第 49 卷 第 1 期
2022 年 1 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 1
January 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.1.28

Study of Co1 and BIK BCL2 Gene Analysis in Gorontalo Local Chicken

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Abstract: Analysis studies of Co1 and BIK BCL2 genes in local Gorontalo chickens were carried out using real-time PCR. This study aims to detect the presence of Co1 and BIK BCL2 genes in local Gorontalo chickens so that they can be used as information on gene diversity that can be used as genetic markers to study the diversity of local chicken species in Indonesia. The method used in this study is the SYBR green real-time PCR method. The research conducted found that the concentration values of the extracted samples were in the range of 68.15-68.30 ng/μL. Meanwhile, the purity values measured at wavelength A260/A280 were obtained with purity ranges between 1.867-1.923. The amplification results of the Co1 target gene show that the Ct value is in the range of 20.90-21.20, while for Tm, it is 84.10-84.20. In the BIK BCL2 target gene analysis, the Ct value was in the range of 16.15-16.20, while for Tm, it was 91.10-91.20. From these results, it can be concluded that all research samples were sampled from six different regions detected with CT and Tm values from the real-time analysis carried out obtained homogeneous values and no more than 2 degrees of difference between samples. The research novelty was using Nisi chicken as the native, one of the local chickens in Indonesia as the object of study. Besides, we used a BIK BCL 2 gene as the marker to determine the genetic diversity of the local chicken. A suggestion for further research is to explore more diverse genes to obtain more data on genetic diversity.

Keywords: chicken, Co1, BIK BCL2, PCR.

哥倫打羅地方雞細胞色素氧化酶 和比科爾乙細胞淋巴瘤2 基因分析研究

摘要：使用實時聚合酶鏈反應對當地哥龍打洛雞中的細胞色素氧化酶和比科爾B細胞淋巴瘤2基因進行分析研究。本研究旨在檢測哥倫打洛當地雞中細胞色素氧化酶和比科爾B細胞淋巴瘤2基因的存在，以便將它們作為基因多樣性的信息，作為遺傳標記來研究印度尼西亞當地雞物種的多樣性。本研究使用的方法是協同品牌綠色實時聚合酶鏈反應方法。研究發現，提取樣品的濃度值在68.15-
68.30納克/微升範圍內。同時，在一个260/一个280波長處測得的純度值在1.867-
1.923的純度範圍內。細胞色素氧化酶靶基因的擴增結果表明，循環閾值值在20.90-
21.20之間，而熔點溫度在84.10-
84.20之間。在比科爾乙細胞淋巴瘤2靶基因分析中，循環閾值值在16.15-
16.20之間，而熔點溫度在91.10-
91.20之間。從這些結果可以得出結論，所有研究樣本均來自六個不同區域的樣本，通過實時分析獲得的电脑断层扫描和Tm值檢測得到均勻的值，樣本之間的差異不超過2度。這項研究

Received: October 16, 2021 / Reviewed: November 14, 2021 / Accepted: December 21, 2021 / Published: January 28, 2022

Fund Project: PUTI 2020 (Hibah Publikasi Terindeks Internasional with contract no. NKB-4265/UN2.RST/HKP.05.00/2020), Universitas Indonesia (Research Grant NKB-1678/UN2.RST/HKP.05.00/2020)

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的新穎之處是使用尼西雞作為本地雞，印度尼西亞當地的一隻雞作為研究對象。此外，我們使用比科爾B細胞淋巴瘤2基因作為標記來確定當地雞的遺傳多樣性。進一步研究的建議是探索更多樣化的基因以獲得更多關於遺傳多樣性的數據。

关键词：雞，細胞色素氧化酶，比科爾乙細胞淋巴瘤2，聚合酶鏈反應。

1. Introduction

Gorontalo local chicken is a native Indonesian chicken that is also one of the biological resources to be preserved. One of the efforts to maintain this sustainability is to conduct a study to obtain sufficient information about the existence of this species so that it can assist in further study efforts. Initial studies in this study have been carried out by looking at the morphometric analysis of six different regions [1]. In this study, further studies were carried out to see the diversity of its genes.

Molecular analysis to see diversity or conduct indigenous studies has been carried out [2-10]. This research has been carried out using several genetic markers commonly used, namely Cyt B [8], CO1 [2, 7, 8], IGF-1 [11], and Microsatellite [3-6]. So far, the information obtained about the molecular analysis carried out in several of these studies is still using endpoint PCR analysis (conventional PCR), so there is a need for new technology in carrying out this analysis using real-time PCR. Several studies were carried out using real-time PCR for testing the DNA of new chicken species in the form of analysis to detect viruses in chickens [12-15], bacteria in chickens [16], as well as detection of species DNA in processed food products of chicken meat [17]. Studies on local chickens have been carried out on several types of chickens in Indonesia, such as laughing chickens, pelung chickens, balenggek kokok chickens, and many more. The information found is that there are at least 36 local Indonesian chicken lines [1, 11, 18-24].

The selection of the Co1 gene in this study is because this gene will be used as a confirmation gene that is already commonly used, while the BIK BCL gene is a specific gene whose presence can be used for further studies for further research that wants to develop this genetic marker. Research using the BIK BCL2 genetic marker is still very limited because this gene is an Interacting Killer gene. Several studies on this gene have been carried out to see the functional characterization of the Bcl-2 gene family in zebrafish. The involvement of Bcl-2 family proteins in AKT-regulated cell survival in cisplatin-resistant epithelial ovarian cancer, In-silico analysis of the BCL2 gene using multiple bioinformatics tools to identify the most lethal mutations that are crucial for its structural and functional integrity, and Translating cell death discoveries into novel cancer therapies [25].

So based on this background, this study was conducted to provide the latest information regarding the analysis of the Co1 and BIK BCL2 genes in local Gorontalo chickens. This study is an initial study to explore local Gorontalo chickens so that the information obtained from this study can benefit the diversity of chicken species in Indonesia. The novelty aspect raised in this study is the use of BIK BCL2 genetic markers, which are still limited, requiring more information so that a comprehensive study can be carried out further.

2. Methodology

2.1. Sample

The sample was used in the form of 30 roosters taken from a young tail feather and then extracted. The number of 30 tails was sampled from six regions in Gorontalo Province, namely: City, BoneBol, Kabgor, Gorut, Boalemo, and Pohuwato, the number of which was adjusted to the availability of samples where the total sample that had to be fulfilled was 30 individuals representing the 6 regions.

2.2. DNA Isolation

DNA isolation was carried out using the Dneasy Mericon Food [Qiagen] kit, isolated using Qiacube [Qiagen] automatic DNA extraction. The working stage begins by weighing a sample weighing 1 g, then put in a 2 mL centrifuge tube and adding 800 µL of lysis buffer and 20 µL of proteinase K. After this addition, and it is followed by incubation at 70°C for 60 minutes. After the incubation process is complete, the next step is followed by centrifugation at a speed of 14000 rpm for 5 minutes. After this process is complete, the solution in the tube will be divided into two layers, take the supernatant, put it in a new 2 mL centrifuge tube, add 800 µL of chloroform, then vortex for 1 minute, and then centrifuged again at 14000 rpm for 10 minutes when this step is complete, pipette 350 µL of supernatant and insert it into a new 1.5 mL tube. Turn on Qiacube and set the standard Dneassy maricon Food method with a final elution of 100 µL. Inserting tube containing 350 µL of supernatant into the Qiacube according to the method protocol has been made. After this stage is complete, the isolated DNA can be used immediately or stored in a freezer at a temperature of -20℃.

2.3. Analysis of Isolated DNA

The isolated DNA was then measured for its quality by looking at the concentration and purity values using the NP80 nano photometer (IMPLEN). The methods used were Nucleic acid, dsDNA type, nano volume mode, 2 L sample volume, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1,000 [27, 28].

2.4. Primer Used

The primer used in this study was independently designed from the NCBI site with a 113 bp Co1 gene primer sequence consisting of the Forward primer sequence: 5'-TAT CGG AGG CCT AAC GGG AA-3' and the Reverse primer sequence: 5'- AAA CTG CCC CCA TTG AGA GG -3'. Likewise, the BIKBCL2 primer has a sequence length of 186 bp consisting of forwarding primers: 5'- GGC GAA GCA GGA GGA AAT AAG CG -3' and Reverse primer sequence: 5'- CGC ACA CCA GAA AAC CTT CG -3'.

2.5. Master Mix Setup

In this study, the QuantiNova SYBR Green master mix was used with the primer and master mix set up as follows: mix 15 µL of the master mix, then add 2 µL of forwarding primer and 2 µL of reverse primer, and 8 L of DNA template then fill with nucleotide-free water 3 µL for a final volume of 30 µL. This master mic mix is entered in real-time PCR and ready for analysis.

2.6. Setup Real-Time PCR

The method used in conducting Real-time PCR analysis is a two-step method with the following setup: Pre-denaturation at 95°C for 15 minutes for 1 cycle, denaturation at 95°C for 30 seconds for 40 cycles, and annealing at 60°C for 60 seconds for 40 cycles. After that, set the melt curve analysis: ramp from 50°C to 90°C, hold for 90 s on the 1st step and further for 5 s.

3. Result and Discussion

3.1. Analysis of Isolated DNA

The isolated DNA was analyzed for purity and concentration using a nano photometer, and the results were obtained as presented in Table 1. From the table, it can be seen that the concentration values of the extracted samples are 68.15-68.30 ng/µL, while the purity values measured at wavelength A260/A280 are obtained with a purity range 1.867-1.92.

Table 1 DNA isolation analysis result

Sample	Nanophotometer results	
	Purity A260/A280	Concentration (ng/ui)
Kota	1.875	68.20
Kabgor	1.888	68.15
Bonebol	1.867	68.30
Boalemo	1.923	68.10
Gorut	1.901	68.20
Pohuwato	1.890	68.20

Note: The sample used is the average of 30 samples

If viewed from (Table 1) above, it can be seen that the data on the purity and concentration values are quite uniform that happened because the samples used were weighed with the same weight and isolated using an automatic isolation device. This system produces a more stable insulation power because most of the steps are carried out by a robotic system. DNA isolation techniques have resulted in several types of automatic isolation technologies with a spin column system or magnetic beads. These techniques have their respective advantages so that their use can be adjusted to the needs of the PCR method that will be used to perform the analysis.DNA analysis results from good isolation are in the range of purity values from 1.7 to 2.2 with a more than 20 ng concentration. Some indications that the DNA isolated from the results did not give maximum results can be seen from the purity of the isolated DNA, where if the purity value is below 1.7, it can be suspected that the isolated DNA is contaminated with a protein. In contrast, if it is above 2.2, it can be suspected that the isolated DNA is contaminated with RNA [11, 26, 27]. In conducting DNA isolation, it is very important to know that each step must be carried out properly according to the work instructions in the manual kit used. The use of several extraction tools will also be very helpful in producing good-quality DNA isolation. Isolated DNA was analyzed for purity and concentration using a nano photometer, and the results were obtained as presented in (Table 1). From the table, it can be seen that the concentration values of the extracted samples are in the range of 68.15-68.30 ng/µL, while the purity values measured at wavelength A260/A280 are obtained with a purity range 1.867-1.92.

The lysis stage in the DNA isolation process is an important step where at this stage the cell will be lysed using the proteinase K enzyme. This enzyme works by digesting or destroying the proteins that make up the cell so that lysis occurs. Proteinase K is an enzyme actively working when incubated at a temperature of 65-70°C. Several studies have shown that optimizing the incubation period of this enzyme can work from 30 minutes to 3 hours. Therefore, if using this technique in the incubation, it is better to start with the optimization. If there is a failure during isolation, one of the steps that must be optimized is the initial lysis stage [26, 27].

In addition to proteinase K, in the lysis process, there is also cetyl trimethyl ammonium bromide (CTAB) or SDS, which works requiring a reducing agent such as B mercaptoethanol, which helps in denaturing proteins by breaking the disulfide bonds between residual cysteine and removing tannins and polyphenols present in the sample. Chelating agents such as EDTA bind to magnesium ions required for DNase activity. Buffers used are Tris at pH 8 and salts such as sodium chloride, which aid in precipitation by

neutralizing the negative charge on DNA so that the molecules can stick together.

The most common precipitation used in DNA isolation is 70-96% alcohol in the precipitation stage. The addition of alcohol to the DNA isolation solution blinds the nucleic acid will spontaneously precipitate on the spin column filter so that the separation of inhibitors can be carried out by centrifugation. In some types of kits, the centrifugation rotation speed may be different. Washing of salts can be done using 70% alcohol. The most common salts used in DNA washing are 0.3 M sodium acetate, 0.2 M sodium chloride, 2-2.5 M ammonium acetate, 0.8 M lithium chloride, and potassium chloride. All these salt ingredients are then diluted using alcohol or isopropyl.

The final stage of the isolation process is elution. The DNA pellet bound to the silicon spin column membrane will be eluted at this stage. The generally used buffer is nucleate-free water or tris HCl 1 mM EDTA.

3.2. Real-Time PCR Analysis

Table 2 shows the results of the real-time PCR analysis. The amplification results of the Co1 target gene show that the Ct value is in the range of 20.90 – 21.20, while for Tm, it is 84.10 – 84.20. In the BIK BCL2 target gene analysis, the Ct value was in the range of 16.15 – 16.20, while for Tm, it was 91.10 – 91.20.

Table 2 Real-time PCR amplification result

Sampling Location	Target genes			
	Co1		BIKBCL2	
	Ct	Tm	Ct	Tm
Kota	21.20	84.10	16.20	91.20
Kabgor	21.10	84.15	16.15	91.10
Bonebol	20.90	84.10	16.20	91.20
Boalemo	20.80	84.20	16.15	91.10
Gorut	21.15	84.10	16.20	91.20
Pohuwato	20.95	84.10	16.20	91.15
NTC Control	Undetermined			

Generally, two types of real-time PCR analysis methods are often used, namely the specific probe method and the green SYBR method. Both of these methods have their respective advantages and disadvantages compared to their use. The specific probe method has the advantage of detecting specific genes, while the green SYBR method is more economical in terms of the price of the kit used in conducting research. In this study, the method used is the SYBR green method, wherein the data produced are in the form of Ct (Cycling-Threshold) and Tm (Melting Temperature).

When viewed from (Table 2), the results obtained show uniformity because all the templates used in real-time PCR analysis are balanced in concentration to have the same concentration value when the analysis is carried out. In the Ct analysis, the resulting value is influenced by the concentration, while in the Tm analysis, the resulting value is influenced by the concentration of the G-C primer used. This is in line with research conducted by [28, 29], which states that in studies using real-time PCR, the concentration of template and primer sequences used can affect the Ct and Tm values in the sample.

3.3. Analysis of Co1 Genes

The Co1 gene (Cytochrome Oxidase 1) is one of the mitochondrial DNA genes often used as a DNA barcode in molecular analysis. In some studies, the use of this gene is still used using the endpoint PCR technique so that the primary sequence used is still longer compared to the primary sequence in this study.

In a study conducted by [2], the Co1 gene was tested for its reliability in conducting studies on indigenous local chickens in China by looking at its diversity using the DNA barcode method with the CO1 target gene. In this study, the primer used was 590 bp in size. This study found that using the CO1 gene as a genetic marker gave good results for conducting this study that is in line with research [4]. It studied new chicken species (Danzhou chicken) in China, found in 2014. This study found that Danzhou chickens had less variation in the Co1 gene when compared to chickens. Other Chinese locals are Onion Chicken and Wenchang Chicken.

Therefore, this study uses the Co1 gene so that in the future, a thorough study can be carried out using specific genes and DNA barcodes in conducting analytical studies of Gorontalo local chickens so that complete genetic information can be obtained to be used as analytical material in identifying the presence of local chickens. Gorontalo is one of the genetic resources that kinship with other local Indonesian chickens. The use of real-time PCR in this study is also expected to provide other information in conducting studies so that molecular analysis does not only use endpoint PCR but can also use real-time PCR to perform further analysis for detection of specific genes.

The results of the real-time PCR analysis in this study using the genetic marker of the CO1 gene as the target gene can be seen in Fig. 1. The results of the cycling amplification (Fig. 1) show that the Co1 gene was detected at a Ct value of 20.80 – 21.20. In real-time PCR analysis, the homogeneity value of Ct can be shifted by more than 2 degrees from the set value so that if the resulting Ct value is still in the range of more than two correction values, it can be concluded that the CO1 genes analyzed are the same and not significantly different. Likewise, with the Tm value, the resulting data shows the range of detected Tm in 84.10 – 84.20. This value also has a difference of not less than 2 degrees.

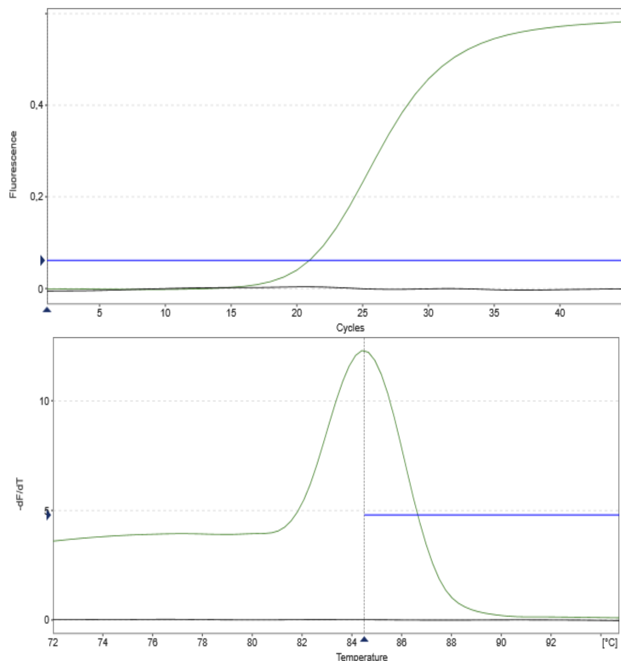

Fig. 1 CO1 gen gene real-time PCR analysis

The Co1 gene in chickens varies depending on the primary sequence used. In a study conducted by [7], the CO1 gene has a size of 565 bp, while in study conducted by [4], the CO1 gene has a size of 651 bp. Another study also produced different sequences with a length of 590 bp [2]. The primers used from the above studies are primers used for analysis using endpoint PCR, while for real-time PCR analysis, the primers used must be designed with a length between 85-300 bp so that they can be properly amplified by real-time PCR.

3.4. BIK BCL2 Gene Analysis

Analysis of the BIK BCL 2 gene is shown in Fig. 2. The BIK BCL 2 gene is a specific gene that encodes the interacting killer system in animals. Information about this gene in diversity analysis studies or its use as a target gene for study analysis is still very limited, so this study was conducted to identify the presence of this gene in local chicken species. In the future, it is hoped that the presence of this gene can be used as initial information for a comprehensive study of the characteristics and 3D composition of the BIK BCL 2 gene so that we can recognize its function and use in conducting species DNA studies.

The BIK BCL 2 gene was designed from the NCBI site, and several primary candidates were used. In this study, the primers used were chosen by considering several things, including:

From the real-time PCR analysis that was carried out, it was known that the BIK BCL2 gene in chickens was detected at Ct 16.15 – 16.20 with a Tm value of 91.10 – 91.20. Similar to the Co1 analysis in the discussion above, the difference in Ct and Tm values , which are less than 2 degrees Ct and Tm numbers, indicates no difference in BIK BCL2 gene diversity Gorontalo local chickens even though they come from six different regions.

In the melt culture analysis, which is marked by the formation of a melting point at a certain temperature, this value is influenced by the primary sequence content in the form of GC content. The primer used for this study has a molecular weight of 6250.1 µg/µmole with µg/OD of 25.17 with % GC content of 55%, the value of Tm (1M Na+) is 86 and the value of Tm (50 mM Na+) is 64.

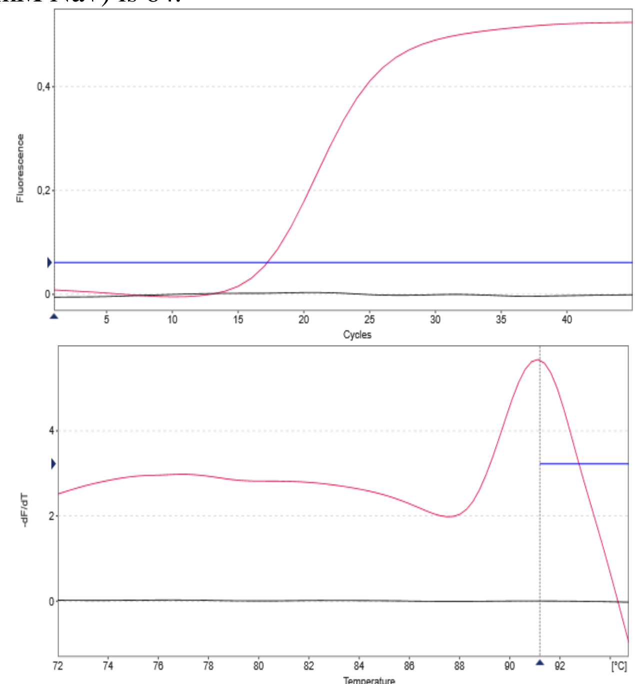

Fig. 2 Real-time PCR analysis of BIK BCL2 gene

The limited information regarding the use of the BIK BCL 2 gene is a challenge in itself in this study so that one of the benefits that can be obtained from the use of BIK BCL2 genetic markers in this study is as one of the information that can be used in further research for a more comprehensive study.

4. Conclusion

This study concludes that in all research samples sampled from six different regions, the Co1 and BIK BCL 2 genes were detected where the CT and Tm values from the real-time analysis were obtained that were homogeneous and not more than 2 degrees of difference between samples. We used the Nisi chicken as the object of study, and BIK BCL 2 gene for determining the chicken diversity was the novelty of this research and the perspective research in the future. The limitation of this study was sample collection in the pandemic era. A suggestion for further research is to explore more diverse genes so that more molecular data can be obtained on the research sample's chicken species.

References

[1] SOPHIAN A., ABINAWANTO, NISA U.C., and FADHILLAH. Morphometric analysis of Gorontalo

(Indonesia) native chickens from six different regions. *Biodiversitas*, 2021, 22(4): 1757-1763. DOI: 10.13057/bio div/d220420.

[2] CUI H., IBTISHAM F., XU C., HUANG H., and SU Y. DNA barcoding of Chinese native chicken breeds through COI gene. *Thai Journal of Veterinary Medicine*, 2017, 47(1): 123-129.

[3] OKUMU O.N., NGERANWA J., BINEPAL Y., KAHI A., BRAMWEL W.W., ATEYA L.O., and WEKESA F. Genetic diversity of indigenous chickens from selected areas in Kenya using microsatellite markers. *Journal of Genetic Engineering & Biotechnology*, 2017, 15(2): 489-495. DOI: 10.1016/j.jgeb.2017.04.007.

[4] PENG W., YANG H., CAI K., ZHOU L., TAN Z., and WU K. Molecular identification of the Danzhou chicken breed in China using DNA barcoding. *Mitochondrial DNA Part B Resources*, 2019, 4(2): 2459-2463. DOI: 10.1080/23802359.2019.1638321.

[5] ROH H.J., KIM S.-C., CHO C.-Y., LEE J., JEON D., KIM D.-K., KIM K.-W., AFRIN F., KO Y.-G., LEE J.-H., BATSAIKHAN S., SUSANTI T., HEGAY S., KONGVONGXAY S., AMATYA N. GORKHALI, THI L.A.N., THAO T.T.T., and MANIKKU L. Estimating genetic diversity and population structure of 22 chicken breeds in Asia using microsatellite markers. *Asian-Australasian Journal of Animal Sciences*, 2020, 33(12): 1896-1904. DOI: 10.5713/ajas.19.0958.

[6] NXUMALO N., CECCOBELLI S., CARDINALI I., LANCIONI H., LASAGNA E., and KUNENE N.W. Genetic diversity, population structure and ancestral origin of KwaZulu-Natal native chicken ecotypes using microsatellite and mitochondrial DNA markers. *Italian Journal of Animal Science*, 2020, 19(1): 1277-1290. DOI: 10.1080/1828051X.2020.1838350.

[7] KARP-TATHAM E., KÜSTER T., ANGELOU A., PAPADOPOULOS E., NISBET A.J., XIA D., TOMLEY F.M., and BLAKE D.P. Phylogenetic Inference Using Cytochrome C Oxidase Subunit I (COI) in the Poultry Red Mite, *Dermanyssus gallinae* in the United Kingdom Relative to a European Framework. *Frontiers in Veterinary Science*, 2020, 7(8): 1-13. DOI: 10.3389/fvets.2020.00553.

[8] DAVE A.R., CHAUDHARY D.F., P.M.MANKAD, KORINGA P.G., and RANK D.N. Genetic diversity among two native Indian chicken populations using cytochrome c oxidase subunit I and cytochrome b DNA barcodes. *Veterinary World*, 2021, 14(5): 1389-1397. DOI: 10.14202/vetworld.2021.1389-1397.

[9] MALOMANE D.K., WEIGEND S., SCHMITT A.O., WEIGEND A., REIMER C., and SIMIANER H. Genetic diversity in global chicken breeds in relation to their genetic distances to wild populations. *Genetics Selection Evolution*, 2021, 53(1): 1-11. DOI: 10.1186/s12711-021-00628-z.

[10]SABRY A., MOHAMED A.A., and HASSEN M. Genetic Diversity of Indigenous Chicken (Gallus Gallus domesticus) from Ecozones of Egypt and Kingdom of Saudi Arabia. *Open Journal of Applied Sciences*, 2021, 11(06): 775-787. DOI: 10.4236/ojapps.2021.116057.

[11] ABINAWANTO, SOPHIAN A., LESTARI R., BOWOLAKSONO A., EFENDI P.S., and AFNAN R. Analysis of IGF-1 gene in Ayam ketawa (*Gallus gallus domesticus*) with dangdut and slow type vocal characteristics. *Biodiversitas*, 2019, 20(7): 2004-2010. DOI: 10.13057/bio div/d200729.

[12] CONG F., ZHU Y., WANG J., LIAN Y., LIU X., XIAO L., HUANG R., ZHANG Y., CHEN M., and GUO P. A multiplex xTAG assay for the simultaneous detection of five chicken immunosuppressive viruses 11 Medical and Health Sciences 1108 Medical Microbiology 06 Biological Sciences 0605 Microbiology. *BMC Veterinary Research*, 2018, 14(1): 1-8. DOI: 10.1186/s12917-018-1663-1.

[13] BOO S.Y., TAN S.W., ALITHEEN N.B., C.L. HO, OMAR A.R., and YEAP S.K. Identification of Reference Genes in Chicken Intraepithelial Lymphocyte Natural Killer Cells Infected with Very-virulent Infectious Bursal Disease Virus. *Scientific Reports*, 2020, 10(1): 1-9. DOI: 10.1038/s41598-020-65474-3.

[14] XIANG Y., LI L., LIU P., YAN L., JIANG Z., YU Y., LI Y., CHEN X., and CAO W. Rapid detection of avian leukosis virus subgroup J by cross-priming amplification. *Scientific Reports*, 2021, 11: Article number: 10946. DOI: 10.1038/s41598-021-90479-x.

[15] LI Q., ZHANG Y., MENG F., JIANG H., XU G., DING J., ZHANG Y., DONG G., TIAN S., CHANG S., and ZHAO P. A New Strategy for the Detection of Chicken Infectious Anemia Virus Contamination in Attenuated Live Vaccine by Droplet Digital PCR. *BioMed Research International*, 2019: 2750472. DOI: 10.1155/2019/2750472.

[16] KUCHIPUDI S.V., YON M., NAIR M.S., BYUKUSENGE M., BARRY R.M., NISSLY R.H., WILLIAMS J., PIERRE T., MATHEWS T., WALNER-PENDLETON E., DUNN P., BARNHART D., LOUGHREY S., DAVISON S., KELLY D.J., TEWARI D., and JAYARAO B.M. A Highly Sensitive and Specific Probe-Based Real-Time PCR for the Detection of Avibacterium paragallinarum in Clinical Samples From Poultry. *Frontiers in Veterinary Science*, 2021, 8(4). DOI: 10.3389/fvets.2021.609126.

[17] DOLCH K., ANDRÉE S., and SCHAWAGELE F. Comparison of Real-Time PCR Quantification Meat Products. *Foods*, 2020, 9(1049): 3-15.

[18] ABINAWANTO A., and EFFENDI P.S. The bioacoustics analysis and the morphometric study of the gaga's chicken (Ayam Ketawa) from Pinrang and Kebayoran Lama. *AIP Conference Proceedings*, 2018, 2023(1). DOI: 10.1063/1.5064134.

[19] ABINAWANTO, SOPHIAN A., EFFENDI P., and SISWANTINING T. Short communication: Variation in vocal cord morphometric characters among dangdut type and the slow type gaga chicken. *Biodiversitas*, 2018, 19(5): 1902-1905. DOI: 10.13057/bio div/d190542.

[20] ARLINA F., RUSFIDRA, ANDRIANO D., and SUMATRI C. Short communication: The type and sound diversity of kukuak balenggek chicken (gallus gallus domesticus) reared in West Sumatra, Indonesia. *Biodiversitas*, 2020, 21(5): 1914-1919. DOI: 10.13057/bio div/d210518.

[21] MUSTOFA F., FATHONI A., SARI A.P.Z.N.L., SASONGKO H., and MAHARANI D. Bodyweight and body size measurement of five Indonesian local chicken. *IOP Conference Series: Earth and Environmental Science*, 2021, 788(1). DOI: 10.1088/1755-1315/788/1/012016.

[22]BUGIWATI S.R.A., DAGONG M.I.A., and TOKUNAGA T. Crowing characteristics of native singing chicken breeds in Indonesia. *IOP Conference Series: Earth and Environmental Science*, 2020, 492(1). DOI: 10.1088/1755-1315/492/1/012100.

[23]DARYONO B.S., MUSHLIH M., and PERDAMAIAN A.B.I. Crowing sound and inbreeding coefficient analysis of Pelung chicken (*Gallus Gallus domesticus*). *Biodiversitas*, 2021, 22(5): 2451-2457. DOI: 10.13057/bio div/d220501.
[24]ABINAWANTO, ZULISTIANA T., LESTARI R., DWIRANTI A., and BOWOLAKSONO A. The genetic diversity of ayam ketawa (*Gallus gallus domesticus*, Linneaus, 1758) in bangkalan district, madura island, indonesia. *Biodiversitas*, 2021, 22(6): 3145-3155. DOI: 10.13057/biodiv/d220617.
[25]GHANI U., ALI Y., GUL K., ULLAH R., KHAN A., and WEI D.-Q. In-silico analysis of BCL2 gene using multiple bioinformatics tools to identify the most lethal mutations that are crucial for its structural and functional integrity. 2021: 1-12 [Online]. Available from: https://doi.org/10.21203/rs.3.rs-310735/v1
[26]DAI Y., JIN S., LI X., and WANG D. The involvement of Bcl-2 family proteins in AKT-regulated cell survival in cisplatin-resistant epithelial ovarian cancer. *Oncotarget*, 2017, 8(1): 1354-1368. DOI: 10.18632/oncotarget.13817.
[27]DELBRIDGE A.R.D., GRABOW S., STRASSER A., and VAUX D.L. Thirty years of BCL-2: Translating cell death discoveries into novel cancer therapies. *Nature Reviews Cancer*, 2016, 16(2): 99-109. DOI: 10.1038/nrc.2015.17..
[28]SOPHIAN A., PURWANINGSIH R., MUINDAR, IGIRISA E., and AMIRULLAH M. Short Communication: Analysis of purity and concentration of DNA extracted from intron patho gene-spin extraction on crab processed food product samples. *Asian Journal of Tropical Biotechnology*, 2021, 18(1). DOI: 10.13057/biotek/c180103.
[29]SOPHIAN A. Short Communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. *Asian Journal of Natural Product Biochemistry*, 2021, 19(1): 21-24. DOI: 10.13057/biofar/f190104.

参考文:

[1] SOPHIAN A.、ABINAWANTO、NISA U.C. 和 FADHILLAH。來自六個不同地區的哥倫打洛（印度尼西亞）本土雞的形態計量分析。生物多樣性, 2021, 22(4): 1757-1763。 DOI：10.13057/bio div/d220420。
[2] CUI H., IBTISHAM F., XU C., HUANG H., 和 SU Y. 通過 COI 基因對中國本土雞品種進行 DNA 條形碼分析。泰國獸醫雜誌, 2017, 47(1): 123-129.
[3] OKUMU O.N., NGERANWA J., BINEPAL Y., KAHI A., BRAMWEL W.W., ATEYA L.O. 和 WEKESA F. 使用微衛星標記從肯尼亞選定地區的本土雞的遺傳多樣性。基因工程與生物技術雜誌, 2017, 15(2): 489-495. DOI：10.1016/j.jgeb.2017.04.007。
[4] PENG W., YANG H., CAI K., ZHOU L., TAN Z., 和 WU K. 利用 脫氧核糖核酸 條形碼對中國儋州雞品種進行分子鑑定。線粒體 脫氧核糖核酸 B 部分資源，2019，4(2)：2459-2463。DOI：10.1080/23802359.2019.1638321。
[5] ROH HJ、KIM S.-C.、CHO C.-Y.、LEE J.、JEON D.、KIM D.-K.、KIM K.-W.、AFRIN F.、KO Y.-G.、LEE J.-H.、BATSAIKHAN S.、SUSANTI T.、HEGAY S.、KONGVONGXAY S.、AMATYA N. GORKHALI、THI LAN、THAO TTT 和 MANIKKU L. 估計22隻雞的遺傳多樣性和種群結構使用微衛星標記在亞洲繁殖。亞洲-澳大利亞動物科學雜誌, 2020, 33(12): 1896-1904。 DOI：10.5713/ajas.19.0958。
[6] NXUMALO N.、CECCOBELLI S.、CARDINALI I.、LANCIONI H.、LASAGNA E. 和 KUNENE N.W.使用微衛星和線粒體 脫氧核糖核酸 標記的誇祖魯-納塔爾原生雞生態型的遺傳多樣性、種群結構和祖先起源。意大利動物科學雜誌, 2020, 19(1): 1277-1290。DOI：10.1080/1828051X.2020.1838350。
[7] KARP-TATHAM E.、KÜSTER T.、ANGELOU A.、PAPADOPOULOS E.、NISBET A.J.、XIA D.、TOMLEY F.M. 和 BLAKE D.P. 使用細胞色素 C 氧化酶亞基 I在英國家禽紅螨中的系統發育推斷，相對於歐洲框架。獸醫科學前沿, 2020, 7(8): 1-13. DOI：10.3389/fvets.2020.00553。
[8] DAVE A.R.、CHAUDHARY D.F.、P.M.MANKAD、KORINGA P.G. 和 RANK D.N. 使用細胞色素 c 氧化酶亞基 I 和細胞色素 b 脫氧核糖核酸 條形碼在兩個印度本土雞種群中的遺傳多樣性。獸醫世界，2021 年，14（5）：1389-1397。DOI：10.14202/vetworld.2021.1389-1397。
[9] MALOMANE D.K.、WEIGEND S.、SCHMITT A.O.、WEIGEND A.、REIMER C. 和 SIMIANER H. 全球雞種的遺傳多樣性與其與野生種群的遺傳距離有關。遺傳學選擇進化，2021，53（1）：1-11。DOI：10.1186/s12711-021-00628-z。
[10] SABRY A.、MOHAMED A.A. 和 HASSEN M. 來自埃及和沙特阿拉伯王國生態區的本土雞（捷拉斯家禽）的遺傳多樣性。應用科學開放雜誌, 2021, 11(06): 775-787. DOI：10.4236/ojapps.2021.116057。
[11] ABINAWANTO, SOPHIAN A., LESTARI R., BOWLAKSONO A., EFENDI P.S. 和 AFNAN R. 雞笑了(捷拉斯家禽)的胰島素樣生長因子-1基因分析，具有當都和慢速型聲音特徵。生物多樣性, 2019, 20(7): 2004-2010。DOI：10.13057/bio div/d200729。
[12] CON F., ZHU Y., WANG J., LIAN Y., LIU X., XIAO L., HUANG R., ZHANG Y., CHEN M., 和GUO P.檢測五種雞免疫抑制病毒 11 醫學與健康科學 1108 醫學微生物學06生物科學0605微生物學。生物醫學中心獸醫研究，2018，14(1): 1-8. DOI：10.1186/s12917-018-1663-1。
[13] BOO S.Y., TAN S.W., ALITHEEN N.B., C.L. HO、OMAR A.R. 和 YEAP S.K.高毒力傳染性法氏囊病病毒感染雞上皮內淋巴細胞自然殺傷細胞中參考基因的鑑定。科學報告，2020 年，10(1)：1-9。DOI：10.1038/s41598-020-65474-3。
[14] XIANG Y., LI L., LIU P., YAN L., JIANG Z., YU Y., LI Y., CHEN X., 和 CAO W.

通過交叉檢測快速檢測禽白血病病毒J亞群啟動擴增。科學報告，2021年，第11期：文章編號：10946。DOI：10.1038/s41598-021-90479-x。

[15] LI Q, ZHANG Y., MENG F., JIANG H., XU G., DING J., ZHANG Y., DONG G., TIAN S., CHANG S., 和ZHAO P.
新戰略用於通過微滴式數字聚合酶鏈反應檢測減毒活疫苗中的雞傳染性貧血病毒污染。國際生物醫學研究，2019：2750472。DOI：10.1155/2019/2750472。

[16] KUCHIPUDI S.V.、YON M.、NAIR M.S.、BYUKUSENGE M.、BARRY RM、NISSLY RH、WILLIAMS J.、PIERRE T.、MATHEWS T.、WALNER-PENDLETON E.、DUNN P.、BARNHART D.、LOUGHREY S.、DAVISON S.、KELLY D.J.、TEWARI D. 和 JAYARAO B.M.基於探針的高靈敏度和特異性實時聚合酶鏈反應檢測家禽臨床樣品中的副雞禽桿菌。獸醫學前沿，2021，8(4)。DOI：10.3389/fvets.2021.609126。

[17] DOLCH K.、ANDRÉE S. 和 SCHAWAGELE F. 實時聚合酶鏈反應定量肉類產品的比較。食品, 2020, 9(1049): 3-15.

[18] ABINAWANTO A. 和 EFFENDI P.S. 品讓和老凱巴約蘭的嘎嘎雞(笑雞)的生物聲學分析和形態測量研究。 美國物理研究所 會議論文集，2018 年，2023 年（1）。DOI：10.1063/1.5064134。

[19] ABINAWANTO, SOPHIAN A., EFFENDI P., 和 SISWANTINING T.
短交流：當都型和慢速型嘎嘎雞聲帶形態特徵的變化。
生物多樣性，2018，19（5）：1902-1905。
DOI：10.13057/bio div/d190542。

[20] ARLINA F.、RUSFIDRA、ANDRIANO D. 和 SUMATRI C.
短通訊：在印度尼西亞西蘇門答臘飼養的指甲是歪的雞(捷拉斯家禽) 的類型和聲音多樣性。生物多樣性，2020, 21(5): 1914-1919。 DOI：10.13057/bio div/d210518。

[21] MUSTOFA F.、FATHONI A.、SARI A.P.Z.N.L.、SASONGKO H. 和 MAHARANI D.
五隻印度尼西亞當地雞的體重和體型測量。
美國物理研究所會議系列：地球與環境科學，2021，788 (1)。DOI：10.1088/1755-1315/788/1/012016。

[22] BUGIWATI S.R.A.、DAGONG M.I.A. 和 TOKUNAGA T.
印度尼西亞本土唱歌雞品種的打鳴特徵。美國物理研究所會議系列：地球與環境科學，2020年，492(1)。DOI：10.1088/1755-1315/492/1/012100。

[23] DARYONO B.S.、MUSHLIH M. 和 PERDAMAIAN A.B.I.佩隆雞（捷拉斯家禽）的叫聲和近親繁殖係數分析。生物多樣性, 2021, 22(5): 2451-2457。
DOI：10.13057/bio div/d220501。

[24] ABINAWANTO、ZULISTIANA T.、LESTARI R.、DWIRANTI A. 和 BOWOLAKSONO A. 印度尼西亞馬杜拉島邦卡蘭區雞笑了（捷拉斯家禽，LINNEAUS，1758）的遺傳多樣性。生物多樣性, 2021, 22(6): 3145-3155。 DOI：10.13057/biodiv/d220617。

[25] GHANI U.、ALI Y.、GUL K.、ULLAH R.、KHAN A. 和 WEI D.-Q。使用多種生物信息學工具對B細胞淋巴瘤2
基因進行計算機分析，以確定對其結構和功能完整性至關重要的最致命的突變。2021：1-12
[在線]。可從：https://doi.org/10.21203/rs.3.rs-310735/v1

[26] DAI Y., JIN S., LI X., 和 WANG D. Bcl-2家族蛋白在順鉑耐藥的上皮性卵巢癌中參與AKT調節的細胞存活。腫瘤靶點，2017，8（1）：1354-1368。
DOI：10.18632/oncotarget.13817。

[27] DELBRIDGE A.R.D.、GRABOW S.、STRASSER A. 和 VAUX D.L.
B細胞淋巴瘤2三十年：將細胞死亡發現轉化為新型癌症療法。自然評論癌症，2016，16（2）：99-109。 DOI: 10.1038/nrc.2015.17..

[28] SOPHIAN A.、PURWANINGSIH R.、MUINDAR、IGIRISA E. 和 AMIRULLAH M.
短溝通：分析從蟹加工食品樣品中提取的內含子病理基因自旋提取的 脫氧核糖核酸的純度和濃度。亞洲熱帶生物技術雜誌, 2021, 18(1)。
DOI：10.13057/biotek/c180103。

[29] SOPHIAN A. 短溝通：鹹魚加工食品中提取的脫氧核糖核酸純度和濃度分析。亞洲天然產物生物化學雜誌, 2021, 19(1): 21-24. DOI：10.13057/biofar/f190104。