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Identification of Diversity and Genetic Distance of Indonesian Local Chicken Strains Based on Myostatin Gene

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Abstract: Indonesian local chickens are essential genetic resources with significant potential for advancing the national poultry industry due to their high genetic diversity, reflecting adaptation to tropical environments and traditional farming systems. This study analyzed the genetic diversity of Kampung, Sentul, KUB, SenSi, and Broiler chickens based on the MSTN g.4842T>G locus. Blood samples from 150 chickens underwent PCR-RFLP analysis using the BsrI restriction enzyme targeting exon 2 of the MSTN gene. Parameters such as observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC), and Hardy-Weinberg equilibrium were assessed. Genetic distances were calculated using PHYLIP, and a dendrogram was constructed with UPGMA. The study identified two alleles (G and T) and three genotypes (GG, GT, TT), with the T allele being dominant, particularly in Broiler chickens (78.3%). Ho was lower than He in all populations, indicating a heterozygosity deficit. PIC values ranged from 0.282 (Broiler) to 0.367 (SenSi), classifying the MSTN locus as moderately polymorphic. Kampung and KUB chickens showed the closest genetic relationship, while Broiler chickens were the most distinct. These findings underscore the potential of local chickens in breeding programs for adaptability and productivity. Further research with additional markers is recommended to enhance sustainable poultry farming.

Keywords: Genetic diversity; Genetic distance; Indonesian local chickens; MSTN gene.

Introduction

Indonesian local chickens are a vital genetic resource with significant potential for developing the national poultry industry. Their genetic diversity reflects adaptation to tropical environments, traditional farming practices, and natural selection over the years (Sumantri et al., 2020). This diversity is crucial for preserving biodiversity and enhancing the productivity and sustainability of local poultry breeds. However, the utilization of local chickens is often constrained by limited information on their genetic characteristics and the factors influencing their performance (Ariza et al., 2021; Kpomasse et al., 2023). One important genetic factor that may impact the performance of local chickens is the myostatin gene, which plays a crucial role in regulating muscle growth.

The myostatin gene is a key regulator of muscle growth in poultry. It acts as an inhibitor of muscle cell proliferation and differentiation, meaning that variations or mutations in this gene can significantly affect the growth performance of chickens (Kim et al., 2020; Shoyombo et al., 2022). Previous studies have shown that polymorphisms in the myostatin gene are closely associated with carcass traits and growth performance, making it a primary focus in genetic breeding research (Lee et al., 2024; Lu et al., 2024). Genetic diversity analysis based on the myostatin gene can also determine the genetic distance among local chicken strains, supporting the classification of strains based on their genetic advantages (Sumantri et al., 2020; Rodgers & Ward, 2021).

Research on Indonesian local chickens has received insufficient attention, particularly in the context of genetic analysis. In contrast, similar studies in other countries have demonstrated that genetic mapping using the myostatin gene provides valuable insights into improving productivity and ensuring the sustainability of local strains (Rexroad et al., 2019; Nawaz et al., 2024). Through molecular approaches, the genetic diversity of local chickens can be identified more accurately, ultimately supporting conservation efforts and enhancing the economic value of local poultry.

This study aims to identify the diversity and genetic distance of several Indonesian local chicken strains by analyzing the myostatin gene. This approach is expected to provide in-depth information about the genetic potential of Indonesian local chickens, which can be used in breeding programs, conservation efforts, and developing superior local chicken strains. Furthermore, this analysis is anticipated to be a foundation for integrating genetic knowledge into policies and poultry farming practices in Indonesia.

Material and Method

Experimental Animals

Blood samples from 150 chickens consisting of Kampung chickens, Sentul chickens, Kampung Unggul Balitbangtan chickens (KUB), Sentul Terseleksi chickens (SenSi), and Broilers were obtained from the Animal Breeding and Genetics Laboratory, Faculty of Animal Science, University of Mataram, Indonesia.

Polymorphism Study

DNA was extracted using the Genomic DNA Mini Kit Geneid (ISO 9001:2008 QMS). To ensure enough DNA for downstream applications, a NanoDrop spectrophotometer quantified it. To retain integrity until analysis, measured DNA was kept at -20°C (Bhoyar et al., 2024). Based on exon 2 of the chicken MSTN genomic sequence (GenBank: AF346599.2), forward (5-AACGGTGTTTGTGCAGATCC-3) and reverse (5-CAATCCATCTTCACCCGGTCC-3) primers were generated for PCR amplification. PCR was performed in a 25 µL reaction volume with a 10 µL DNA template, 12.5 µL PCR master mix, 1 µL forward and reverse primers, and 0.5 µL Taq DNA polymerase. The PCR process consisted of predenaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 20 seconds, and extension at 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes. RFLP was used for genotyping. The 247-bp MSTN exon 2 PCR product was digested with 3 U of BsrI restriction enzyme (Thermo Fisher Scientific, Waltham, MA). The PCR product (5 µL) was digested overnight at 37 °C. RFLPs were detected in 150 hens using electrophoresis on a 2% agarose gel in 0.5X TBE, added 2.5 µL of ethidium bromide, and examined with a UV transilluminator (AlphaImager®EP). The T allele has four bands (99, 64, 45, and 39 bp), and the G allele has three bands (144, 64, and 39).

Genotype and allele frequencies, heterozygosity (Ho and He), Hardy-Weinberg equilibrium, polymorphism information content (PIC), and fixation index (Fst) were studied using Popgene 1.31.

Genetic Distance

The calculation of genetic distances was performed using the PHYLIP software package, specifically the GENDIST program, which computes genetic distances from gene frequency data (Grzegorczyk et al., 2021; Nei, 1972). Subsequently, a dendrogram was constructed employing the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, facilitated by the MEGA (Molecular Evolutionary Genetics Analysis) software (Tamura et al., 2021).

Results and Discussion

Polymorphism of The MSTN Gene

The partial fragment of the myostatin gene in all individuals was successfully amplified, producing a DNA fragment of 247 bp. Genotyping of the myostatin gene using the

BsrI restriction enzyme successfully identified two alleles. The T allele was represented by four bands (99, 64, 45, and 39 bp), while the G allele was represented by three bands (144, 64, and 39 bp). These two alleles resulted in three genotypes: TT, GG, and GT (Figure 1). Genotype distribution, allele frequency, and genetic parameters at the MSTN g.4842T>G locus in Indonesian Local Chickens are displayed in Table 1.

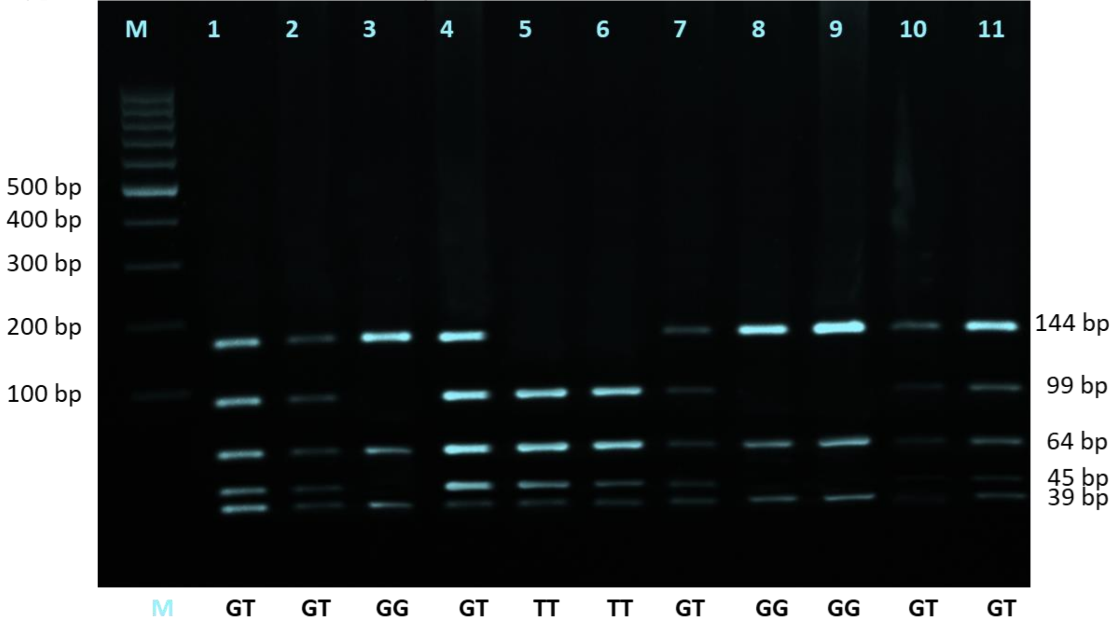

Figure 1. PCR-RFLP Patterns of the MSTN g.4842T>G Locus in Indonesian Local Chickens Using *Bsr*I Restriction Enzyme on a 2% Agarose Gel

Table 1 presents the genotype distribution, allele frequency, and genetic parameters observed at the MSTN g.4842T>G locus in various populations of Indonesian local chickens. These parameters are crucial for understanding the genetic diversity and inheritance patterns associated with muscle growth in local chicken breeds.

Table 1. Genotype Distribution, Allele Frequency, and Genetic Parameters at the MSTN g.4842T>G Locus in Indonesian Local Chickens

Locus	**Restriction enzyme**	**Parameter**		**Breed**				
				Kampung	**Sentul**	**KUB**	**SenSi**	**Broiler**
g.4842T>G	*Bsr*I	Genotype	GG	0.200	0.233	0.167	0.267	0.133
		Frequency	GT	0.267	0.333	0.300	0.300	0.167
			TT	0.533	0.433	0.533	0.433	0.700
		Allele	G	0.333	0.400	0.317	0.417	0.217
		Frequency	T	0.667	0.600	0.683	0.583	0.783
		Ho		0.267	0.333	0.300	0.300	0.167
		He		0.444	0.480	0.433	0.486	0.340
		PIC		0.345	0.365	0.339	0.367	0.282
		p-value		0.091	0.247	0.245	0.111	0.021
		Chi^2		4.790	2.800	2.810	4.390	7.740

Ho= observed heterozygosity. He= expected heterozygosity. PIC= polymorphism information content. Chi^2 = Chi-square.

The genotype distribution showed significant variation across five Indonesian local chickens at the MSTN g.4842T>G locus, restricted using the BsrI enzyme. The observed genotype distribution (GG, GT, TT) varied among breeds. The GG genotype (homozygous for the G allele) had the highest frequency in SenSi chickens (26.7%) and the lowest in Broiler chickens (13.3%). The GT genotype (heterozygous) showed moderate frequency in most breeds, with the highest frequency in Sentul chickens (33.3%). The TT genotype (homozygous for the T allele) was dominant in Broiler chickens (70%) but was lower in SenSi and Sentul chickens (43.3%). This variation in genotype distribution reflects differences in genetic influence among populations. Previous studies have also indicated that genotype distribution at loci associated with growth, such as MSTN, is strongly influenced by both artificial and natural selection (Xu et al., 2020).

The allele frequency data showed that the T allele was more dominant than the G allele across all populations, with the highest T frequency observed in Broiler chickens (78.3%) and the lowest in SenSi chickens (58.3%). Conversely, the G allele had the highest frequency in SenSi chickens (41.7%) and the lowest in Broiler chickens (21.7%). Related studies suggest that the dominance of specific alleles, such as T in Broiler chickens, is often linked to the effects of genetic selection for traits such as growth efficiency or meat quality (Gu et al., 2004; Lu et al., 2024).

The Ho value represents the proportion of heterozygous individuals in the population. The highest Ho value was found in Sentul chickens (33.3%), while the lowest was in Broiler chickens (16.7%). The He value, which reflects the potential genetic diversity within a population, was higher than Ho in all populations, indicating a heterozygosity deficit. The highest He value was observed in SenSi chickens (48.6%), suggesting high allele diversity. This heterozygosity deficit may be associated with genetic bottlenecks in specific populations, especially Broiler chickens, which tend to have higher homozygous genotypes due to intensive selection (Talebi et al., 2020; Yuan et al., 2022).

The PIC values illustrate the ability of the locus to detect genetic variation. Based on the results, all PIC values fell into the moderately polymorphic category ($0.25 < \mathrm{PIC} < 0.50$). The highest PIC value was found in SenSi chickens (0.367), while the lowest was in Broiler chickens (0.282). The moderate PIC values support using the MSTN g.4842T>G locus as a genetic marker in population genetics studies and breeding programs (De La Parra et al., 2023; Habimana et al., 2020).

The highest $\chi 2$ value was observed in Broiler chickens (7.74), indicating a significant deviation from Hardy-Weinberg equilibrium. This result is consistent with the significant p-value (p =0.021). For other populations, p-value were not significant ($p > 0.05$), indicating they were in Hardy-Weinberg equilibrium. These results suggest that the Broiler chicken population is genetically more homogeneous, whereas other local chicken populations exhibit more extraordinary genetic diversity (Ariza et al., 2021; Chu et al., 2023).

The genetic variation at the MSTN g.4842T>G locus provides insight into the genetic diversity of Indonesian local chickens. Broiler chickens, which show a dominance of the TT genotype and low heterozygosity, tend to experience bottleneck effects due to intensive breeding selection (Bortoluzzi et al., 2019; Gao et al., 2023). Local chicken populations, such as Kampung, Sentul, KUB, and SenSi, exhibit higher genetic diversity, supporting adaptive capabilities and genetic improvement potential (Chebo et al., 2022; Mogano et al., 2024; Wu et al., 2024).

Genetic Distance Among Chicken Populations

The genetic distance shows that Indonesian local chicken populations have closer genetic relationships with each other compared to Broiler chickens, which have undergone intensive selection. The genetic distance matrix information is presented in Table 2. The UPGMA dendrogram representation based on genetic distance is shown in Figure 2.

Table 2. Genetic Distance Matrix of Indonesian Local Chicken Populations Based on the MSTN g.4842T>G Locus

	Kampung	**Sentul**	**KUB**	**SenSi**	**Broiler**
Kampung	0.000	0.006	0.000	0.013	0.022
Sentul	0.006	0.000	0.011	0.007	0.019
KUB	0.000	0.011	0.000	0.013	0.025
SenSi	0.013	0.007	0.013	0.000	0.021
Broiler	0.022	0.019	0.025	0.021	0.000

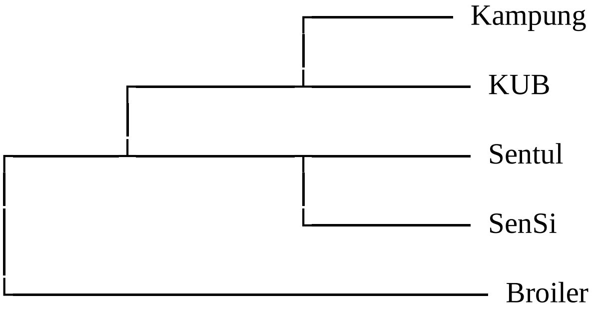

Figure 2. UPGMA dendrogram representation based on genetic distance in Indonesian local chicken populations at the MSTN g.4842T>G locus

Kampung and KUB chickens are located on the same branch, indicating a very close genetic relationship (*D* = 0.000). Sentul and SenSi chickens form a separate branch but remain close to Kampung and KUB, reflecting a relatively close genetic relationship. Broiler chickens are on the most distant branch, demonstrating a more significant genetic differentiation than other populations due to intensive selection for commercial purposes.

As shown in Table 2, Kampung and KUB chicken populations have a genetic distance of *D* = 0.000, reflecting an almost identical genetic similarity. This may be attributed to a similar genetic origin or a high level of gene flow between the two populations. This genetic similarity supports the hypothesis that Indonesian local chickens share the same ancestry, as supported by previous studies showing genetic uniformity among local chickens in Southeast Asia (Sumantri et al., 2020). A close relationship is also observed between Sentul and SenSi chickens (*D* = 0.007), indicating a significant genetic connection. These two populations likely share similar genetic characteristics due to their domestication history or intensive gene flow in the past.

Broiler chicken populations exhibit the most significant genetic distance from other populations, particularly with KUB (*D* = 0.025) and Kampung chickens (*D* = 0.022). This indicates significant genetic differentiation due to stringent artificial selection in Broiler chickens for specific traits, such as rapid growth and feed efficiency (Fontanesi et al., 2015). This differentiation can also be explained by reduced genetic diversity in Broiler chickens due to bottleneck effects during domestication (Wang et al., 2021).

Genetic diversity in local chicken populations, such as Kampung, Sentul, KUB, and SenSi, reflects good adaptability to local environments. This contrasts with Broiler chickens, which tend to be more genetically homogeneous due to intensive selection. Other studies indicate that Indonesian local chicken populations have high levels of genetic diversity, especially at loci associated with environmental adaptation and disease resistance (Mariandayani et al., 2023; Xie et al., 2024). Therefore, these local chicken populations have great potential to be used in crossbreeding programs to improve production efficiency while maintaining resilience to tropical environmental conditions.

Based on genetic distance, Kampung x KUB chickens are ideal for crossbreeding to maintain local adaptive traits due to their very close genetic relationship. Sentul x SenSi chickens are suitable for crossbreeding to combine the superior characteristics of both populations, such as larger body size and environmental resilience. Moderate genetic differences (*D* = 0.025) make KUB x Broiler chickens a promising option for improving growth performance without losing local adaptive advantages. The genetic distance among populations indicates that Indonesian local chickens, particularly Kampung, Sentul, KUB, and SenSi, have close genetic relationships, supporting local adaptation and high genetic diversity. Broiler chickens, on the other hand, show significant genetic differentiation, reflecting the impact of intensive artificial selection. This genetic diversity is a foundation for strategic crossbreeding to improve production efficiency while maintaining adaptive resilience. Further studies using additional genetic markers are necessary to support the sustainable management of these genetic resources (Restoux et al., 2022; Xie et al., 2024).

Conclusion

This study demonstrates that genetic variation at the MSTN g.4842T>G locus provides valuable insights into the genetic diversity of Indonesian local chickens. The observed genotypes (GG, GT, TT) and allele frequencies (G, T) reveal significant differences among Kampung, Sentul, KUB, SenSi, and Broiler chicken populations. Observed heterozygosity (Ho) was lower than expected (He) across all populations, indicating a deficit of heterozygotes, particularly in Broiler chickens. The moderate PIC values suggest the potential of this locus as a genetic marker for breeding programs. Genetic distance analysis shows a very close genetic relationship between Kampung and KUB chickens (D = 0.000), while Broiler chickens exhibit the most significant genetic differentiation from the other populations. These findings indicate that Indonesian local chickens possess high genetic diversity, supporting their potential for local adaptation and crossbreeding programs aimed at improving productivity and resilience in tropical environments. Conversely, Broiler chickens exhibit high genetic homogeneity due to intensive selection. This research underscores the importance of conserving and utilizing local genetic resources to ensure the sustainability of the poultry industry in Indonesia.

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References

Ariza, A. G., Arbulu, A. A., González, F. J. N., Baena, S. N., Bermejo, J. V. D., & Vallejo, M. E. C. (2021). The Study of Growth and Performance in Local Chicken Breeds and Varieties: A Review of Methods and Scientific Transference. *Animals*, *11*(9), 2492. https://doi.org/10.3390/ani11092492

Bhoyar, L., Mehar, P., & Chavali, K. (2024). An overview of DNA degradation and its implications in forensic caseworks. *Egyptian Journal of Forensic Sciences*, *14*(1). https://doi.org/10.1186/s41935-024-00389-y

Bortoluzzi, C., Bosse, M., Derks, M. F. L., Crooijmans, R. P. M. A., Groenen, M. a. M., & Megens, H. (2019). The type of bottleneck matters: Insights into the deleterious variation landscape of small managed populations. *Evolutionary Applications*, *13*(2), 330–341. https://doi.org/10.1111/eva.12872

Chebo, C., Betsha, S., & Melesse, A. (2022). Chicken genetic diversity, improvement strategies and impacts on egg productivity in Ethiopia: a review. *World S Poultry Science Journal*, *78*(3), 803–821. https://doi.org/10.1080/00439339.2022.2067020

Chu, J., Ma, Y., Song, H., Zhao, Q., Wei, X., Yan, Y., Fan, S., Zhou, B., Li, S., & Mou, C. (2023). The genomic characteristics affect phenotypic diversity from the perspective of genetic improvement of economic traits. *iScience*, *26*(4), 106426. https://doi.org/10.1016/j.isci.2023.106426

De La Parra, M. D., Inostroza, K., Alcalde, J. A., Larama, G., & Bravo, S. (2023). Characterization of the genetic diversity, structure, and admixture of 7 Chilean chicken breeds. *Poultry Science*, *103*(2), 103238. https://doi.org/10.1016/j.psj.2023.103238

Gao, C., Du, W., Tian, K., Wang, K., Wang, C., Sun, G., Kang, X., & Li, W. (2023). Analysis of Conservation Priorities and Runs of Homozygosity Patterns for Chinese Indigenous Chicken Breeds. *Animals*, *13*(4), 599. https://doi.org/10.3390/ani13040599

Grzegorczyk, J., Gurgul, A., Oczkowicz, M., Szmatoła, T., Fornal, A., & Bugno-Poniewierska, M. (2021). Single Nucleotide Polymorphism Discovery and Genetic Differentiation Analysis of Geese Bred in Poland, Using Genotyping-by-Sequencing (GBS). *Genes*, *12*(7), 1074. https://doi.org/10.3390/genes12071074

Gu, Z., Zhang, Y., Shi, P., Zhang, Y., Zhu, D., & Li, H. (2004). Comparison of avian myostatin genes. *Animal Genetics*, *35*(6), 470–472. https://doi.org/10.1111/ j.1365-2052.2004.01194.x

Habimana, R., Okeno, T. O., Ngeno, K., Mboumba, S., Assami, P., Gbotto, A. A., Keambou, C. T., Nishimwe, K., Mahoro, J., & Yao, N. (2020). Genetic diversity and population structure of indigenous chicken in Rwanda using microsatellite markers. *PLoS ONE*, *15*(4), e0225084. https://doi.org/10.1371/journal.pone.0225084

Kim, D., Choi, Y. M., Suh, Y., Shin, S., Lee, J., Hwang, S., & Lee, K. (2020). Research Note: Association of temporal expression of myostatin with hypertrophic muscle growth in different Japanese quail lines. *Poultry Science*, *99*(6), 2926–2930. https://doi.org/ 10.1016/j.psj.2019.12.069

Kpomasse, C. C., Kouame, Y. a. E., N'nanle, O., Houndonougbo, F. M., Tona, K., & Oke, O. E. (2023). The productivity and resilience of the indigenous chickens in the tropical environments: improvement and future perspectives. *Journal of Applied Animal Research*, *51*(1), 456–469. https://doi.org/10.1080/09712119.2023.2228374

Lee, J., Kim, D., & Lee, K. (2024). Myostatin gene role in regulating traits of poultry species for potential industrial applications. *Journal of Animal Science and Biotechnology/Journal of Animal Science and Biotechnology*, *15*(1). https://doi.org/10.1186/s40104-024-01040-5

Lu, T., Gibril, B. a. A., Xu, J., & Xiong, X. (2024). Unraveling the Genetic Foundations of Broiler Meat Quality: Advancements in Research and Their Impact. *Genes*, *15*(6), 746. https://doi.org/10.3390/genes15060746

Mariandayani, H. N., Darwati, S., Khaerunnisa, I., & Prasasty, V. D. (2023). Growth performance of Indonesian three-breed cross chicken associated with growth hormone and insulin-like growth factor 2 genes. *Veterinary World*, 2471–2478. https://doi.org/10.14202/vetworld.2023.2471-2478

Mogano, R. R., Mpofu, T. J., Mtileni, B., & Hadebe, K. (2024). South African indigenous chickens' genetic diversity, and the adoption of ecological niche modelling and landscape genomics as strategic conservation techniques. *Poultry Science*, *104*(1), 104508. https://doi.org/10.1016/j.psj.2024.104508

Nawaz, A. H., Setthaya, P., & Feng, C. (2024). Exploring Evolutionary Adaptations and Genomic Advancements to Improve Heat Tolerance in Chickens. *Animals*, *14*(15), 2215. https://doi.org/10.3390/ani14152215

Nei, M. (1972). Genetic Distance between Populations. *The American Naturalist*, *106*(949), 283–292. https://doi.org/10.1086/282771

Restoux, G., Rognon, X., Vieaud, A., Guemene, D., Petitjean, F., Rouger, R., Brard-Fudulea, S., Lubac-Paye, S., Chiron, G., & Tixier-Boichard, M. (2022). Managing genetic diversity in breeding programs of small populations: the case of French local chicken breeds. *Genetics Selection Evolution*, *54*(1). https://doi.org/10.1186/s12711-022-00746-2

Rexroad, C., Vallet, J., Matukumalli, L. K., Reecy, J., Bickhart, D., Blackburn, H., Boggess, M., Cheng, H., Clutter, A., Cockett, N., Ernst, C., Fulton, J. E., Liu, J., Lunney, J., Neibergs, H., Purcell, C., Smith, T. P. L., Sonstegard, T., Taylor, J., . . . Wells, K. (2019). Genome to Phenome: Improving Animal Health, Production, and Well-Being – A New USDA Blueprint for Animal Genome Research 2018–2027. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/ fgene.2019.00327

Rodgers, B. D., & Ward, C. W. (2021). Myostatin/Activin Receptor Ligands in Muscle and the Development Status of Attenuating Drugs. *Endocrine Reviews*, *43*(2), 329–365. https://doi.org/10.1210/ endrev/bnab030

Shoyombo, A. J., Abdulmojeed, Y., Alabi, O. O., Popoola, M. A., Okon, E. M., & Arije, D. O. (2022). Applications of Myostatin in Poultry and Aquaculture - A Review. *The Open Agriculture Journal*, *16*(1). https://doi.org/10.2174/18743315-v16-e2208010

Sumantri, C., Khaerunnisa, I., & Gunawan, A. (2020). The genetic quality improvement of native and local chickens to increase production and meat quality in order to build the Indonesian chicken industry. *IOP Conference Series Earth and Environmental Science*, *492*(1), 012099. https://doi.org/10.1088/1755-1315/ 492/ 1/012099

Talebi, R., Szmatoła, T., Mészáros, G., & Qanbari, S. (2020). Runs of Homozygosity in Modern Chicken Revealed by Sequence Data. *G3 Genes Genomes Genetics*, *10*(12), 4615–4623. https://doi.org/10.1534/g3.120.401860

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/ molbev/msab120

Wang, M., Zhang, J., Guo, X., Li, M., Meyer, R., Ashari, H., Zheng, Z., Wang, S., Peng, M., Jiang, Y., Thakur, M., Suwannapoom, C., Esmailizadeh, A., Hirimuthugoda, N. Y., Zein, M. S. A., Kusza, S., Kharrati-Koopaee, H., Zeng, L., Wang, Y., . . . & Zhang, Y. (2021). Large-scale genomic analysis reveals the genetic cost of chicken domestication. *BMC Biology*, *19*(1). https://doi.org/ 10.1186/s12915-021-01052-x

Wu, S., Chen, Z., Zhou, X., Lu, J., Tian, Y., Jiang, Y., Liu, Q., Wang, Z., Li, H., Qu, L., & Zhang, F. (2024). Analysis of genetic diversity and genetic structure of indigenous chicken populations in Guizhou Province based on genome-wide single nucleotide polymorphism markers. *Poultry Science*, *103*(12), 104383. https://doi.org/10.1016/j.psj. 2024.104383

Xie, X., Shi, L., Zhong, Z., Wang, Z., Pan, D., Hou, G., & Xiao, Q. (2024). Danzhou chicken: a unique genetic resource revealed by genome-wide resequencing data. *Poultry Science*, *103*(8), 103960. https://doi.org/10.1016/j.psj.2024.103960

Xie, X., Wang, Z., Zhong, Z., Pan, D., Hou, G., & Xiao, Q. (2024). Genome-wide scans for selection signatures in indigenous chickens reveal candidate genes associated with local adaptation. *Animal*, *18*(5), 101151. https://doi.org/10.1016/ j.animal.2024.101151

Xu, K., Han, C. X., Zhou, H., Ding, J. M., Xu, Z., Yang, L. Y., He, C., Akinyemi, F., Zheng, Y. M., Qin, C., Luo, H. X., & Meng, H. (2020). Effective MSTN Gene Knockout by AdV-Delivered CRISPR/Cas9 in Postnatal Chick Leg Muscle. *International Journal of Molecular Sciences*, *21*(7), 2584. https://doi.org/10.3390/ijms21072584

Yuan, J., Li, S., Sheng, Z., Zhang, M., Liu, X., Yuan, Z., Yang, N., & Chen, J. (2022). Genome-wide run of homozygosity analysis reveals candidate genomic regions associated with environmental adaptations of Tibetan native chickens. *BMC Genomics*, *23*(1). https://doi.org/ 10.1186/s12864-021-08280-z