreased from 42,052 to 39,000, and the number of individuals decreased to 436.

GWAS

GWAS was performed using PLINK 1.9 software, using a linear mixed model. Prior to this, the same software was used to build the kinship matrix. The following mathematical models were used in the analysis:

$$y = W\alpha + x\beta + u + \varepsilon$$

In this expression, y represents the phenotype value, W refers to the fixed effects matrix, α represents the vector of correlation coefficients constituting the intercept, x represents the genotype, β represents the effect of the genotype, u is the vector of random effects, and ε is the vector of random residuals.

Bonferroni multiple comparison correction was performed on the GWAS results, and the corrected threshold was calculated according to the following formula:

$$\alpha_{corrected} = \frac{\alpha}{N}$$

α is the original significance level (0.05), N is the number of SNP (39,000 in this study), and the corrected threshold is calculated to be 1.28×10^{-6}. This threshold is greater than the commonly used 5×10^{-8}, mainly because the chip has difficulty detecting some rare mutations. According to the research of Uffelmann et al., the genomic significance threshold has a certain degree of flexibility (Uffelmann, et al., 2021). Therefore,

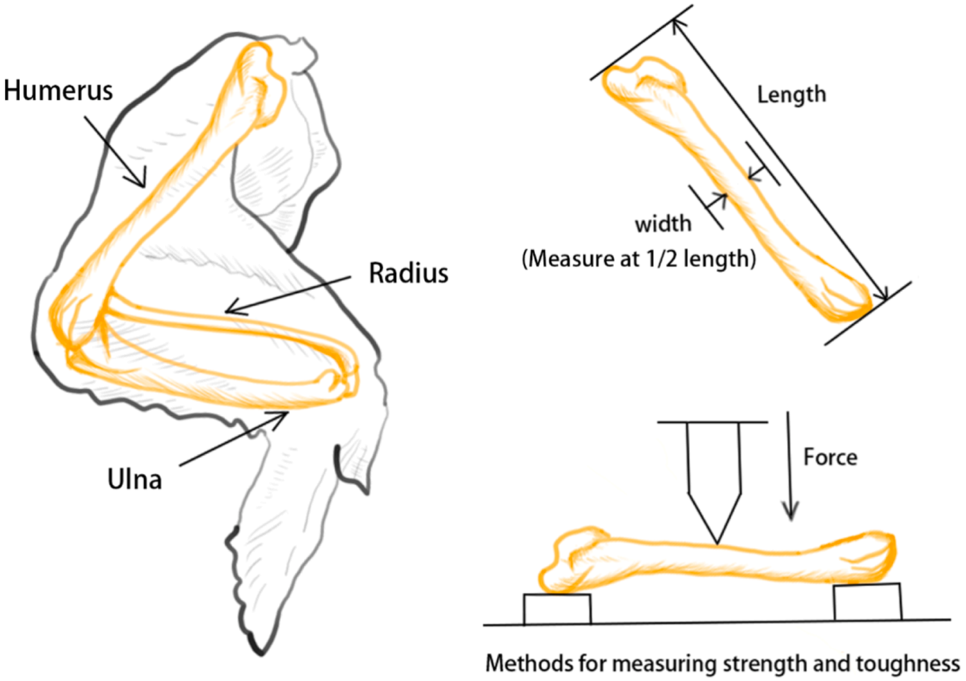

Fig. 1. Wing bone sample collection and measurement of bone traits.

the threshold obtained based on the Bonferroni correction method is reasonable. SNP with a P-value less than this threshold were considered as significant association loci.

GWAS results visualization, including Manhattan figure and QQ figure drawing, was performed by "CMplot" package (https://cran.rproject.org/web/packages/CMplot/index.html) in R (4.4.0).

Analysis of gene annotation, phenotypic variation explained (PVE) and heritability

To select genes that may be associated with bone traits, we used the variant effect predictor from Ensembl (https://www.ensembl.org/Tools/VEP) to annotate the SNP. The PVE of SNP was calculated using Tassel 5. Finally, Kyoto encyclopedia of genes and genomes (**KEGG**) and Gene ontology (**GO**) analysis were performed on the annotated genes to obtain the possible related biological pathways. The Yang method in GCTA 1.94.1 software was used to construct the genetic relationship matrix, and then the heritability of all traits was calculated.

Statistical analyses

The mean value, standard deviation and coefficient of variation of traits were calculated using SPSS 21.0.0. "ASReml" package (https://cran.r-project.org/web/packages/biometryassist/vignettes/installing-asreml-r.html#inst Alling-asml -r) in R (4.4.0) was used to calculate phenotypic correlation and genetic correlation.

Results

The price of broiler parts in East Asia

As Table 1 shows, in China, Japan and South Korea, the price of refrigerated chicken wings is higher than that of drumsticks and breasts. In China, for example, the average selling prices of chicken wings, drumsticks and breasts on the three online fresh platforms were $5.7, $3.1 and $2.7 per kg, respectively, and chicken wings contributed a significant portion of the value of chicken carcasses.

Table 1
Prices of broiler chicken parts in East Asia (US Dollar/kg).

Country	Online Store	Broiler Products		
		Wing	Leg	Breast
China	DingDong Maicai	6.4 ± 2.0	2.5 ± 0.1	3.4 ± 0.8
	Jingdong	4.9 ± 0.4	3.3 ± 0.7	2.2 ± 0.3
	Meituan	5.9 ± 1.1	3.5 ± 1.3	2.4 ± 0.7
	Average	5.7 ± 1.0	3.1 ± 1.0	2.7 ± 0.9
Japan	Amazon	8.6 ± 1.3	5.9 ± 0.4	5.3 ± 0.2
South Korea	Coupang	9.1 ± 0.6	7.3 ± 1.3	5.9 ± 2.0

Basic statistics of traits

Among the 436 individuals, the number of ulna, radius and humerus abnormalities, including deformity and fracture, was 46, 23 and 36, accounting for 10.6 %, 5.3 % and 8.3 % of the total, respectively. Since the traits of wing bones have not been selected, as can be seen in Fig. 2, the coefficient of variation for some traits is large, such as weight, strength and toughness of ulna, weight and toughness of radius, weight, strength and toughness of humerus and toughness of tibia, which is greater than 20 %. It showed that the dispersion degree of traits was high and the individual differences were significant. As can be seen from Fig. 3, the coefficient of variation for growth traits and breast muscle traits is small, because these traits have been mainly selected.

Genetic parameters

The heat maps of genetic and phenotypic correlations of traits are shown in Fig. 4, with the upper right triangle representing phenotypic correlations and the lower left triangle representing genetic correlations. It can be seen that there was a significant positive correlation between wing bone traits and tibial traits, for example, the phenotypic correlation between radius strength and tibial toughness was 0.8. There was also a significant correlation between wing bone traits and growth traits, and the phenotypic correlation between humerus width and weight at 6 weeks of age was 0.49. It is worth noting that the phenotypic and genetic correlations between breast muscle traits and other traits were weak.

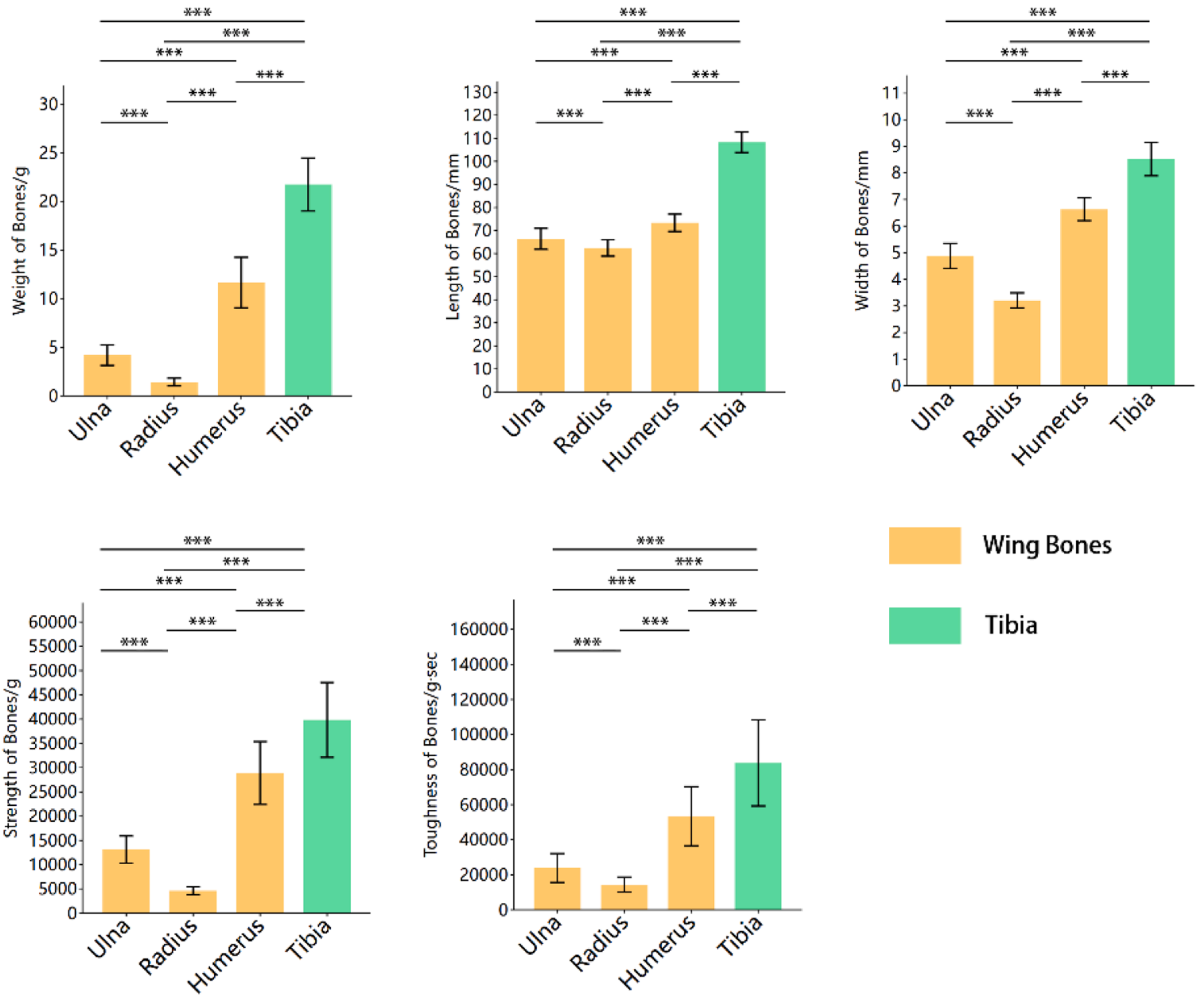

Fig. 2. Bar charts comparing the wing bone traits and tibial traits. ***: ($P < 0.001$).

Heritability is shown in Fig. 4. In addition to weight of ulna, weight of radius, length of radius, width of ulna, width of radius and thickness of the tibial cortex, the other traits had moderate heritability, among which feed consumption of 3 to 6 weeks intake, residual feed intake, breast yield and breast muscle rate had high heritability, reaching more than 0.4, indicating that these traits were significantly influenced by genetic factors.

GWAS results

The Manhattan plot and QQ plot containing the 27 SNP loci initially screened are shown in Fig. 5. The Manhattan plot shows that among the 17 bone traits, significant SNP loci were initially screened out in 8 traits.

The basic data of the selected significant SNP are shown in Table 2. These SNP were located on chromosomes 1, 2, 3, 4, 5, 10 and 11. Among the 27 loci initially screened out, the P values of rs144986584, rs144809012, rs40454963, and rs40505483 were less than the corrected threshold. The most significant SNP was rs144986584, which affected the width of the humerus, located on chromosome 1 with a P-value of 2.263×10^{-7}. Given that the Bonferroni multiple comparison correction is relatively conservative, the research annotates all loci with $P < 10^{-5}$. The PVE of SNP ranged from 0.0257 % to 4.526 %. Among them, rs41125821, rs15765520, rs5371226, rs5261546, rs5320321, rs139837227, rs40454963, rs40505483, rs41146061, rs34151319, rs34235318, rs34302790, rs34083141, rs34292113, rs34138954, rs34182331, rs34190543, rs34264353, rs34093507, rs4438116 had a large PVE (>1 %), suggesting that these SNP may be important loci in regulating bone strength traits. Eight bone-related candidate genes were annotated from 27 significant loci. KEGG and GO analysis of these genes showed no significant pathway. However, in GO analysis, FERM, ARH/RhoGEF and pleckstrin domain protein 1 (***FARP1***) and dedicator of cytokinesis 9 (***DOCK9***) genes enriched the guanyl-nucleotide exchange factor activity at a potentially significant level ($P = 0.11$), which was related to osteoclast polarity and membrane recycling process, and may affect bone traits and bone development.

Discussion

Importance of studying chicken wing bones

Cornish White chickens are commonly used as broiler sires and are widely used for breast and leg meat production. In broiler production, the significance of bone traits selection is to increase muscle load while maintaining the integrity of breast and leg (de Almeida Mallmann, et al., 2019). In some countries, wing integrity is also important, and the wing bones determine part of the economic performance of broiler chickens

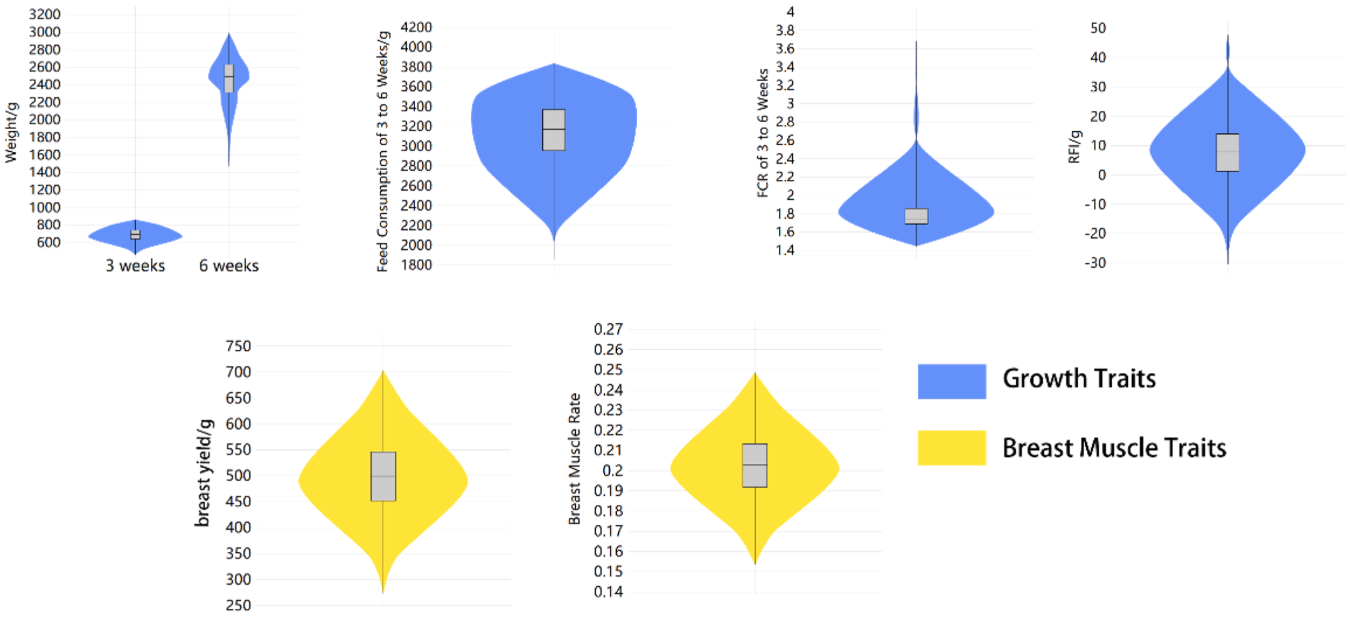

Fig. 3. Violin plots of growth traits and breast muscle traits.

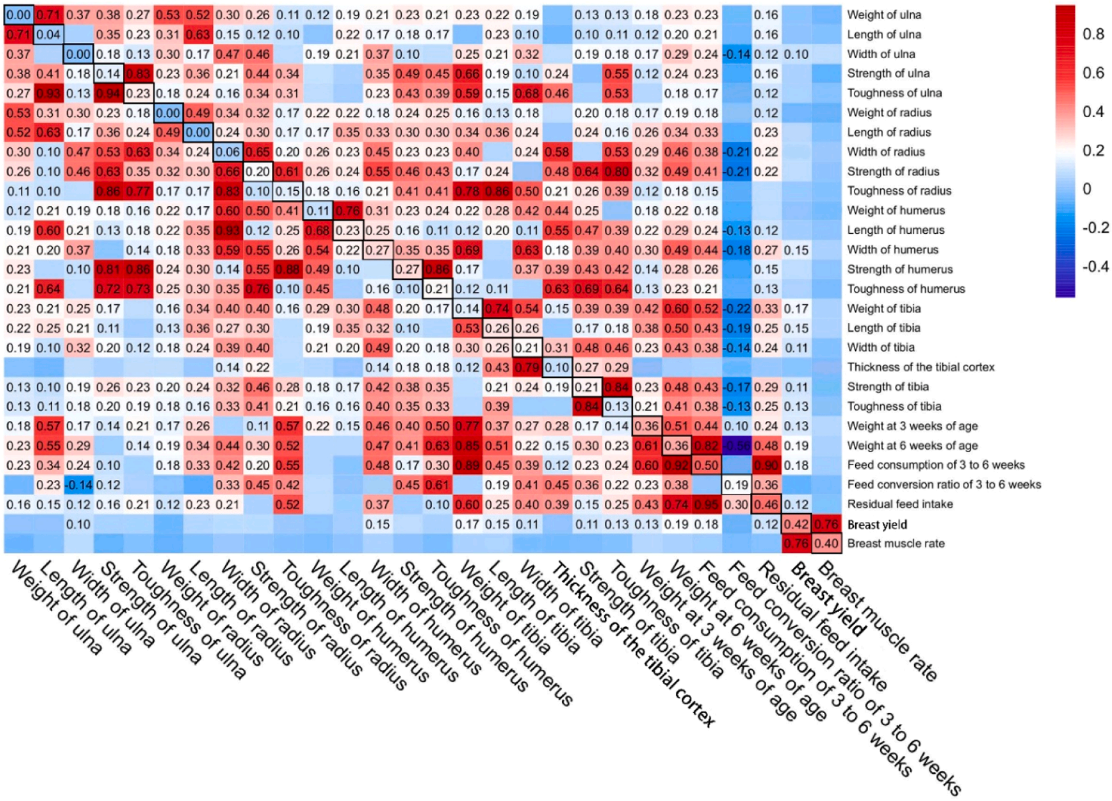

Fig. 4. Heat map of correlation coefficients of different traits. The square showing the value indicates that the significance test of the correlation coefficient passes ($P < 0.05$). The upper right triangle represents phenotypic correlation. The lower left triangle represents genetic correlation. The value on the diagonal is heritability.

(Wu, et al., 2022). Taking DingDong Maicai, Jingdong and Meituan, three Chinese online stores, as an example, the average price per kilogram of refrigerated chicken drumsticks and chicken breasts was \$3.1 and \$2.7, respectively, while the average price per kilogram of chicken wings was \$5.7, which was several times the price of chicken drumsticks and chicken breasts. On Amazon in Japan, the average prices for chicken wings, drumsticks and breasts were \$8.6, \$5.9 and \$5.3 per kilogram, respectively, while on Coupang in South Korea, they were \$9.1, \$7.3 and \$5.9 per kilogram, respectively. It can be seen that chicken wings were more valued in East Asian countries. In 2024, the market size of chicken wing products in China will reach 100 billion US dollars, which can be converted to a weight of 2 to 3 million tons (Book118, 2025). The considerable scale makes research on wing bones even more necessary.

The bone formation of broilers is a complex process involving

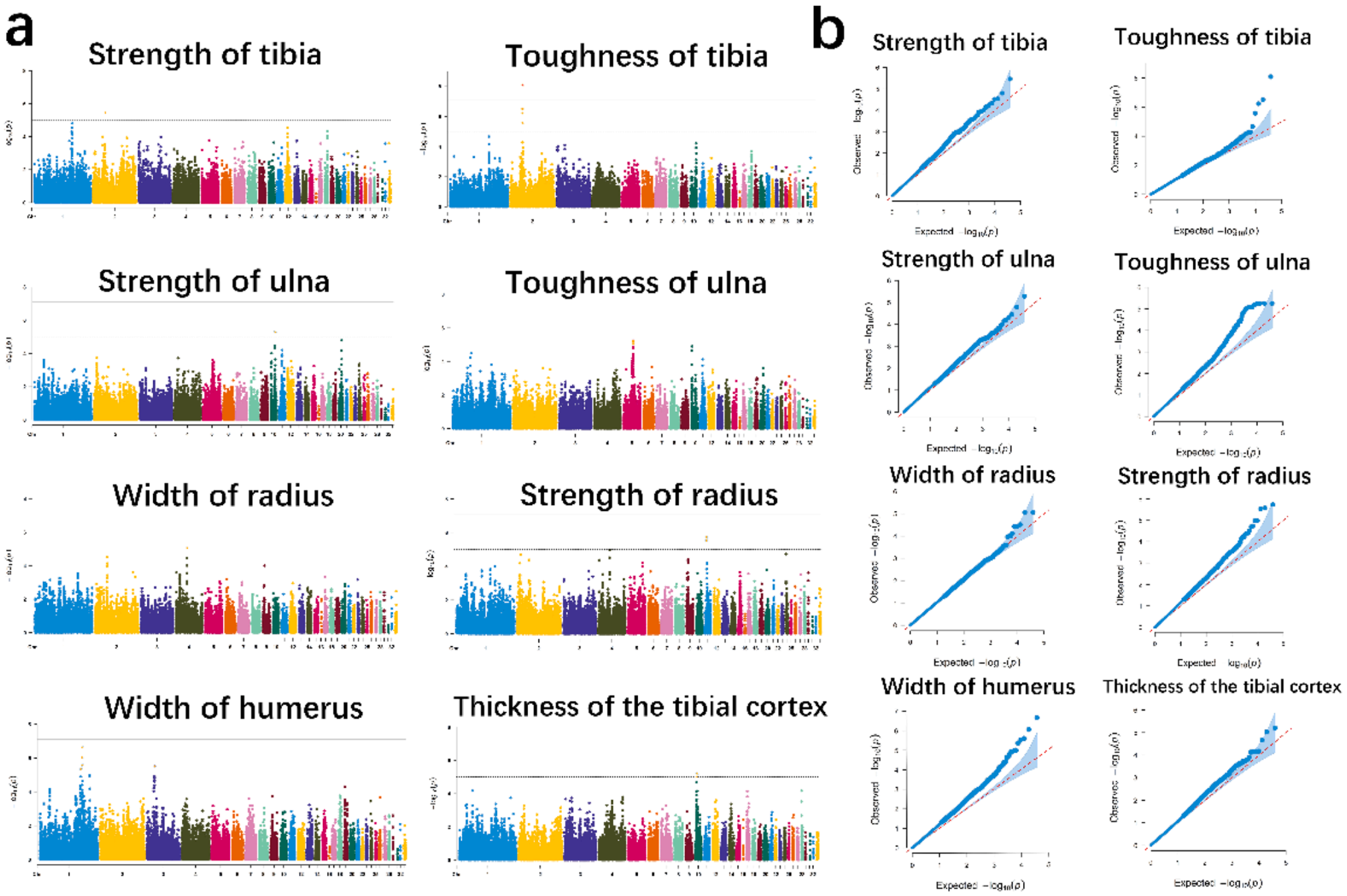

Fig. 5. Manhattan plot and QQ plot of 8 bone traits. a. Manhattan plot of 8 bone traits, with orange dots representing $P < 10^{-5}$ and red dots representing $P < 10^{-7}$. b. QQ plot of 8 bone traits.

multiple systems working together. Osteoblasts are the main cells responsible for bone formation. These cells secrete extracellular matrix proteins, and multiple osteoblasts interact to form a bone unit. Calcium is deposited in the form of hydroxyapatite along with type I collagen to provide structural support for bones (Salhotra, et al., 2020). The main components of bone are collagen fibers and hydroxyapatite. Most of the bones in broilers are developed from pillars of cartilage cells, the destruction of which reduces bone strength and increases the risk of fracture, increases body weight or bone load, and leads to mechanical injury and lameness (Prisby, et al., 2014). Therefore, the bone formation mechanism of broilers determines their weaknesses, and it is urgent to study the genetic mechanism of broiler bones.

Genetic factors affecting bone quality

Some studies in the past have explored the influence of genetic factors on bone traits. The research by Cao et al. found that the expression of genes such as Dentin matrix acidic phosphoprotein 1 (***DMP1***) has a moderate correlation with the traits of broiler tibia. The correlation coefficient can reach up to 0.689 (mRNA expression of *DMP1* and tibial mineral content) (Cao, et al., 2021). A GWAS study identified 21 candidate genes that might regulate the growth and development of chicken bones. Among them, the Slit guidance ligand 2 gene plays a role in regulating the differentiation of osteoblasts in vitro and can inhibit osteoclast generation and bone resorption (Mebratie, et al., 2019). For the traits involved in this study, epigenetic factors are also worthy of being included in the discussion. The research by Zhou et al. found that the short tandem repeats and methylation in the promoter region affect the expression of the cystathionine β -synthase gene in laying hens, and this enzyme affects the bone strength of laying hens (Zhou, et al., 2019).

Characteristics of Cornish chicken wing bone

The quality of ulna, humerus and radius determines the development of wings, and the loss of wings will affect the carcass value and welfare of broilers. In this study, the abnormality rate of wing bones ranged from 5.3 % to 10.6 %. Combined with the study of Zheng et al., the abnormality rate of broiler tibia was relatively high (13 %) (Zheng, et al., 2023). In Liu's study, tibia diameter decreased significantly in spent laying hens, which was about 10 % lower than the peak (Liu, 2016). Ma et al. compared the tibia weight of WOD168, WOD178, WOD188 and AA broilers. As the proportion of blood source of laying hens increased, the tibia weight decreased, but there was no significant difference in the ratio of tibia weight to body weight, which meant that the relative bone load of medium-sized broilers was not too large (Ma, et al., 2021). In the study of Khan et al., the average tibial fracture strength of Ross 308 large-sized white feathered broilers was 30,198 g, which was lower than that in this study (39,841 g). The mean tibia length and weight of Ross 308 were 110.69 mm and 23.28 g, respectively, compared to 108.30 mm and 21.73 g for Cornish White chickens in this study. This indicated that the bone strength of Cornish White chicken had certain advantages (Khan, et al., 2023). By comparing the descriptive statistics and correlation results with spent laying hens or other broilers, some characteristics of Cornish White chicken can be revealed. Although the bones of Cornish White chickens are small in size and weight, they are strong and less prone to fracture.

The coefficient of variation of some bone traits was high, which may be due to the fact that the traits were controlled by multiple genes, or there was significant allelic heterogeneity, and the genetic variation was abundant in the population. It may also be that the trait is more susceptible to environmental or epigenetic factors, which is consistent with

Table 2
Basic information of significant SNP.

Trait	SNP	Variant type	Chromosome	Physical location (bp)	MAF	β value	P value	PVE (%)	Gene
Strength of tibia	rs41125821	intergenic variant	2	41125821	0.449	2531	3.452×10^{-6}	1.424	
Strength of ulna	rs15765520	Intron variant	10	15765520	0.43	891.4	5.159×10^{-6}	2.496	*NR2F2***
Strength of radius	rs5371226	Intron variant/non coding transcript variant	11	5371226	0.112	438.6	1.837×10^{-6}	2.695	
	rs5261546	intergenic variant	11	5261546	0.119	414.6	2.667×10^{-6}	2.763	
	rs5320321	Intron variant/non coding transcript variant	11	5320321	0.122	410.2	2.999×10^{-6}	2.78	
Width of radius	rs38492128	Intron variant/non coding transcript variant	4	38492128	0.192	0.1129	8.484×10^{-6}	0.0257	
	rs38531741	Regulatory variant	4	38531741	0.192	0.1129	8.484×10^{-6}	0.0257	
Width of humerus	rs144986584**	Regulatory variant	1	144986584	0.384	0.1598	2.263×10^{-7}	0.0118	*FARP1***
	rs144809012**	Downstream gene variant	1	144809012	0.405	0.1517	9.153×10^{-7}	0.16	*DOCK9***
	rs144914683	Downstream gene variant	1	144914683	0.452	0.1392	2.605×10^{-6}	0.409	*STK24*** *FARP1***
	rs25375656	Intron variant	3	25375656	0.252	0.1599	3.099×10^{-6}	0.577	*CAMKMT***
	rs139837227	Upstream gene variant	1	139837227	0.471	0.1419	4.442×10^{-6}	1.332	
Toughness of tibia	rs40454963**	Intron variant	2	40454963	0.49	−8796	3.092×10^{-7}	3.715	*TRIM71*
	rs40505483**	intergenic variant	2	40505483	0.498	8471	5.836×10^{-7}	3.755	
	rs41146061	Downstream gene variant	2	41146061	0.344	8220	2.648×10^{-6}	3.003	*GGCT NOD1***
Toughness of ulna	rs34151319	Intron variant	5	34151319	0.2	3298	5.476×10^{-6}	4.523	*ARHGAP5*
	rs34235318	Intron variant/non coding transcript variant	5	34235318	0.2	3298	5.476×10^{-6}	4.523	
	rs34302790	Intron variant	5	34302790	0.2	3298	5.476×10^{-6}	4.523	*AKAP6***
	rs34083141	Downstream gene variant	5	34083141	0.196	3298	5.698×10^{-6}	4.454	*NUBPL*
	rs34292113	Intron variant	5	34292113	0.201	3242	6.596×10^{-6}	4.526	*AKAP6***
	rs34138954	Intron variant	5	34138954	0.197	3239	8.389×10^{-6}	4.305	*ARHGAP5*
	rs34182331	Intron variant	5	34182331	0.197	3239	8.389×10^{-6}	4.305	*ARHGAP5*
	rs34190543	Intron variant	5	34190543	0.197	3239	8.389×10^{-6}	4.305	*ARHGAP5*
	rs34264353	intergenic variant	5	34264353	0.194	3239	8.714×10^{-6}	4.237	
	rs34093507	Downstream gene variant	5	34093507	0.213	3114	9.56×10^{-6}	4.461	
Thickness of the tibial cortex	rs6668301	Intron variant	10	6668301	0.293	0.114	6.23×10^{-6}	0.378	*MYO1E***
	rs4438116	Intron variant	10	4438116	0.499	0.101	9.224×10^{-6}	1.088	*C2CD4A*

[*] Genes that have been shown to be associated with bone traits.

[**] The loci reached the genomic significance level.

the low heritability of this part of traits. The traits with a large coefficient of variation often have a large space for genetic improvement. In future GWAS studies on these traits, the sample size should be increased to improve the signal-to-noise ratio, so as to prevent genetic effects from being covered up by environmental effects (Berry, 2018; Wray, et al., 2019).

Genetic parameters

Compared with this study, in Zheng's study, the heritability of leg bone disease was calculated to be 0.16, which was low to medium heritability (Zheng, et al., 2023). In present study, the genetic parameters of ulna, radius and humerus traits were calculated. Unfortunately, the heritability of these traits was low, indicating that the traits were greatly influenced by acquired factors and the environment, and could be improved through reasonable feeding and management. In the heritability results, bone strength and bone toughness traits were mostly low and medium heritability traits, with heritability ranging from 0.1 to 0.3, indicating that there was still room for breeding of bone strength traits. The heritability of bone weight, length and width ranged from 0 to 0.2, and the variation range is large, indicating that the selection effect for these traits may be poor. The heritability of weight and width of ulna and weight and length of radius was 0, indicating that these traits were almost entirely determined by the environment. Considering the positive genetic correlations between bone strength and toughness and bone weight, length and width, future breeding can be based on bone strength and toughness. During the process of selection, to adapt to the lower heritability, the selection method can be optimized. Marker-assisted selection or genomic selection can be utilized to enhance the accuracy of breeding. At the same time, the population size can be expanded to improve the accuracy of phenotypic collection, and the long-term breeding strategy can be optimized by combining the phenotypic and genomic information of multiple generations (Speed, et al., 2022). Most of the traits with high heritability were growth traits and breast muscle traits, which was basically consistent with the research of Cao and He, in which the heritability of breast yield was almost the same (0.423 and 0.49) (Cao, 2024; He, 2024).

In this study, the phenotypic and genetic correlation coefficients between the bone traits of the wings and legs were calculated. Bone strength has been shown to correlate with cortical bone thickness, which is an important indicator of bone development and quality (Santos, et al., 2022). In this study, the bone weight, length, width, strength and toughness of different parts showed significant positive phenotypic and genetic correlations, indicating that the growth of wings and legs was closely linked during the development of Cornish White chicken. As bone weight, length and width increased, bone strength and toughness increased, i.e., tibia > humerus > ulna > radius, which was consistent with the laws of mechanics and the results of Li 's study, indicating that the results had a certain reliability (Li, 2017). Body weight, feed intake and residual feed intake were positively correlated with bone traits. The phenotypic correlation coefficient between body weight and tibial weight at 6 weeks was up to 0.6. The lower the feed conversion rate, the lower the feed consumption, the greater the weight gain, the larger the body size and the greater the bone strength. In de Almeida Mallmann's study, broilers attacked by pectoral muscle disease had lower bone mineralization intensity (de Almeida Mallmann, et al., 2019). This reflects the consistency of bone development and meat production.

Chicken is one of the most important sources of animal protein in human nutrition. In the study of Ma et al., the breast muscle rate of broilers of different breeds could reach 19.92 % to 31.21 %(Ma, et al., 2022). The size and growth rate of breast muscle have been increasing due to intensive genetic selection, but at the same time, breast muscle abnormalities affecting nearly 90 % of broilers have appeared (Huang and Ahn, 2018). It is worth noting that the correlations between breast muscle traits and bone traits are very weak in present study. It may indicate that the development of wings and legs and the development speed of breast muscle are almost independent of each other, which further indicates the possibility of breeding in the future. The study by Bailey et al. found a low phenotypic correlation between breast yield and body weight (Bailey, et al., 2020), and in the study of Hu et al., there were no significant genetic correlations between breast muscle rate of broilers and pre-mortem body weight, eviscerated weight and half-eviscerated weight (Hu, et al., 2024), which was consistent with this study. Erdal et al. 's study found that for both 1500 g and 2000 g chickens, the level of total dietary energy did not affect the bone strength ($P > 0.05$) (Erdal, et al., 2012). Waldenstedt argued that reducing growth rates by reducing feed intake was not easily compatible with welfare needs, and that rapid growth and high body weight did not necessarily lead to leg disorders (Waldenstedt, 2006).

GWAS

The P values of rs144986584, rs144809012, rs40454963, and rs40505483 were less than the corrected threshold. Therefore, these results are considered relatively reliable. The P values of the remaining loci were all greater than the corrected significance threshold, suggesting that genomic significance was not achieved and there was a risk of false positivity. False positives may lead to incorrect associations between traits and loci (Marigorta, et al., 2018). Given that Bonferroni is more likely to filter out true associations, the study discussed all loci with $P < 10^{-5}$.

The GWAS result annotation produced 13 genes in total. Among them, eight genes that have been reported to be related to bone health are *FARP1, DOCK9* and serine/threonine kinase 24 (***STK24***), nucleotide binding oligomerization domain containing 1 (***NOD1***), calmodulin-lysine N-methyltransferase (***CAMKMT***), A-Kinase anchoring protein 6 (***AKAP6***), myosin 1E (***MYO1E***) and nuclear receptor subfamily 2 group F member 2 (***NR2F2***), respectively located on chromosomes 1, 1, 1, 2, 3, 5, 10 and 10. In previous studies, mutations in some genes may affect bone traits. For example, in the study of Soyocak et al., rs5743336 SNP mutation of *NOD1* gene increased the incidence of human osteoporosis (Soyocak, et al., 2020).

The new traits of the wing bone have annotated two genes, *NR2F2* and *AKAP6*. The *NR2F2* gene was first discovered as a new member of the steroid receptor superfamily in 1991 (Ladias and Karathanasis, 1991). In the study by Gao et al., through lentivirus transfection experiments, it was found that it might be involved in the mechanism of cartilage differentiation of mesenchymal stem cells under hypoxic culture (Gao, et al., 2017). The formation of cartilage in mesenchymal stem cells with high expression of the *NR2F2* gene was significantly enhanced. Proteins encoded by A kinase-anchored protein are highly expressed in various regions of the brain as well as in the heart and skeletal muscles. *AKAP6* in its family is the main gene regulated by activity-dependent neuroprotective proteins and is also involved in cognition and bone maintenance (Gozes, et al., 2017). It can be seen that the effects of NR2F2 and AKAP6 on bones are highly likely to contribute to the strength and toughness of the wing bones. The annotation results provide a genetic basis for improving the quality of the wing bones.

Four genes were annotated on the traditional traits of the wing bone. Among them, the *FARP1* and *DOCK9* genes have downstream gene variations, which may include variations located in enhancers that affect the expression intensity of the genes. For the pair of genes *FARP1* and *STK24*, chimerism of *FARP1-STK24* was detected in osteosarcoma, indicating that this pair of genes is expected to become a target for bone traits (Ali, et al., 2019). Sabik et al. determined that DOCK9 is one of the core modules of bone mineral density in mice by integrating co-expression networks and GWAS data (Sabik, et al., 2020). This gene has a high correlation with in vitro mineralization ($R = 0.71$). Another study has shown that interferon regulatory factor 4 inhibits osteogenic differentiation of bone marrow mesenchymal stem cells by transcriptional activation of the miR-636/DOCK9 axis (Zhang, et al., 2022). Several other studies have shown that DOCK9 regulates osteoclast differentiation and ossification (Ralston and de Crombrugghe, 2006; Ralston and Uitterlinden, 2010). The *CAMKMT* gene encodes Class I protein methyltransferase and may play a role in calcium-dependent signal transduction. Its RNA expression level affects the zygomatic arch length of mice (Percival, et al., 2018). It can be seen from this that the research on the functions of these genes is conducive to clarifying the mechanism of bone growth and development.

The tibial traits annotated two genes, *NOD1* and *MYO1E*. A large number of studies have revealed the important role of the *NOD1* gene in bone health. NOD1 is highly expressed in osteocytes. The NOD1-RANKL axis in osteocytes may be the key mechanism for inducing osteoclast generation and bone resorption (Kittaka, et al., 2023). The bone resorption activity of osteoclasts in individuals with activated NOD1 increased, which was reflected in the increased expression of lysosomal protease cathepsin K (Plantinga, et al., 2013). However, some other studies have shown that the absence of NOD1 significantly aggravates bone resorption induced by Gram-negative bacteria, accompanied by an increase in the number of osteoclasts (Chaves de Souza, et al., 2016). In conclusion, the role of NOD1 in regulating bone strength and health remains to be further studied. Nakamura et al. conducted siRNA -mediated *MYO1E* knockdown in macrophages. The results showed that cells with a smaller number of nuclei increased by approximately 1.8 times after knockdown, indicating that MYO1E is essential for osteoclast differentiation (Nakamura, et al., 2020). These results support the possible influence of NOD1 and MYO1E on tibial traits.

The functional studies of the above-mentioned genes mainly applied methods such as gene knockdown and gene overexpression. However, in this study, through GWAS research, these candidate genes were screened and annotated, providing evidence for previous functional verification experiments. The research also innovatively discovered genes such as tripartite motif containing 71 that had never been reported to be related to bone traits before, providing a theoretical basis for future functional verification of them. The above-mentioned studies mostly focused on mammals such as mice, while this GWAS study used flocks of white Cornish chickens. The unique physiological characteristics of birds may lead to the inapplicability of the results of the above-mentioned functional studies (Sabater González, 2019). It is necessary to conduct in-depth research to further explore the effects of each candidate gene on the bone strength and health of broilers, thereby enhancing the strength of wing bones and leg bones and improving the welfare of poultry (Sauka-Spengler and Barembaum, 2008). The results of this study have certain reference value for broiler chicken farmers and researchers.

In the subsequent study, three groups of high, medium and low bone quality can be selected. The expression levels of the candidate genes screened out in this study can be determined by qPCR, the correlation between them and bone traits can be calculated, and regression analysis can be conducted, so as to predict bone quality based on the expression levels of the candidate genes (Taylor, et al., 2019).

Nevertheless, the limitations of GWAS analysis led to the possible existence of potentially significant and unidentified genetic variations, which could also be targets of wing bone strength and await further research for confirmation (Tam, et al., 2019). Most of the mutations detected in this study were intron mutations. Although they might change the regulatory region, they did not directly alter the protein sequence, and the significance of these mutations was limited (Ohno, et al., 2018).

GO enrichment results

The guanyl-nucleotide exchange factor (**GEF**) in the result of GO enrichment was used to initiate signal transduction by catalyzing the exchange from G protein-bound GDP to GTP (Bos, et al., 2007). FERM, ARH/RhoGEF and pleckstrin domain protein 2 (***FARP2***), which belonged to the same family as *FARP1*, activated Rac family small GTPase 1 or cell division cycle 42 in response to upstream signals in this pathway, thereby regulating processes such as neuronal axonal guidance and bone homeostasis (He, et al., 2013). The regulatory mechanism of GEF on *FARP2* is still unclear, and the study of this pathway has great prospects for improving the bone growth performance of broiler chickens.

The influence of other factors on the strength of the wing bones

Apart from genetic factors, nutrition, diseases and management also affect the bone strength and health of poultry. A study shows that increasing the dietary leucine content of broilers from 10 g/kg to 17.2 g/kg at 21 days of age can increase the tibial fracture strength by 14.7 %, and increasing the valine content from 6 g/kg to 9 g/kg can increase the tibial fracture strength by 23.6 %(Ospina-Rojas, et al., 2018). Taking tibial chondrodysplasia as an example of the disease, its symptoms are manifested as irregular, unmineralized and unvascularized cartilage hyperplasia near the growth plate, eventually leading to poor calcification of the cartilage matrix, apoptosis of chondrocytes and cessation of bone development (Jahejo and Tian, 2021). Rocchi et al. found that the degree of bone mineralization in chickens under heat stress was lower. On the 42nd day, the fracture strength of their tibia decreased to 22.37 kg compared with 39.71 kg in the control group. The total ash content of the tibia decreased to 51.33 % compared with 57.78 % of the control group (Rocchi, et al., 2022). The factors influencing the bone strength and health of poultry are complex, and a composite strategy should be adopted to deal with possible bone fractures or bone diseases.

Conclusion

This study investigated the wing bone traits of Cornish White chickens, measured the weight, size, strength and toughness of the wing bones, the growth traits and breast muscle traits of the chickens, and performed GWAS analysis on these traits. Eight bone-related genes located on 5 different chromosomes were annotated and 1 pathway was enriched, providing a theoretical basis and reference for the genetic selection of skeletal traits of Cornish White chicken.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

Acknowledgments

This work was financially supported by the National Key Research and Development Program of China (2021YFD1200803), the China Agriculture Research Systems (CARS-40), the National Key Research and Development Program of China (2022YFD1300100), and the Natural Science Foundation of Sichuan Province (Grant No. 2024NSFSC0389).

References

Ali, N.M., Niada, S., Brini, A.T., Morris, M.R., Kurusamy, S., Alholle, A., Huen, D., Antonescu, C.R., Tirode, F., Sumathi, V., Latif, F., 2019. Genomic and transcriptomic characterisation of undifferentiated pleomorphic sarcoma of bone. J. Pathol. 247, 166–176.

Bailey, R.A., Souza, E.., Avendano, S., 2020. Characterising the influence of genetics on breast muscle myopathies in broiler chickens. Front. Physiol. 11, 1041.

Berry, D.P., 2018. Symposium review: breeding a better cow-will she be adaptable? J. Dairy Sci. 101, 3665–3685.

Book118. 2025. Research report on the market development prospect, trend and investment strategy of China's wing industry in 2025. Accessed Apr. 2025. https://m.book118.com/html/2025/0117/8024114075007022.shtm.

Bos, J.L., Rehmann, H., Wittinghofer, A., 2007. GEFs and GAPs: critical elements in the control of small G proteins. Cell 129, 865–877.

Cao, S., Li, T., Shao, Y., Zhang, L., Lu, L., Zhang, R., Hou, S., Luo, X., Liao, X., 2021. Regulation of bone phosphorus retention and bone development possibly by related hormones and local bone-derived regulators in broiler chicks. J. Anim. Sci. Biotechnol. 12, 88.

Cao, Z. 2024. Evaluation of key indicators for rapid breeding of carcass appearance in yellow feather broilers. Master.

Chaves de Souza, J.A., Frasnelli, S.C., Curylofo-Zotti, F.A., Ávila-Campos, M.J., Spolidório, L.C., Zamboni, D.S., Graves, D.T., Rossa Jr., C., 2016. NOD1 in the modulation of host-microbe interactions and inflammatory bone resorption in the periodontal disease model. Immunology 149, 374–385.

de Almeida Mallmann, B., Martin, E.M., Soo Kim, K., Calderon-Apodaca, N.L., Baxter, M. F.A., Latorre, J.D., Hernandez-Velasco, X., Paasch-Martinez, L., Owens, C.M., Dridi, S., Bottje, W.G., Greene, E.S., Tellez-Isaias, G., 2019. Evaluation of bone marrow adipose tissue and bone mineralization on broiler chickens affected by wooden breast myopathy. Front. Physiol. 10, 674.

Erdal, R., Richardson, I., Ljøkjel, K., Haug, A., 2012. Sensorial quality and bone strength of female and male broiler chickens are influenced by weight and growth rate. Br. Poult. Sci. 53, 616–622.

Gao, G., Zhang, X., Hubbell, K., Cui, X., 2017. NR2F2 regulates chondrogenesis of human mesenchymal stem cells in bioprinted cartilage. Biotechnol. Bioeng. 114, 208–216.

Gozes, I., Van Dijck, A., Hacohen-Kleiman, G., Grigg, I., Karmon, G., Giladi, E., Eger, M., Gabet, Y., Pasmanik-Chor, M., Cappuyns, E., Elpeleg, O., Kooy, R.F., Bedrosian-Sermone, S., 2017. Premature primary tooth eruption in cognitive/motor-delayed ADNP-mutated children. Transl. Psychiatry 7, e1043.

He, L., Duan, Y., Zhang, N., Qi, W., He, M., Cao, J., Han, J., 2023. Effects of 25-hydroxycholecalciferol on growth performance, bone development and phosphate transporter gene expressions in the small intestine of broiler chickens. Acta Ecol. Anim. Domest. 44, 24–28.

He, X., Kuo, Y., Rosche, T.J., Zhang, X., 2013. Structural basis for autoinhibition of the guanine nucleotide exchange factor FARP2. Structure 21, 355–364.

He, Y., 2024. PhD. Yunnan Agricultural University.

Hu, X., Liu, J., Liu, X., Chen, K., Qu, X., 2024. Slaughtering performance, meat quality and muscle nutrients of Huanglang chicken and their correlation analysis. Chin. J. Anim. Sci. 60, 203–206. +211.

Huang, X., Ahn, D.U., 2018. The incidence of muscle abnormalities in broiler breast meat - A review. Korean J. Food Sci. Anim. Resour. 38, 835–850.

Ikegawa, S., 2012. A short history of the genome-wide association study: where we were and where we are going. Genomics Inform 10, 220–225.

Jahejo, A.R., Tian, W.X., 2021. Cellular, molecular and genetical overview of avian tibial dyschondroplasia. Res. Vet. Sci. 135, 569–579.

Khan, K., Kaya, M., Fidan, E.D., Kilimci, F.S., 2023. Impact of litter on femur and tibial morphology, bone biomechanics, and leg health parameters in broiler chickens. Anim. Biosci. 36, 1393–1402.

Kittaka, M., Yoshimoto, T., Levitan, M.E., Urata, R., Choi, R.B., Teno, Y., Xie, Y., Kitase, Y., Prideaux, M., Dallas, S.L., Robling, A.G., Ueki, Y., 2023. Osteocyte RANKL drives bone resorption in mouse ligature-induced periodontitis. J. Bone Miner. Res. 38, 1521–1540.

Ladias, J.A., Karathanasis, S.K., 1991. Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. Science 251, 561–565.

Li, Q. 2017. The mechanisms of age and letrozole on bone metabolism in laying hens. Master.

Liu, K.L., He, Y..F., Xu, B.W., Lin, L.X., Chen, P., Iqbal, M.K., Mehmood, K., Huang, S.C., 2023. Leg disorders in broiler chickens: a review of current knowledge. Anim. Biotechnol. 34, 5124–5138.

Liu, S., 2016. Calcium and phosphorus and bone metabolism of cage layers and the regulation mechanism. Master.

Ma, M., Mao, H., Wang, K., Qu, L., Dou, T., Guo, J., Wang, X., Hu, Y., 2021. Analysis and comparison on the development of body size traits of different broilers. China Poult 43, 111–115.

Ma, M., Wang, K., Qu, L., Dou, T., Guo, J., Wang, X., Hu, Y., Lu, J., 2022. Determination and analysis of slaughter performance, chest muscle quality and composition of different chicken breeds. Chin. Agric. Sci. Bull. 38, 137–142.

Marigorta, U.M., Rodríguez, J..A., Gibson, G., Navarro, A., 2018. Replicability and prediction: lessons and challenges from GWAS. Trends Genet 34, 504–517.

Mebratie, W., Reyer, H., Wimmers, K., Bovenhuis, H., Jensen, J., 2019. Genome wide association study of body weight and feed efficiency traits in a commercial broiler chicken population, a re-visitation. Sci. Rep. 9, 922.

Nakamura, S., Masuyama, R., Sakai, K., Fukuda, K., Takeda, K., Tanimura, S., 2020. SH3P2 suppresses osteoclast differentiation through restricting membrane localization of myosin 1E. Genes Cells 25, 707–717.

Ohno, K., Takeda, J.I., Masuda, A., 2018. Rules and tools to predict the splicing effects of exonic and intronic mutations. Wiley Interdiscip. Rev. RNA 9, e1451.

Ospina-Rojas, I.C., Murakami, A..E., Duarte, C.R.A., Sakamoto, M.I., Aguihe, P.C., Pozza, P.C., Santos, T.C., 2018. Tibiotarsus bone characteristics and tibial dyschondroplasia incidence of broilers fed diets supplemented with leucine and valine. J. Anim. Physiol. Anim. Nutr. (Berl.) 102, e768–e776.

Percival, C.J., Green, R., Roseman, C.C., Gatti, D.M., Morgan, J.L., Murray, S.A., Donahue, L.R., Mayeux, J.M., Pollard, K.M., Hua, K., Pomp, D., Marcucio, R., Hallgrímsson, B., 2018. Developmental constraint through negative pleiotropy in the zygomatic arch. EvoDevo 9, 3.

Plantinga, T.S., Fransen, J.., Knevel, R., Netea, M.G., Zwerina, J., Helsen, M.M., van der Meer, J.W., van Riel, P.L., Schett, G., van der Helm-van Mil, A.H., van den Berg, W. B., Joosten, L.A., 2013. Role of NOD1 polymorphism in susceptibility and clinical progression of rheumatoid arthritis. Rheumatology (Oxford) 52, 806–814.

Prakash, A., Saxena, V.K., Kumar, R., Tomar, S., Singh, M.K., Singh, G., 2021. Differential gene expression in liver of colored broiler chicken divergently selected for residual feed intake. Trop. Anim. Health Prod. 53, 403.

Prisby, R., Menezes, T., Campbell, J., Benson, T., Samraj, E., Pevzner, I., Wideman Jr., R. F., 2014. Kinetic examination of femoral bone modeling in broilers. Poult. Sci. 93, 1122–1129.

Ralston, S.H., de Crombrugghe, B., 2006. Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev 20, 2492–2506.

Ralston, S.H., Uitterlinden, A.G., 2010. Genetics of osteoporosis. Endocr. Rev. 31, 629–662.

Rocchi, A., Ruff, J., Maynard, C.J., Forga, A.J., Señas-Cuesta, R., Greene, E.S., Latorre, J. D., Vuong, C.N., Graham, B.D., Hernandez-Velasco, X., Tellez Jr., G., Petrone-Garcia, V.M., Laverty, L., Hargis, B.M., Erf, G.F., Owens, C.M., Tellez-Isaias, G., 2022. Experimental cyclic heat stress on intestinal permeability, bone mineralization, leukocyte proportions and meat quality in broiler chickens. Animals (Basel) 12, 1273.

Sabater González, M., 2019. Skeletal bone structure and repair in small mammals, birds, and reptiles. Vet. Clin. North Am. Exot. Anim. Pract. 22, 135–147.

Sabik, O.L., Calabrese, G..M., Taleghani, E., Ackert-Bicknell, C.L., Farber, C.R., 2020. Identification of a core module for bone mineral density through the integration of a Co-expression network and GWAS data. Cell Rep 32, 108145.

Salhotra, A., Shah, H.N., Levi, B., Longaker, M.T., 2020. Mechanisms of bone development and repair. Nat. Rev. Mol. Cell Biol. 21, 696–711.

Santos, M.N., Widowski, T..M., Kiarie, E.G., Guerin, M.T., Edwards, A.M., Torrey, S., 2022. In pursuit of a better broiler: tibial morphology, breaking strength, and ash content in conventional and slower-growing strains of broiler chickens. Poult. Sci. 101, 101755.

Sauka-Spengler, T., Barembaum, M., 2008. Gain- and loss-of-function approaches in the chick embryo. Methods Cell Biol 87, 237–256.

Soyocak, A., Özgen, M., Turgut Coşan, D., Kurt, H., Doğaner, F., Armağan, O., Değirmenci, İ., Şahin Mutlu, F., 2020. Genetic variation in NOD1/CARD4 and NOD2/CARD15 immune sensors and risk of osteoporosis. Biosci. Rep. 40, BSR20192313.

Speed, D., Kaphle, A., Balding, D.J., 2022. SNP-based heritability and selection analyses: improved models and new results. Bioessays 44, e2100170.

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., Meyre, D., 2019. Benefits and limitations of genome-wide association studies. Nat. Rev. Genet. 20, 467–484.

Tan, X., Liu, R., Li, W., Zheng, M., Zhu, D., Liu, D., Feng, F., Li, Q., Liu, L., Wen, J., Zhao, G., 2022. Assessment the effect of genomic selection and detection of selective signature in broilers. Poult. Sci. 101, 101856.

Tan, X., Liu, R., Zhao, D., He, Z., Li, W., Zheng, M., Li, Q., Wang, Q., Liu, D., Feng, F., Zhu, D., Zhao, G., Wen, J., 2024. Large-scale genomic and transcriptomic analyses elucidate the genetic basis of high meat yield in chickens. J. Adv. Res. 55, 1–16.

Tang, X., Zheng, J., Luo, N., Ying, F., Zhu, D., Li, S., Liu, D., An, B., Wen, J., Zhao, G., Li, H., 2024. Genetic mechanism of broiler leg disease based on genome-wide association analysis. Acta Vet. Zootech. Sin. 55, 99–109.

Taylor, S.C., Nadeau, K.., Abbasi, M., Lachance, C., Nguyen, M., Fenrich, J., 2019. The ultimate qPCR experiment: producing publication quality, reproducible data the first time. Trends Biotechnol 37, 761–774.

Uffelmann, E., Huang, Q.Q., Munung, N.S., de Vries, J., Okada, Y., Martin, A.R., Martin, H.C., Lappalainen, T., Posthuma, D., 2021. Genome-wide association studies. Nat. Rev. Methods Prim. 1, 59.

USDA. 2025. Foreign Agricultural Service. Accessed Jan. 2025. https://apps.fas.usda.gov/gats/ExpressQuery1.aspx.

Waldenstedt, L., 2006. Nutritional factors of importance for optimal leg health in broilers: a review. Anim. Feed Sci. Technol. 126, 291–307.

Wray, N.R., Kemper, K..E., Hayes, B.J., Goddard, M.E., Visscher, P.M., 2019. Complex trait prediction from genome data: contrasting EBV in livestock to PRS in humans: genomic prediction. Genetics 211, 1131–1141.

Wu, J., Wang, H., XU, X., 2022. Rapid detection technology for broken-winged broiler carcass based on machine vision. Trans. Chin. Soc. Agric. Eng. 38, 253–261.

Zhang, X., Zhang, Y., Yang, L., Wu, Y., Ma, X., Tong, G., Ban, Z., Zhao, H., 2022. IRF4 suppresses osteogenic differentiation of BM-MSCs by transcriptionally activating miR-636/DOCK9 axis. Clinics (Sao Paulo) 77, 100019.

Zheng, J., Liu, D., Tang, X., He, Z., Li, Q., Liu, R., Wen, J., Zhu, D., Ying, F., Zhao, G., 2023. Classification of leg health traits and estimation of heritability in white feather broiler by X-ray detection. China Poult. 45, 1–5.

Zhou, R., de Koning, D.J., McCormack, H., Wilson, P., Dunn, I., 2019. Short tandem repeats and methylation in the promoter region affect expression of cystathionine beta-synthase gene in the laying hen. Gene 710, 367–374.