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Research Article

Genetic External Characteristics of Tukong: Indonesian Rumpless Indigenous Chicken

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ABSTRACT

Tukong chicken, also known as rumpless chicken due to the absence of a tailbone, is one of the indigenous chicken breeds in Indonesia currently at risk of extinction. Due to its scarcity, genetic information on the Tukong chicken remains limited, and its potential has not been fully explored. Therefore, this study aimed to determine the genetic external characteristics of the Tukong chickens. Data were collected from 205 chickens (52 males; 153 females) raised in Landak and Bengkayang Regency, West Kalimantan. The varieties are based on feather color, plumage color, feather pattern, feather flicker, comb shape, and shank color. The data were used to analyze the rate of the original gene, the frequency of autosomal genes, sex-linked genes, feather pattern, introgression rate, gene frequency, and expected heterozygosity. The majority of the Tukong chickens had colored feathers (88.78%), columbian feather type (47.80%), plain feather pattern (61.46%), silver feather flicker (54.63%), yellow-white shank (62.44%), and pea comb (77.07%), which were controlled by ii, ee, bb, SS, IdId, and P_ genes, respectively. The introgression rate from exotic breeds into Tukong chicken is 53.2%, with the original gene at 46.8%, while the average expected heterozygosity was 0.416±0.025, indicating a high level of genetic diversity of the traits. This observed variation in qualitative morphological characteristics might benefit both the conservation and utilization of the Tukong chicken.

1. Introduction

Indigenous poultry genetic resources contribute significantly to poverty alleviation and household food security in many developing countries. In Indonesia, small-scale and commercial chicken production significantly contributes to poor households' livelihood and food security (Maharani *et al*. 2021). The country is rich in poultry biodiversity, with over 31 indigenous breeds of chickens raised by rural farmers across regions (Hidayat and Asmarasari 2015). Indigenous chickens are mostly reared in the

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backyard with low biosecurity measures, particularly in rural areas, where households raise small groups of local chickens for family food. Consequently, indigenous chickens are considered to significantly contribute to food security and the economic sustainability of rural households. Compared to commercial chickens, indigenous chickens are found in all habitable agroecological zones and are diverse in morphology and characteristics, which may affect their genetic makeup. Asmara *et al*. (2019) observed homogeneity in terms of comb type in Pelung chicken from different agroecologies, whereas Maharani *et al*. (2021) observed variation in both qualitative and quantitative traits of indigenous chickens.

Characterization is an essential task in designing effective conservation and improvement programs. It involves identifying genetic and phenotypic attributes and production systems (Biscarini *et al*. 2015). For instance, phenotypic and genetic characterization has been performed in indigenous poultry breeds. The results can inform the level of phenotypic and genetic variation, the potential economically essential traits, and the rate of inbreeding in a population (Cabarles *et al*. 2012; Hariyono *et al*. 2019; Maharani *et al*. 2019). Phenotypic characterization involves the identification of qualitative and quantitative morphological characteristics. The quantitative traits observed in chickens include body weight and measurements. In contrast, the qualitative characteristics include plumage color, feather color, earlobe color, shank color, comb type, earlobe color, and eye color (Maharani *et al*. 2021). Several studies of the phenotypic diversity of indigenous chicken populations have been done separately for different countries, including Indonesia (Arlina *et al*. 2014; Asmara *et al*. 2019; Maharani *et al*. 2021), Thailand (Laenoi *et al*. 2015), Algeria (Dahloum *et al*. 2016), and Sri Lanka (Liyanage *et al*. 2015). Liyanage *et al*. (2015) observed varying plumage colors in indigenous chickens from Sri Lanka, which is a form of adaptation to the living environment, such as camouflage against predators a breeding strategy. Meanwhile, Thai indigenous chicken in the highland region forms a very heterogeneous character; they exhibit wide variation in color, plumage, comb type, skin color, eye color, shank color, and beak color (Laenoi *et al*. 2015), as also reported in Indonesian indigenous chicken (Maharani *et al*. 2021).

Generally, the characterization of animal genetic resources involves three types of information (phenotypic, genetic, and historical). However, limited characterization of indigenous chicken exacerbates significant threats to indigenous breeds, such as uncontrolled crossbreeding and habitat destruction. For instance, Tukong chicken, a breed of poultry from West Kalimantan, is among the indigenous breeds considered at risk of extinction without any information on their potential and phenotypic attributes. The breed has specific characteristics like the absence of a tailbone or rump; thus, it is also known as a rumpless chicken. We previously evaluated the productive performances of Tukong chickens; they can reach a body weight of 1.65 kg (male) and 1.42 kg (female) at adult age (Tribudi *et al*. 2020). Tukong chickens are primarily raised for cockfighting and as a source of meat and eggs, but their rumplessness is considered a defect, making them unsuitable for traditional and religious ceremonies (Tribudi *et al*. 2023). Despite their importance, no literature is available regarding their phenotypic, genetic, and historical characterization due to a lack of support from the local government. This condition also causes the absence of accurate population data for the breed. Therefore, this study aimed to identify the genetic external characteristics of the Tukong chicken as a basis for designing its conservation and improvement strategies.

2. Materials and Methods

2.1. Ethical Statement

All methods involving animals in the study followed national (the Indonesian Animal Welfare Act No 18 of 2009) or institutional criteria for animal care and use and the institution's or practice's ethical standards. This study has been authorized and approved with the Ethical Clearance Certificate of The Institutional Animal Care and Use Committee (IACUC) of Brawijaya University, with approval number 155-KEP-UB-2022.

2.2. Study Sites and Animal Experimental

This study was conducted in Landak and Bengkayang regency in the West Kalimantan province of Indonesia. It is located in the northeastern Pontianak regency at 0.4237°N 109.7592°E (Figure 1). A total of 205 Tukong chickens (Figure 1), consisting of 53 roosters and 152 hens, were raised for one month in West Kalimantan (Landak and Bengkayang Regencies). The sample size was determined based on recommendations from The Food and Agriculture Organization (2012), which state that the number of chicken samples for characterization should range from 100 to 300 hens and 10-30 roosters. A map of the studied location is presented in Figure 2. This research utilized the survey method and intensive direct examination. Purposive sampling was employed for sample selection, and Tukong chickens over 8 months of age were sourced from their native habitats in Landak and Bengkayang Regencies, West Kalimantan. The variation in base feather color, plumage color, feather pattern, feather flicker, shank color, and comb shape of Tukong chickens was identified based on

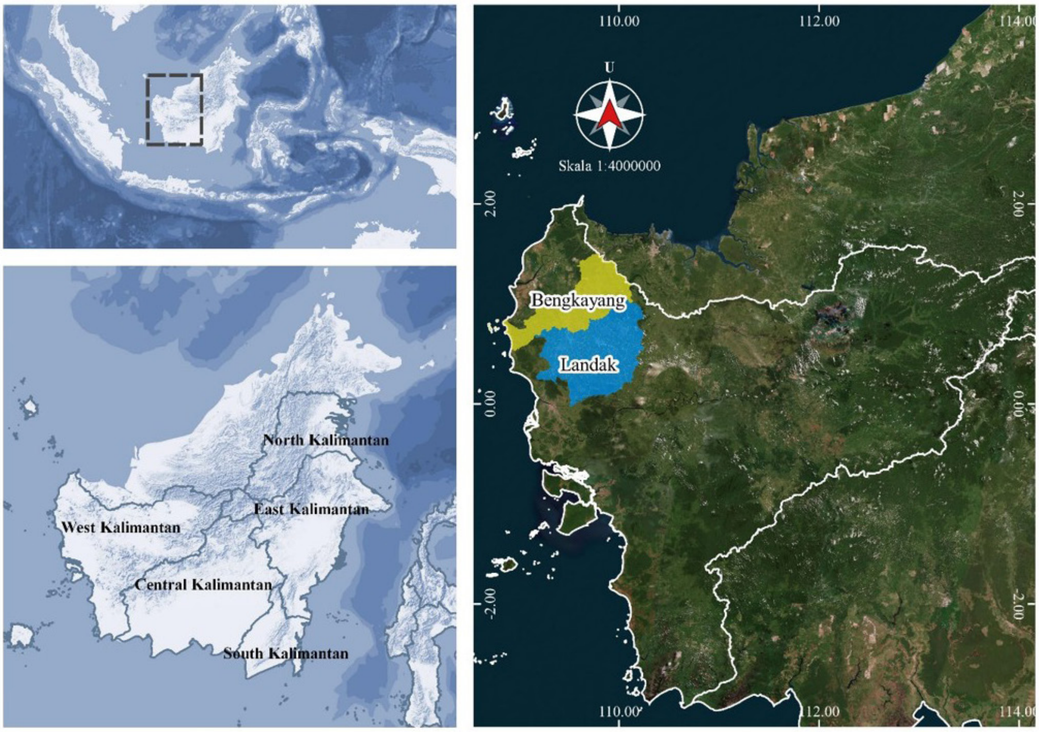

Figure 1. Map of the study site, Landak and Bengkayang Regency

Figure 2. The Tukong chicken

the classifications outlined by Hutt (2003) and Soomes (1988).

2.3. Data Analysis

The frequencies of autosomal genes (plumage color and comb shape), sex-linked genes (variation in base feather color, feather flicker, and shank color), and feather pattern were quantified based on the methods outlined by Nishida *et al*. (1980), and Stanfield (1982). Introgression rates from exotic breeds (Rhode Island Red, White Leghorn, and Barred Plymouth Rock) and original genes of Tukong chicken were calculated (Nishida *et al*. 1988). Expected heterozygosity per individual was estimated following the method described by Nei and Kumar (2000). The formula was as follows:

$$h = 1 - \sum_{i}^{n} qi^2$$

$$H = \frac{\sum h}{r}$$

Description:

qi : gene frequency
r : number of loci observed
H : average expected heterozygosity
h : expected heterozygosity value

3. Results

3.1. Variation in Qualitative Morphological Traits

Variation in all the qualitative morphological characteristics of the Tukong chickens was presented in Table 1. Two variants for feather color were observed: white and colored (11.22% vs. 88.78%). For feather color type, the majority of the chickens exhibited the columbian type (47.80%), followed by black (36.59%) and wild (15.61%). Two variants in feather pattern (striated and plain) were observed, in which plain (61.46%) was the most common feather pattern in the observed chickens. Silver was the most predominant feather flicker (54.63%), while 45.37% of the chickens had a gold feather flicker. Most chickens had white/yellow shanks (Id_; 62.44%), while the remainder had black shanks (idid, 37.56%). Two variants for comb shape were observed: pea (77.07%) and single (22.93%). The columbian feather color type and the plain feather pattern may have provided adaptive advantages like improved camouflage or resistance to environmental stressors. The pea comb over the single comb may have been selected for heat tolerance due to better heat dissipation. In general, the qualitative morphological characteristics of the chickens were predominantly controlled by ii (feather color), ee (feather color type), bb (feather pattern), SS (feather flicks), IdId (shank color), and P_ (comb type) genes.

3.2. Introgression Rate from Exotic Breeds

The rate of introgression from Rhode Island Red, White Leghorn, and Barred Plymouth Rock on the phenotypic traits of Tukong chickens was presented in Table 2. The introgression rate ranged from 5.8% (White Leghorn) to 22.20% (Rhode Island Red), while approximately 25.3% introgression from the Barred Plymouth Rock was observed. The introgression rate from the three exotic breeds reached 53.20%, indicating approximately 46.80% of the purity gene in Tukong chickens. The introgression rate of 53.20% from exotic breeds like Rhode Island Red, White Leghorn, and Barred Plymouth

Table 1. Variation in all the qualitative morphological characteristics of the Tukong chickens

Traits	Locus	Phenotype (allele)	N	Percentage (%)	Gene frequency
Feather color	I_i	White (qI)	23	11.22	0.058
		Colored (qi)	182	88.78	0.942
Plumage color	E_e+_e	Black (qE)	75	36.59	0.204
		Wild type (qe+)	32	15.61	0.105
		Columbian (qe)	98	47.80	0.691
Feather pattern	B_b	Striated (qB)	79	38.54	0.310
		Plain (qb)	126	61.46	0.690
Feather flicker	S_s	Silver (qS)	112	54.63	0.447
		Gold (qs)	93	45.37	0.553
Shank color	Id_id	Yellow/white (qId)	128	62.44	0.532
		Black/grey (qid)	77	37.56	0.468
Comb shape	P_pp	Pea (P-)	158	77.07	0.521
		Single (pp)	47	22.93	0.479

Note: N = number of observation

Table 2. Introgression rate (Q) from Rhode Island Red, White Leghorn, and Barred Plymouth Rock into Tukong chickens

Source of introgression	Introgression rate
QSR	0.222
QWL	0.058
QBR	0.253
QSR+QWL+QBR	0.532
Original gene	0.468
1–(QSR+ QWL+ QBR)	46.80%

Note: QWL= White Leghorn chicken introgression value, QSR= Rhode Island Red chicken introgression value, QBR= Barred Plymouth Rock's chicken introgression value

Rock meant that more than half of the Tukong chicken gene pool now contained foreign genetic material, which could have improved productivity traits like egg-laying and growth rate but also diluted indigenous genetic characteristics. The remaining 46.80% purity of the gene pool suggested that conservation efforts should have focused on maintaining Tukong chickens' genetic identity while balancing introgression. Since some genes, like S and Id, remained dominant, selective breeding programs could have reinforced original genetic traits and prevented further diversity loss.

3.3. Frequency of Original Genes Controlling Morphological Traits

The frequency of original genes controlling the morphological characteristics in Tukong chickens is shown in Table 3. A range value was observed from 0.011 (p gene, controlling single comb) to 0.757 (S gene, controlling silver feather flicks). The feather color showed little genetic variety, while the other features showed moderate diversity. The S gene (associated with silver feather flicks, frequency = 0.757) and the IdId gene (linked to white/yellow shank, 62.44%) were highly prevalent in the Tukong chicken population, indicating that these traits had become dominant. Genetic drift was defined as random fluctuations in gene frequencies over generations, particularly in small populations. Low-frequency alleles, such as the p gene (which governs single comb, at 0.011), indicate that genetic drift had caused some qualities to disappear, reducing variety.

3.4. Expected Heterozygosity per Individual

Table 4 presents the expected heterozygosity per individual for each qualitative morphological characteristic. The expected heterozygosity ranged from 0.109 (feather color) to 0.499 (comb shape), with an average of 0.416. The findings indicated a low level of genetic diversity in feather color, while the remaining traits showed moderate diversity.

4. Discussion

The morphological traits of chickens are important not only for their economic value but also for their significant cultural and religious roles. Therefore, specific characteristics must be carefully identified when developing breeding programs. In the case of Tukong chickens in Indonesia, data on their qualitative traits has been minimal until now. This study aimed to identify the qualitative traits of the Tukong chicken population in West Kalimantan, providing baseline data that can be used for their conservation and potential development. As shown in Table 1, the Tukong chickens predominantly exhibited colored plumage, including black, brown, grey, yellow, and various mixtures, with white feathers being less common. This finding is in line with two previous studies, which both reported a predominance of colored feathers (black, white, and brown) in Indonesian indigenous chickens (Asmara *et al*. 2019; Maharani *et al*. 2021).

Plumage color is known to be an important economic trait in poultry due to its significant effect on egg production and quality traits (Lan *et al*. 2021). The pigmentation differences in plumage color are

Table 3. Frequency of original genes in Tukong chickens

Original gene frequency	Value
qE(N) = qE – qB	0.514
qE+(N) = qE+	0.105
qe(N) = qE – QSR	0.470
qS(N) = qS- qB	0.757
qs(N) = qs- QSR	0.332
qid(N) = qid	0.468
qP(N) = qP	0.521
qp(N) = qp – qid	0.011

Note: qE = black; qE+ = wild, qe = colombian, qS = silver, qs = gold, qP = pea, qp = single, qid = black/grey

Table 4. Expected heterozygosity per Individual for each morphological trait

Trait	Expected heterozygosity
Feather color	0.109±0.035
Plumage color	0.469±0.026
Feather pattern	0.428±0.024
Feather flicker	0.494±0.021
Shank color	0.498±0.021
Comb shape	0.499±0.021
Average	0.416±0.025

attributable to the presence of melanin, which produces a variety of colors in chickens. Specifically, melanin pigments such as trichochrome are related to feather color and indicate genetic differences among specific plumage colors (Smyth 1990). Melanin comes in two types: eumelanine and pheomelanine. Eumelanin is responsible for the black and blue feather colors, while pheomelanin contributes to the red-brown, salmon, and dark yellow hues (Brumbraugh and Moore 1968).

However, this population's relatively low frequency of white plumage (Table 1) may be due to cultural and religious factors. In many communities, especially in West Kalimantan, white chickens, particularly cocks, are important in traditional spiritual ceremonies. As such, these chickens are often sold quickly, which could explain their reduced presence in the population. On the genetic side, this study encountered difficulties in identifying the heterozygous and homozygous genotypes of the Tukong chickens in the field. The primary challenge was the lack of proper breeding records maintained by the owners. This poor documentation hampers the ability to trace genetic lines accurately and makes it difficult to assess the true genetic diversity within the breed. Furthermore, the observed variability in plumage color suggests this trait is not under active selection within the Tukong chicken breed. Without systematic breeding practices and genetic selection, the population remains highly diverse regarding physical characteristics.

Variants in feather color types (e.g., columbian, black, and wild) observed in this study were under the findings in indigenous chickens in Southeast Sulawesi of Indonesia (Pagala *et al*. 2021). Two variants in feather pattern (striated and plain) observed in this study were also marked by Pagala *et al*. (2021). Several genes that control the phenotypic variation of feather patterns have been identified, such as melanocortin-1 receptor (MC1R) (Schwochow *et al*. 2021), endothelin receptor B2 (EDNRB2) (Kinoshita *et al*. 2014), and cyclin-dependent kinase inhibitor 2A (CDKN2A) (Schwochow Thalmann *et al*. 2017). Genetic polymorphism in the MC1R gene and the interaction between the MC1R receptor and agouti signaling protein genes (ASIP) has been reported to affect feather pigmentation patterns in chicken (Schwochow *et al*. 2021).

The strip-based color of the feathers was observed when the distribution of melanin in the secondary feathers was blocked. The variety of base colors in feathers was determined to be a sex-linked trait, which was identified as ZBW in females and ZBZB or ZBZb in males, respectively (Hutt 2003). This research was closely related to the research of Arlina *et al*. (2014), Jakaria *et al*. (2012), Ulfah *et al*. (2012), and Mansjoer (1985), who described native chickens in Indonesia as being closely related to the Sumatran red junglefowl (*Gallus gallus gallus*). The variability in feather patterns emphasized that this trait was not subject to selection within the Tukong chicken breed, contributing to their adaptation and survival under local conditions. The extensive variation in plumage color within the indigenous chicken population indicated the absence of conscious selection efforts. Arlina *et al*. (2014) and Ensminger (1992) stated that plumage color and pattern were inherited through single pairs of genes that could influence consumer preferences. However, limited data have been available to suggest that variation in plumage color among native chickens in Indonesia resulted primarily from a lack of conscious selection or breeding programs targeting color preferences. In this study, gold was identified as the most chickens' most predominant feather flick color, aligning with previous reports on other indigenous chicken populations in Indonesia (Arlina *et al*. 2014; Pagala *et al*. 2021). Most chickens had white or yellow shanks with bright colors such as yellow and white being influenced by melanin content (Toews *et al*. 2017). The yellow coloration was suggested to be inherited from the gray junglefowl (*Gallus gallus sonneratti*) and the Sri Lankan junglefowl (*Gallus gallus lafayetti*), which were crossed with the red junglefowl (*Gallus gallus*) (Cabarles *et al*. 2012; Lawal and Hanotte 2021).

For comb shape, two variants (pea and single) were also reported in Sensi-1 Agrinak and Kampung chickens of Indonesia (Iskandar 2018; Permadi *et al*. 2020). Robustly developed, combs indicated high production and reproductive performance (Cai *et al*. 2024). In this study, a pea-shaped comb morphology was predominantly exhibited by Tukong chickens. A study by Wright *et al*. (2009) found that pea combs form when a duplicated sequence in intron 1 of the SOX5 gene is amplified quickly. Additionally, chickens with a pea comb phenotype were preferred for cockfighting activities due to the reduced risk of injury when pitted. According to Nishida *et al*. (1980), the gene controlling the pea comb shape (qP) is a gene for the crest shape of native Indonesian chickens that is not influenced by foreign breeds. This is associated with the culture of Indonesians who use local chickens as fighting chickens with a pea comb shape so as not to interfere with the vision of the chickens when fighting or competing.

The occurrence of a red-colored comb in Tukong chickens was comparable to that of other local chicken breeds in Indonesia, such as the Black Kedu, Gaga, Merawang, Nunukan, and Sentul chickens (Maharani *et al*. 2021), as well as the Lurik, Wareng, and Ranupane chickens (Suyatno *et al*. 2023), and Bangkok chickens (Winaya *et al*. 2023). Navara *et al*. (2012) suggested that the red coloration of the comb indicated sperm maturity and quality in roosters. Previous research demonstrated that the genes Hydroxyacid Oxidase 1 (HAO1), Bone Morphogenetic Protein-2 (BMP2), SRY-box transcription factor 5 (SOX5), and androgen receptor (AR) were involved in comb morphology and development (Qi *et al*. 2024).

In general, the qualitative morphological characteristics of the chickens in this study were predominantly controlled by ii (feather color), ee (feather color type), bb (feather pattern), ss (feather flicks), IdId (shank color), and P_ (comb type) genes. These genes have also been observed to affect the qualitative morphological traits in several indigenous chicken populations. (Nishida *et al*. 1988). Meanwhile, the genes controlling the traits in Kokok Balenggek chicken in West Sumatra of Indonesia were ii, e+_, bb, ss, Id_, and PP genes, respectively (Arlina *et al*. 2014), which is almost similar to those in Rhode Island Red (e.g., ii, ee, ss, bb, IdId, and pp genes, respectively). Sartika *et al*. (2006) observed ii, ee, ss, bb, IdId, and pp as the dominant genes controlling morphological traits in Nunukan chicken of Indonesia, similar to Rhode Island Red's controlling genes. The Nunukan is a rumpless chicken breed from North Kalimantan with a smaller body size than the Tukong chicken.

The level of introgression rates of exotic breeds into the Tukong chickens was found to determine their authenticity, with a high content of native genes reflecting a higher level of authenticity. According to Nishida *et al*. (1982), the highest introgression rate of the exotic breeds into Indonesian native chickens was contributed by Rhode Island Red and Plymouth Rock bared, while the introgression from White Leghorn was very low. The influence value (rate of introgression) of European and American chickens was reported to be 84% for Wareng chickens, 25% for Kampung chickens (Sartika *et al*. 2008); Kokok Balenggek chicken was 54,7% (Arlina *et al*. 2014); Legund chicken was 52% (Ulfah *et al*. 2012). In comparison, Tukong chickens exhibited a purity gene level of 46.8%. The results were similar to the report from Arlina *et al*. (2014), Badarudin *et al*. (2022), and Jakaria *et al*. (2012). They found that the purity gene levels for Kokok Balenggek and Legund chickens were 45.3% and 48%, respectively. Nishida *et al*. (1982) identified that the purity level of native genes in Indonesian native chicken, particularly Kampong chicken, ranged from 28 to 55%. Differences in breeding practices aimed at improving productivity, as applied by farmers, were suggested to contribute to these variations. The average heterozygosity value of individual Tukong chickens was relatively diverse, at 41.6%. This result was consistent with observations by Widiastuti (2005), who reported that heterozygosity rates for Kampung chickens were between 26% and 45%. Sartika *et al*. (2008) stated that the genetic variability of Kampung chickens (39%) was higher than that of Wareng chickens (16%). Variations in heterozygosity among native chickens in Indonesia were attributed to the extent of crossbreeding with European and American chickens and the selection practices implemented by farmers. Heterozygosity values are critical indicators of genetic diversity within a population, reflecting the genetic variability that can influence the adaptability and resilience of species to environmental changes. High heterozygosity suggests robust genetic diversity, essential for a population's survival and evolutionary potential. It is generally thought that populations with heterozygosity values higher than 0.5 have a lot of genetic diversity, which helps them adapt to changing environments and lowers the risks of inbreeding (Wei *et al*. 2023).

Higher heterozygosity benefits adaptability because it increases genetic variation within a population, essential for responding to environmental changes. A genetically diverse population has a broader range of potential adaptations to new selective pressures, increasing the likelihood that some individuals will thrive under changing conditions (Lohr and Haag 2015). Additionally, genetic diversity helps prevent inbreeding depression, which is particularly problematic in small populations. Inbreeding depression occurs when closely related individuals mate, increasing the chances of harmful alleles being expressed. The resulting loss of heterozygosity can reduce viability and reproductive success, leading to population decline and a higher risk of extinction (Crossman *et al*. 2024). Small populations are especially vulnerable to genetic drift, which can cause harmful mutations to accumulate more rapidly.

However, maintaining or enhancing genetic diversity can counteract these adverse effects, as research has shown that an influx of new genetic material reduces inbreeding depression and improves survival and reproductive traits (Lamka and Willoughby 2024). Conservation strategies that promote genetic connectivity between fragmented populations have also been effective in mitigating inbreeding depression and supporting adaptive responses to environmental changes by preserving higher heterozygosity levels (Bijlsma and Loeschcke 2012).

The findings of this study revealed a considerable genetic purity of the Tukong chickens. According to the information from the farmers, the Tukong chickens were obtained from other family members or through breeding with other nearby chickens. This can limit the transfer of genetic materials from foreign to local genes. To preserve Tukong chickens, conservation strategies must include breeding programs and habitat protection (Noorai *et al*. 2012). Studies have shown that Tukong chickens possess unique genetic characteristics, including the rumpless phenotype, which plays a role in adaptation, survivability, and productivity. Therefore, one of the primary recommendations is to develop a breeding program that maintains the presence of the rumpless gene as a crucial marker in modern breeding systems. This program can be implemented through DNA-based selection and quantitative evaluation of egg and meat production traits (Husien *et al*. 2024).

In situ conservation is also essential to maintaining the genetic diversity of Tukong chickens. This involves protecting native populations in their natural habitat in West Kalimantan by ensuring adequate food availability and minimizing the threat of crossbreeding with commercial chicken breeds. Ex situ approaches can also be undertaken by preserving Tukong chickens in conservation centers or genetic banks, similar to successful initiatives for other local chicken breeds worldwide.

Effective conservation strategies for other breeds with similar diversity levels, such as Kampung and Araucana chickens, involve comprehensive genetic mapping establishing gene banks (Ragamustari and Sukara 2019). Studies by FAO emphasize that maintaining the genetic diversity of local poultry allows for rapid adaptation to environmental changes and evolving selection objectives. A similar approach has been implemented in conserving Piao chickens in China, where specific genetic mutations contributing to their unique characteristics have been identified (Zhang *et al*. 2020; Gu *et al*. 2020).

The preservation of Tukong chickens also contributes to the broader biodiversity of Indonesian poultry. By safeguarding native genetic resources, this conservation program can support livestock ecosystem stability and enhance national food security. Additionally, the genetic diversity of Tukong chickens can be utilized for research and the development of superior breeds with greater resilience to tropical conditions and diseases. Therefore, collaboration between the government, academics, and farmers is necessary to develop a sustainable and science-based conservation policy.

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