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Gorontalo Local Chicken Diversity Based on IGF-1 (Insulin-Like Growth Factor 1) Gene Analysis

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Abstract: This study aims to examine the diversity of local Gorontalo chicken using the IGF-1 (Insulin-Like Growth Factor 1) gene marker. The sampling was conducted in six different areas, namely Gorontalo City, North Gorontalo (Gorut), Bone Bolango (Bonebol), Boalemo, Pohuwato, and Gorontalo Regency (Kabgor). Furthermore, conventional real-time PCR methods were used, and the sequencing was conducted through the obtained product. The results were analyzed using MEGA 11 software, and the data were genetic distance based on nucleotide and protein composition and phylogeny tree. All samples showed high DNA purity (A260/A280) by 1.910-1.925, and the DNA concentration obtained was 82.10 – 82.60 μL. The real-time PCR test showed the Ct value of 18.20 – 18.50, while the melting temperature or Tm is in the range of 81.20 – 82.10°C. The PCR product sequencing and MEGA 11 analysis showed that the farthest genetic distance between chicken from Gorontalo City (City) and Boalemo Regency was 0.8584. The closest genetic distance is from North Gorontalo Regency (Gorut) by 0.0409. In conclusion, nucleotide and protein composition and phylogenetic tree analysis can be used to examine the diversity of local Gorontalo chicken using IGF1 gene markers by looking at genetic distance. The novelty of this study was that we identified the diversity of local Gorontalo chicken using the IGF-1 gene marker.

Keywords: insulin-like growth factor, genetic distance, Gorontalo local chicken.

基於類胰島素生長因子一 基因分析的戈龍塔洛當地雞多樣性

摘要：本研究旨在使用胰島素樣生長因子一基因標記檢查當地戈龍塔洛雞的多樣性。採樣是在六個不同的地區進行的，即哥倫打洛市、北哥倫打洛（格魯特）、骨博蘭戈（骨寶）、博阿萊莫、波胡瓦托和哥倫打洛攝政（卡布戈爾）。此外，採用常規實時聚合酶链式反应方法，對所得產物進行測序。使用美嘉 11 軟件對結果進行分析，數據為基於核苷酸和蛋白質組成和系統發育樹的遺傳距離。所有樣品均顯示出高脫氧核糖核酸純度（一个 260/一个 280），為 1.910-1.925，所得脫氧核糖核酸濃度為 82.10 – 82.60 ・升。實時聚合酶鏈反應 測試顯示 Ct 值為 18.20 – 18.50，而熔解溫度或 Tm 在 81.20 – 82.10 ° C 範圍內。聚合酶鏈反應產物測序和美嘉 11 分析表明，從戈龍塔洛城市（格魯特）到博阿萊莫攝政的雞之間的最遠遺傳距離為 0.8584。最近的遺傳距離是距北哥倫打洛攝政（格魯特）0.0409。總之，核苷酸和蛋白質組成以及系統發育樹分析可用於通過查看遺傳距離來檢查使用胰島素樣生長因子一基因

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標記的當地戈龍塔洛雞的多樣性。這項研究的新穎之處在於我們使用胰島素樣生長因子一 基因標記確定了當地戈龍塔洛雞的多樣性。

关键词：胰島素樣生長因子，遺傳距離，戈龍塔洛本地雞。

1. Introduction

The local Gorontalo chicken is one of the original genetic resources of Indonesia, and there is not enough information on it. This comprehensive study aims to provide more specific information about its diversity. Gorontalo local chicken has been examined by [1] with kinship analysis based on morphometric studies. In the next step, molecular parameters with genetic markers of the IGF-1 gene will be used for diversity study.

Kinship analysis using the IGF-1 gene was conducted on Ayam Ketawa [2] and local (indigenous) chicken using gene markers CO1 and Cyt B [3-6], as well as DNA microsatellite [7-10]. The diversity was examined using various approaches, including bioacoustic and morphometric analysis [1, 11-17]. Furthermore, the use of genetic markers certainly has its challenges compared to the two types of kinship analysis methods described previously. The use of mitochondrial DNA can be used to trace ancestors based on maternal lineage.

Genetic markers for the IGF-1 gene were selected to enhance previous studies with morphometric analysis. The IGF-1 gene affects the pattern of muscle and bone mass formation; therefore, using these genetic markers should provide a complete description of kinship studies. The difference in sampling area and the variety of samples taken should allow a complete description of diversity studies.

Based on this background, this study was conducted using the IGF-1 gene as a genetic marker. The novelty aspect is using the markers to support morphometric differences in chickens from six different regions of Gorontalo province.

2. Methodology

2.1. Study Area

This study was conducted in the Province of Gorontalo (Indonesia), where samples were obtained from six different regions including City = Gorontalo City, BoneBol = Kabupaten Bone Bolango or Bone Bolango Regency, KabGor = Kabupaten Gorontalo or Gorontalo Regency, GorUt = Kabupaten Gorontalo Utara or North Gorontalo Regency, Boalemo = Kabupaten Boalemo or Boalemo Regency, and Pohuwato = Kabupaten Pohuwato or Pohuwato Regency (Fig. 1).

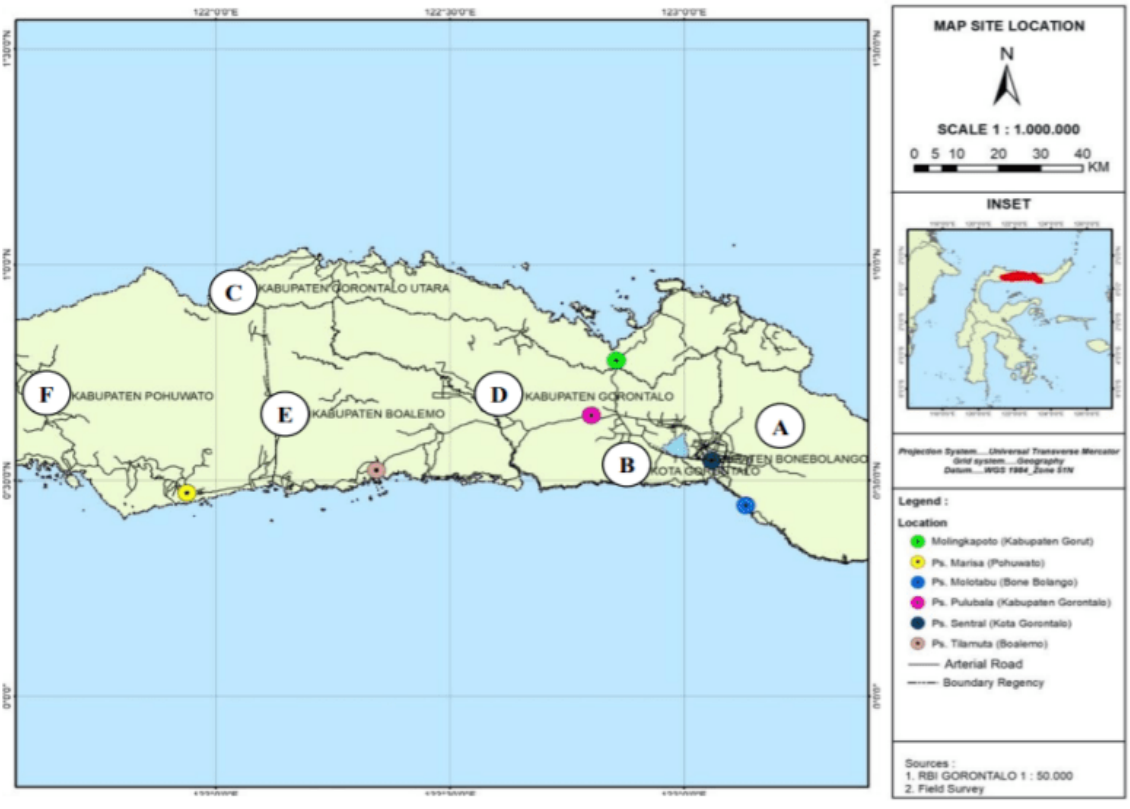

Fig. 1 Sampling location [1]

2.2. Sample

A total of 30 samples were obtained from six regions in Gorontalo Province, Indonesia, namely Gorontalo City (City), North Gorontalo (Gorut), Bone Bolango (Bonebol), Boalemo, Pohuwato, and Gorontalo Regency (Kabgor). Meanwhile, the sample used is the plumage base taken from the tail of a rooster.

2.3. DNA Isolation

DNA isolation was conducted using automatic DNA extraction Qiacube (Qiagen) with the DNA isolation kit Blood Mini (Qiagen). The results were then analyzed for purity and concentration using a nanophotometer [18, 19].

2.4. Master Mix

The master mix used consists of two types, namely for testing through endpoint PCR and real-time PCR.

2.5. Primer

Conventional PCR primers used sequences from [20] with IGF-1F: 5'- GAC TAT ACA GAA AGA ACC CAC -3' IGF-1R: 5'- TAT CAC TCA AGT GGC TCA AGT -3'. Furthermore, primers for real-time PCR were designed from NCBI sites with sequences IGF-1F: 5'- AGA CGC TTA CAC CAC AAG GG -3' IGF-1R: 5'- ACA GAG CGT GCA GAC TTA GG -3'.

2.6. IGF-1 Gene Amplification by PCR

IGF-1 gene amplification by PCR method was conducted using two types, namely real-time and conventional PCR (endpoint PCR).

2.7. Electrophoresis

The PCR products produced will be electrophoresed using automatic electrophoresis Qiaxcel (Qiagen).

2.8. Sequencing

PCR products produced from PCR endpoints will be sequenced at the sequencing service provider institution (Macrogen).

2.9. Bioinformatics Analysis

Bioinformatics analysis was conducted using MEGA 11 to analyze genetic distance, nucleotide composition, and protein components that compile nucleotides.

3. Result and Discussion

3.1. Isolated DNA Analysis

The results of DNA isolation in the sample are shown in Table 1. It shows that the quality of the isolated DNA has a purity in the range (1.910 – 1.925), and the concentration value is in the range of 82.10 – 82.60 μL.

Table 1 Results of DNA isolation on samples

Sampling Location	Nanophotometer Analysis	
	Concentration (ng/uL)	A260/A280
City	82.30	1.925
BoneBol	82.60	1.920
KabGor	82.45	1.910
Gorut	82.10	1.925
Boalemo	82.55	1.920
Pohuwato	82.50	1.915

The data presented in Table 1 shows a fairly constant value of the purity and concentration of DNA since the isolation results are consistent. The consistency of its purity value and concentrations may be caused by the test sample's homogeneity and the isolation process's uniformity. The isolation was conducted using a robotic extraction system; therefore, all isolation stages were controlled and homogeneous. The results of DNA isolation are good when the purity value is in the range of 1.7 – 2.2 and with a concentration value greater than 20 ng/μL [2, 18, 19, 21, 22]. When the purity value is below 1.7, it can be assumed that the isolated DNA is contaminated with a protein. Conversely, when the purity value shows a value above 2.2, it indicates that the isolated DNA is contaminated with RNA.

3.2. Real-Time PCR Analysis

The analysis results with real-time PCR can be seen in Table 2, with amplification results identified at the value of Ct = 18.20 – 18.50, while Tm = 81.20 – 82.10.

Table 2 Real-time PCR analysis results

Sampling Location	Analysis	
	Ct	Tm
City	18.25	82.10
BoneBol	18.20	81.20
KabGor	18.25	81.50
Gorut	18.30	81.20
Boalemo	18.50	82.10
Pohuwato	18.35	82.10

The data in Table 2 shows that all tested samples have adjacent Ct and Tm values. The template concentration influences the Ct value, while the Tm is influenced by the amount of GC base. Two methods are

used for real-time PCR analysis, namely the specific probe and the green SYBR method. The probe method has the advantage of detecting specific genes, while the green SYBR method is more economical [23, 24]. This study uses the green SYBR method to detect the IGF1 target gene, where the data produced are Ct (Cycling-Threshold) and Tm (Melting Temperature) values.

3.3. Electrophoresis Results

The results of PCR amplification (products) of real-time and conventional PCR (endpoint PCR) were then electrophoresed to examine the size of the DNA bands successfully amplified (Fig. 2). Fig. 2A shows real-time PCR product measuring 86 bp, while Fig. 2B shows that the conventional PCR product was 623 bp.

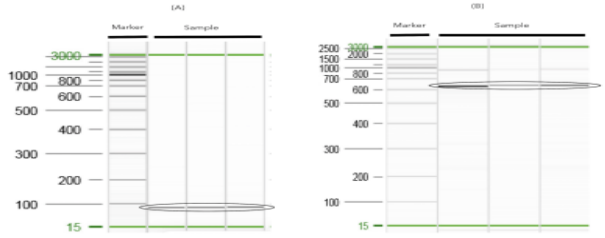

Fig. 2 A) Real-time PCR product electrophoresis results; B) Conventional PCR analysis results

In the data presented in Fig. 1, it can be seen that both analytical techniques using PCR can be amplified, and this is indicated by the electrophoresis results as shown in Fig. 1. It ensures that the two methods can be used for similar analyses. The PCR product will be continued to the sequencing stage to perform a kinship analysis because it is only the initial stage conducted to produce products and amplify DNA templates for the target genes used.

Several studies using RFLP analysis showed that the IGF1 gene in chicken was detected in the range of 621-813 bp; however, polymorphism can be smaller than the amplified gene around 191 – 378 bp [25-28].

The IGF1 gene is a derivative that resembles insulin and plays a role in cell proliferation or metabolism. Therefore, it can be linked to bodyweight analysis or morphometrics in species diversity study [2], [25-29].

3.4. Nucleotide Composition Analysis

The nucleotide composition of the IGF-1 gene sequence can be seen in Table 3, and it shows that the composition of Thymine/Uracil is in the value range of 29.87 – 35.37% with an average of 32.33%. The highest percentage was in chicken sequences originating from Gorontalo City, while the lowest was in BoneBol.

The percentage of cytosine nucleotides is 13.79 – 19.97%, with an average of 15.83%, and the highest was found in chicken sequences originating from the North Gorontalo region. At the same time, the lowest was obtained from Pohuwato and Gorontalo Regency (KabGor).

The percentage of adenine nucleotides ranged from 30.79 – 37.38%, on average 34.76%, and the highest in chicken sequences from the Pohuwato. At the same time, the lowest came from samples obtained from Gorontalo City (City).

Guanine nucleotides have a percentage that ranges from 14.36 – 19.63%, with an average percentage of 17.08%, and the highest was shown in chicken sequences from the Bone Bolango (BoneBol) region, while the lowest was from the North Gorontalo (Gorut).

Table 3 Percentage of nucleotide composition

Sampling Location	Nucleotide Composition (%)				Total (%)
	T(U)	C	A	G	
Gorontalo city	35.75	18.78	30.97	14.50	607.00
BoneBol	29.87	14.60	35.91	19.63	596.00
KabGor	31.40	13.79	36.21	18.60	602.00
Gorut	34.65	19.97	31.02	14.36	606.00
Boalemo	30.83	14.00	37.17	18.00	600.00
Pohuwato	31.40	13.79	37.38	17.44	602.00
Average	32.33	15.83	34.76	17.08	602.17

When the percentage of total nucleotides analyzed is calculated, the results are obtained in the range of 596 – 607%, and samples showed the highest from Gorontalo City (Kota). Preferably, the lowest was obtained from the Bone Bolango (BoneBol).

3.5. Analysis of Protein Composition (Amino Acids)

Based on the composition analysis in the IGF1 gene sequence, 20 types of protein were identified with different percentages (Table 4). The samples from Gorontalo City (City) and Bone Bolango (BoneBol) showed the highest and lowest total amino acids of 187% and 174%, respectively. Meanwhile, the samples from Gorontalo Regency (KabGor) identified the amino acid composition of 177%, and those from North Gorontalo (Gorut) were 186%. The sample from the Boalemo area had a percentage of 177%, while those from the Pohuwato area had 178%.

Table 4 Percentage of amino acid composition

Sampling Location	Types of Amino Acids																			Total
	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	
City	3.2	5.3	5.3	0.5	9.6	1.6	4.3	9.6	5.9	9.1	2.1	6.4	4.3	4.3	2.1	10.2	5.3	4.8	0.5	5.3	187.0
BoneBol	3.4	5.2	3.4	6.3	4.6	5.7	3.4	9.8	9.8	10.3	2.3	3.4	1.1	3.4	2.9	6.9	6.3	4.0	2.3	5.2	174.0
KabGor	4.0	5.1	3.4	6.2	5.1	5.1	2.8	10.2	10.2	10.7	1.7	4.0	0.6	3.4	3.4	6.8	6.2	4.0	2.3	5.1	177.0
Gorut	3.2	4.8	5.9	0.5	8.6	1.1	2.7	10.2	5.4	10.2	1.6	5.9	5.4	5.9	2.2	10.2	5.9	4.8	0.5	4.8	186.0
Boalemo	3.4	5.1	4.0	6.2	4.5	3.4	3.4	10.2	11.9	11.3	1.7	4.5	0.6	3.4	2.8	6.2	6.2	5.1	2.3	4.0	177.0
Pohuwato	3.4	3.9	3.9	6.2	5.6	3.9	2.8	10.7	10.1	10.7	1.7	4.5	0.6	3.4	2.8	6.2	6.7	4.5	2.2	6.2	178.0
Average	3.4	4.9	4.4	4.3	6.4	3.4	3.2	10.1	8.8	10.4	1.9	4.8	2.1	4.0	2.7	7.8	6.1	4.5	1.7	5.1	179.8

Proximity analysis based on protein composition showed that Gorontalo (City) chickens were closest to those from North Gorontalo (Gorut). The next closeness was from Pohuwato, Boalemo, and KabGor, respectively. The chicken farthest from Gorontalo City based on amino acid composition analysis was Bone Bolango by 174%.

3.6. Genetic Distance Analysis

Table 5 shows that the farthest genetic distance is found in chicken from Gorontalo Regency (KabGor) and North Gorontalo (GorUt) of 0.8871. However, the closest was shown between chicken from the Pohuwato area and Boalemo of 0.0308.

Table 5 Genetic distance analysis

	City	BoneBol	KabGor	Gorut	Boalemo	Pohuwato
City						
BoneBol	0.8022					
KabGor	0.8442	0.0414				
Gorut	0.0409	0.8604	0.8871			
Boalemo	0.8584	0.0634	0.0501	0.8864		
Pohuwato	0.8236	0.0470	0.0341	0.8632	0.0308	

Genetic distance analysis showed that chicken from Gorontalo city has the longest distance from the Boalemo area. Furthermore, several factors can be used as reasons, including the geographical conditions of the region. Chicken from the Boalemo has a larger body size when compared to the other five regions since it is an agricultural and transmigrant area rich in feed ingredients for livestock. The information from the community showed that the chicken was brought from Java (Kediri chicken) with a body size larger than the local breed. Another factor is the presence of invasive species such as Bangkok chicken with a large body size. Furthermore, the community prefers to keep chickens with larger body sizes as a better economic source when compared to smaller bodies. Therefore, further study of molecular analysis is recommended to trace the ancestor of Gorontalo local chicken and examine its kinship pattern to other local breeds in Indonesia.

3.7. Phylogeny Tree Analysis

The phylogenetic tree analysis was made using the MEGA 11 application with the neighbor-join tree method and bootstrap 1000 times. Fig. 3 shows that all the resulting bootstrap scores are 71, 86, and 100. The results can be considered valid when the bootstrap value is greater than 55%. However, according to [30], it can be declared valid when greater than 70%. The above is the change value in the sequence of mutations resulting from the analysis of the target gene sequence used to identify kinship relationships between chickens from six different regions. According to [30], MEGA is an application that calculates genetic distances and visualizes data in the form of phylogenetic trees. It is software designed to perform comparative studies of DNA and protein sequences [31]. The phylogenetic tree analysis showed that chickens from Gorontalo City (Kota) have a close relationship with North Gorontalo (Gorut).

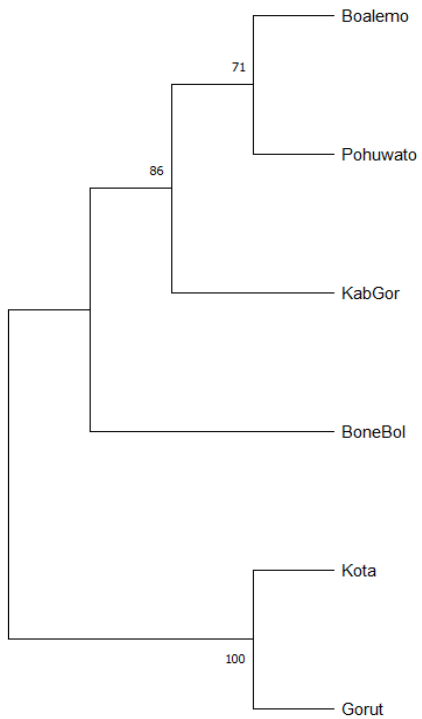

Fig. 3 Phylogeny tree analysis

The results of this phylogenetic tree analysis can support the previous study [1]. In addition, the IGF1 gene plays a role in muscle and bone formation and can be used to perform kinship analysis [2]. Several previous studies were conducted to analyze the

relationship between IGF1 and body morphometrics, and the results showed that this gene has a significant role in body weight and diversity [20, 26-29, 32-34].

4. Conclusion

Following the results, it is reasonable to conclude that the IGF-1 gene marker can be used to study the diversity of Gorontalo local chicken by analyzing their genetic distance based on the composition of nucleotide, amino acids, and phylogenetic trees.

Acknowledgments

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