第 48 卷 第 10 期
2021 年 10 月

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

Vol. 48 No. 10
October 2021

Diversity of Nerve Growth Factor and Progesterone Receptor Genes in Nisi Chicken

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Abstract: The diversity of Nerve Growth Factor (NGF) and Progesterone Receptor (PGR) genes in nisi chickens has been studied. The research aimed to distinguish the diversity of genes in Gorontalo local chicken species. Data analysis was carried out by looking at the Ct (cycling) and Tm (Melt Curve) values in real-time PCR to see the success of the amplification process carried out on the NGF and PGR target genes. Based on molecular analysis, it was found that all samples detected NGF and PGR genes. The results of cycling analysis using real-time PCR for NGF genes were detected with a Ct value of 18.50, while the results of melt curve analysis using real-time PCR for NGF genes were detected with a Tm value of 85.20. For the PGR gene, the results of cycling analysis using real-time PCR were detected with a Ct value of 15.08. In contrast, melt curve analysis using real-time PCR was detected with a Tm value of 85.15. In conclusion, nisi chickens detected NGF and PGR diversity, which can be used as genetic markers in similar studies. The novelty of this study was the use of nisi chickens as the object of study.

Keywords: chicken, genes, nerve growth factor, progesterone receptor.

尼絲雞神經生長因子和孕激素受體基因的多樣性

摘要：研究了尼西雞中神經生長因子和孕激素受體基因的多樣性。該研究旨在區分哥倫打洛當地雞種的基因多樣性。通過查看實時孕激素受體中的循環和熔解曲線值來進行數據分析，以了解對神經生長因子和孕激素受體靶基因進行的擴增過程是否成功。基於分子分析，發現所有樣品均檢測到神经生长因子和孕激素受體基因。使用實時孕激素受體對神經生長因子基因的循環分析結果檢測到騎自行車值為18.50，而使用實時孕激素受體對神經生長因子基因進行熔解曲線分析的結果檢測到熔化曲線值為85.20。對於孕激素受體基因，使用實時孕激素受體檢測循環分析的結果，騎自行車值為15.08。相比之下，使用實時孕激素受體的熔解曲線分析檢測到熔化曲線值為85.15。總之，尼西雞檢測到了神經生長因子和孕激素受體的多樣性，可以作為類似研究中的遺傳標記。本研究的新穎之處在於使用尼西雞作為研究對象。

关键词：雞，基因，神经生长因子，孕激素受体。

1. Introduction

Nisi chicken, also known as "maluo nisi/maluo diti" is a local chicken species native to Gorontalo. This type of chicken has differences from native chickens in general. The most visible difference is the shape of the different body sizes, where the free-range chicken has a larger body when compared to the nisi chicken. Giving the name "nisi / diti" refers to the shape of his small body size. The limited information about this chicken makes this research even more interesting because the research results can provide information about nisi chicken. This native Indonesian genetic resource is important to be preserved. One of the efforts to do is to study the diversity of its genes. According to [1], studies on chicken diversity can be carried out by conducting molecular analysis of target genes used as molecular markers for further studies.

Received: June 12, 2021 / Revised: August 11, 2021 / Accepted: September 5, 2021 / Published: October 30, 2021

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In addition to its effect on the nervous system, NGF also has an important role in various non-neural systems. NGF works by binding to two different membrane receptors, one of which is tyrosine kinase A. The NGF gene is a member of the neurotrophin family [2], which regulates the growth and development of sensory neurons in the sympathetic nervous system [3]. Research conducted by [4] showed that the NGF gene works by influencing the expression related to the regulation of development or cell proliferation that occurs in the early stages of chicken embryo development. The location of the NGF gene on the exon varies between species. In humans, the NGF gene is located on exon 1, while in mice, it is on exon 3 [2]. Research conducted by [5] reported that the NGF gene in chickens is located in exon 4 with a length of 750 bp. According to [6], the paper [7] explained that the NGF gene has the promoter Arg-Ser-Lys-Arg (RSKR), which produces -NGF composed of 118 types of amino acids homodimerically in non-covalent bonds.

Progesterone is sometimes used in the livestock industry in an exogenous hormone mixture that causes a 20% increase in body weight. Since progesterone receptors can be localized to avian mitochondria, the study data suggest mitochondrial muscle metabolism as an important component of phenotypic expression variation in broiler weight. Therefore, the analysis of the progesterone receptor (PRG) gene is often carried out to show changes in progesterone signaling (through the PGR receptor) [8]. The progesterone receptor gene itself is a gene related to the muscle mass transcription system. This gene can be used to conduct studies on the productivity of chickens as pets that have economic value from their use as a source of animal protein.

Based on this background, this research was conducted to detect gene diversity in nisi chickens as part of an effort to conduct ongoing studies to preserve Indonesia's original genetic resources, which are one of the invaluable biological wealth values. The lack of information or research using this gene macro makes this research have a fairly good novelty value as a source of information on similar research that will be carried out in the future.

2. Materials And Methods

2.1. Materials

The ingredients in this study were young nisi chicken feathers, Nuclease free water, Dneasy mericon Food Kit extraction kit (50) paint. 69514 (Qiagen). While the tools used in this study were real-time PCR Rotor-Gene 5 Plex [Qiagen], Qiacube [Qiagen] automatic DNA extraction tool, NanoDrop [implen]. Refrigerant centrifuge [Eppedorf], micro pipet set [Eppendorf].

2.2. Sample Setup

The sample in the base of young nisi chicken feathers was weighed as much as 1 g and added 700 µL of Food Lysis Buffer and 25 µL of proteinase K were then homogenized by vortexing for 1 minute. In order to lyse the sample, it is done by incubation at 70°C for 90 minutes while occasionally shaking it at a speed of 1100 rpm. The next step is to lower the temperature of the sample by allowing it to stand at room temperature for 30 seconds and then put it in an ice block/freezer for 20 minutes. After cooling, the sample was centrifuged at a speed of 2500 x G for 15 minutes. Samples that have undergone the centrifuge process will then form 2 phases, pipette 500 µL of chloroform into a new 2 ml tube carefully, transfer 700 µL of the clear layer without touching the precipitation that occurs at the bottom of the tube. Then it was put in a tube containing 700 µL of chloroform and vortex for 15 seconds, then centrifuge at a speed of 14000 x G for 15 minutes. Take 350 µL of the clear layer. Put it into Qiacube, using the standard method with elution of 60 buffer of buffer EB. The elution DNA can be directly used for the real-time PCR process or stored at a temperature of -20ºC or -80ºC for long storage.

2.3. Purity and Concentration Analysis

Analysis of purity and concentration of isolated DNA was carried out using the NP80 nano photometer (IMPLEN), which was set using the method; Nucleic acid, with dsDNA type, then nano volume mode, the analyzed sample volume was 1-2 µL, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1,000.

2.4. Primers Used

The primer used was designed from NCBI with the target gene, namely Nerve Growth Factor (NGF) with a length of 137 bp, with forwarding primer ‘5-AAA CAG CAA TGT GGT GCG TC -3’ and reverse primer ‘5-AAT CCG GCC ATT AGC ACA CA -3’ and Progesterone Receptor (PGR) target genes with a length of 114 bp, with forwarding primer ‘5- GAA GTC AGA CAG GTG CCG AA -3’ and reverse primer ‘5- CCA GCA CGC ATC AAA ACA CA -3’. In carrying out specific primary designs independently, several things must be considered, namely: Length = 18 – 24, Tm = 58 -60, GC% content = 48 -60, Self-complementary = 0.00 – 4.00, Self 3’ complementary = 0.00 – 4.00 and Initial Bases and Late Bases are not bases that can be paired (A/T – G/C).

2.5. Positive Control

The positive control is the control used to provide comparison results against the interpretation of the results. This analysis did not use a positive control because the primers used in the design were from the same species as the research object.

2.6. Negative Control

The negative control used was nuclease-free water. The function of the negative control is to control the analysis process that is carried out. If the negative control is amplified, it is suspected that the analysis process is contaminated. Another function of the negative control can also be used as a baseline when determining the threshold point for decision making.

2.7. Data Analysis

Data analysis was carried out by looking at the Ct (cycling) and Tm (Melt Curve) values in real-time PCR to see the success of the amplification process carried out on the Nerve Growth Factor (NGF) and Progesterone Receptor (PGR) target genes.

3. Results

3.1. Isolated DNA Analysis

The results of DNA isolation are shown in (Table 1). Analysis of concentration and purity was carried out using a nano photometer. From the table, it can be seen that the concentration values of the extracted samples were in the range of 43,150 ng/µL – 54,750 ng/µL, with an average of 43,970, while the value of purity measured at the wavelength A260/A280 obtained results with a purity range between 1,667 – 1,738. According to [9], a DNA extract purity value above 2 indicates that the DNA extraction results contain protein contamination. In contrast, if the purity results show a value less than 1.8, it indicates that the DNA extract still contains phenol residues and other solvent contaminants.

Table 1 Results of DNA isolation

Sample	Nano Drop Result	
	Concentration (ng/µl)	Purity (A260/A280)
1	43.300	1.718
2	54.750	1.667
3	43.200	1.728
4	43.150	1.733
5	43.300	1.708
6	43.700	1.720
7	43.800	1.738
8	43.600	1.690
9	43.800	1.745
10	43.150	1.733
11	43.300	1.708
12	43.700	1.720
13	43.800	1.738
14	43.600	1.690
15	43.800	1.745
16	43.150	1.733
17	43.300	1.708
18	43.700	1.720
19	43.800	1.738
20	43.600	1.690
21	43.800	1.745
22	43.150	1.733
23	43.300	1.708
24	43.700	1.720
25	43.800	1.738
Average	43.970	1.721

The results of DNA extraction are good if the purity values are in the range of 1.8-2, the concentration is greater than 20 (ng/µl) [10]. However, to conclude that an extracted sample can be tested using real-time PCR, the value of purity and concentration, which is the benchmark for isolation, is said to be good, it can still be ruled out because the development of real-time PCR technology makes it possible if the amplification process can occur with low concentrations even depending on on the sensitivity of a PCR device. Several studies have shown that the results of DNA isolation that are outside the value range of 1.8-2 or concentrations below 10 ng/µl can be carried out for real-time PCR analysis with the smallest detectable value ranging from 0.01-2 p/µL [11-15].

3.2. Real-Time PCR Analysis

Molecular analysis was performed using real-time PCR. The real-time PCR analysis method uses the SYBR Green qualitative method. The samples used in this study were 25 samples of DNA isolation sampled from the base of the young feathers of 25 male nisi chickens. The results of the analysis are as presented in (Table 2).

Table 2 Real-time PCR analysis results

Gen Target	Value	
	Ct	Tm
Negative Control	-	-
Nerve Growth Factor (NGF)	18.50	85.20
Progesteron Receptor (PGR)	15.08	85.15

Note: Ct and Tm values are the average value of 25 data replications.

Based on the molecular analysis conducted on 25 DNA isolated from nisi chickens, it was found that all samples detected nerve growth factor (NGF) and progesterone receptor (PGR) genes. The results of cycling analysis using real-time PCR for nerve growth factor (NGF) genes were detected with a Ct value of 18.50, while the results of melt curve analysis using real-time PCR for nerve growth factor (NGF) genes were detected with a Tm value of 85.20. For the progesterone receptor (PGR) gene, the results of the cycling analysis using real-time PCR were detected with a Ct value of 15.08. In contrast, melt curve analysis using real-time PCR was detected with a Tm value of 80.20. In real-time PCR analysis in this study using a 2 step cycling technique. The use of this technique is quite efficient when compared to the 3 step cycling method. Another advantage of this method is that the higher annealing temperature causes the application process to be faster than the 3 step cycling method. The master mix kit used in this study is the SYBR green kit.

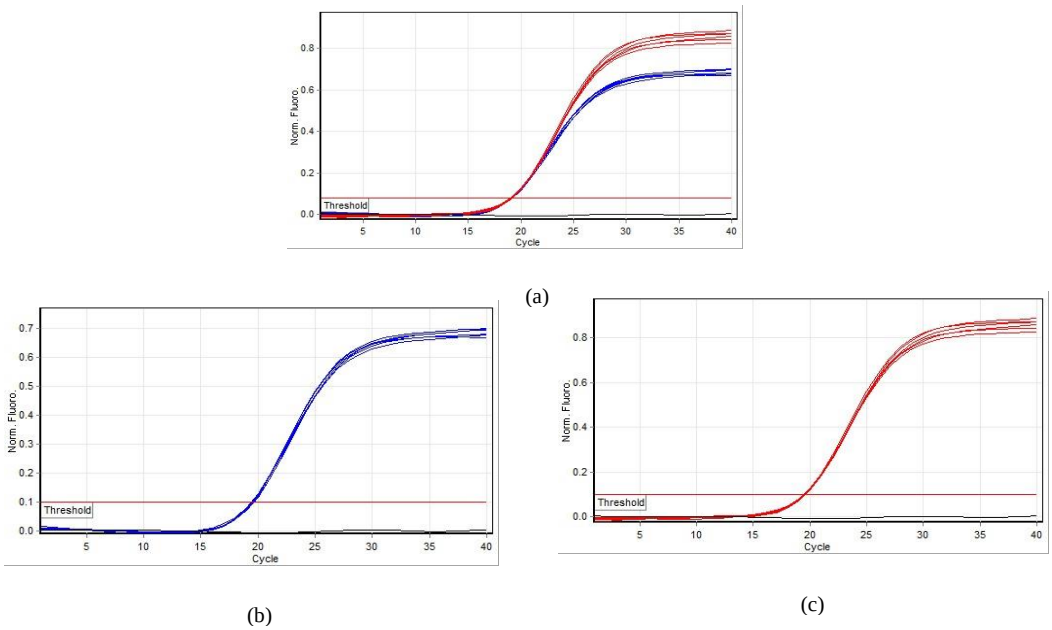

Fig. 1 Results of cycling analysis of targeted NGF and PGR genes

The graph in Fig. 1 shows the results of the cycling analysis of NGF and PGR target gene amplification. The blue color shows the results of amplifying the sigmoid curve of the NGF target gene, and the red color shows the results of amplifying the sigmoid curve of the PGR target sigmoid curve generated from the amplification process that successfully carried out all the amplification stages was influenced by the concentration of the DNA template used. According to [17], cycling is a cycle in which the fluorescence produced in a reaction passes a threshold called the threshold cycle, or Ct was the value. The value of Ct gene. If we look at (Fig. 1) which part (a), which is a curve of two types of genes, namely NGF and PGR, which are combined, it shows that the peaks of the curves separate but the intersection points of the determination of the threshold line are the same because, in the cycling analysis, the shape of the is inversely proportional to the initial size of the DNA template. The threshold is a numerical value set for each cycle to calculate the Ct value for each sample. Thresholds are generally set in the exponential portion of the real-time PCR curve. Usually 10X the standard deviation of Rn for the initial PCR cycle [17].

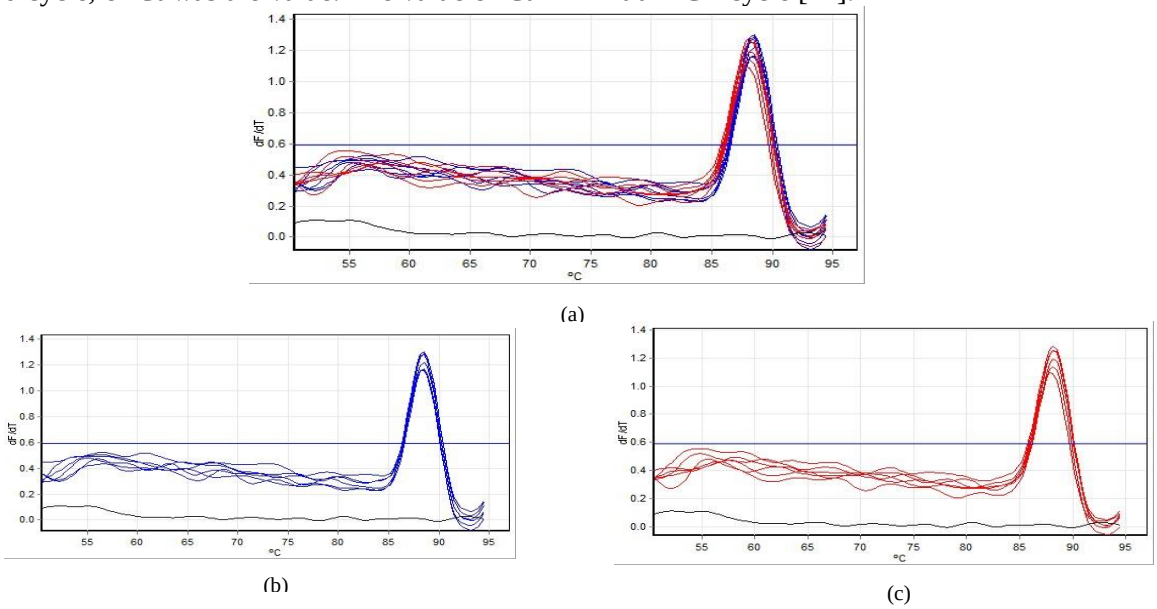

Fig. 2 Results of melt curve analysis of NGF and PGR target genes

The graph in Fig. 2 shows the results of the melt curve analysis of the NGF and PGR target gene amplification. The blue color shows the results of the amplification of the sigmoid curve of the NGF target gene, and the red color shows the results of the amplification of the sigmoid curve of the PGR target gene, the Tm curve in real-time PCR analysis, indicating the amplification process is going well. There are several advantages to using the real-time PCR technique with the green SYBR kit; namely, it can perform screening quickly and simply and is relatively inexpensive compared to probe-based tests.

The cycling analysis with the resulting curve data is not specified in the molecular detection study using the qualitative SYBR green kit method with real-time PCR. This technique is more towards melting curve analysis, where the resulting Tm value will distinguish between species [16]. Moreover, this real-time PCR analysis incorporates fluorescent dyes, increasing the fluorescence signal generated during PCR, which is directly proportional to the amount of PCR product. Here, a fluorescent molecule added to the PCR master mix will produce a fluorescent signal that is detected along with progress in the amplification process [17].

4. Discussion

The detection of PRG genes using real-time PCR was carried out by [8]. Their study used broiler chickens to see that progesterone signaling in broiler skeletal muscle was associated with different feed efficiency. The study of NGF and PGR gene diversity studies attempts to provide a reference for further research on nisi chickens, which are native species of Indonesian genetic resources whose potential must be explored. In this study, the NGF target gene was designed from the NCBI site with a primary sequence length of 137 bp and a PGR gene length of 114 bp. The selection of sequences with these lengths is made by adjusting the primer sequences' length that is good for real-time PCR analysis, which is in the range of 80-250 bp. In carrying out specific primary designs independently, several things must be considered, namely: Length = 18 – 24, Tm = 58 – 60, GC% content = 48 – 60, Self-complementary = 0.00 – 4.00, Self 3' complementary = 0.00 – 4.00 and Initial Bases and Late Bases are not bases that can be paired (A/T – G/C).

So far, research on the NGF gene still focuses on analyzing its three-dimensional structure [18]. The -NGF protein plays a role in neuronal cell survival and embryo differentiation of chickens expressed by the NGF gene. In humans, NGF has a very relevant role in the pathogenesis of psoriasis. NGF affects processes such as keratinocyte proliferation, angiogenesis, T cell activation, expression of adhesion molecules, and upregulation of neuropeptides known to have a role in psoriasis [19, 20]. NGF belongs to the growth factor family. NGF is mitogenic to keratinocytes and recruits degranulating mast cells, d and both of which are early development of psoriatic lesions. NGF activates T lymphocytes, recruits and degranulates inflammatory cells, mitogenic molecules against endothelial cells, and induces (ICAM) intercellular adhesion on endothelial cells. NGF is also known to upregulate neuropeptides. Several studies have shown that NGF has a biological action on the inflammatory process, the immune system, and cell proliferation. In pigeons, -NGF protein binds to the TrkA receptor (tyrosine kinase), which induces dimerization, autophosphorylation, and activation for neuronal cell survival and differentiation [6] is a common receptor bound by -NGF [21], which helps in the growth of neuron cells [7].

Information regarding the PGR (progesterone receptor) gene analysis in chickens is very limited, which is a challenge in this study. Therefore, this study becomes important as a new reference source in using DNA markers with the PGR gene as the target DNA. The progesterone receptor itself is a gene associated with the muscle mass transcription system. This gene can be used to conduct studies on the productivity of chickens as pets that have economic value from their use as a source of animal protein [22].

5. Conclusion

In this study, nisi chickens were detected with Nerve Growth Factor and Progesterone Receptor diversity which can be used as genetic markers in similar studies. The limitation of this study was the difficulty in sampling collection during the COVID-19 pandemic. The research perspective is very interesting because we identified the uniqueness of the local Gorontalo chicken. So, we will get more and more data on this species based on different aspects.

Acknowledgment

The authors are grateful to the Universitas Indonesia for the financial support through the Q2 PUTI Grant with the contract number NKB-1678/UN2.RST/HKP.05.00/2020

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