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Myostatin (MSTN) Gene Detection in Nisi Chicken Using Real-Time PCR

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Abstract: The myostatin (MSTN) gene provides instructions for making myostatin, which helps control the growth and development of tissue throughout the body and limits muscle growth. Myostatin has been studied in mice, cows, and other animals. However, the MSTN gene has not been studied in the nisi chicken. Further, the lack of information using the real-time polymerase chain reaction (PCR) to detect the MTSN gene is still to be explored. Accordingly, this study aimed to detect the MSTN gene in nisi chickens, one of the local-endemic Indonesian chickens, using real-time PCR. DNA of twenty-five male chickens was extracted using an automatic DNA extraction tool, and the MSTN gene was analyzed by real-time PCR Rotor-Gene 5 Plex. Ct and Tm values were indicators of the successful amplification of the MSTN gene. The results showed that the MSTN gene was detected by Ct 16.00 and Tm 80.00, respectively. The study novelty is based on the nisi chicken as the local-endemic chicken from Indonesia as the study objective and the application of the real-time PCR as a method to detect the MSTN gene. From this study, we concluded that real-time PCR is a powerful method to detect the MSTN gene of the nisi chicken. MSTN gene detection using real-time PCR in nisi chickens is a step forward in molecular biology research to identify genetic diversity that can be one of the genetic markers in species DNA research. The aspect that is novel in this study lies in the use of the real-time PCR method in conducting tests to detect DNA species in nisi chickens. The lack of information in the field of research using this technique makes this research essential to be a source of information and reference in similar studies and one of the genetic markers that can apply to other studies in the field of species diversity. This study uses a qualitative testing method with real-time PCR. The interpretation of the results is based on the Ct and Tm values detected during amplification using real-time PCR, where the target gene detected is the MSTN gene. Ct and Tm values were used to see the success of real-time PCR in detecting target genes. Based on the results of real-time PCR, the MSTN gene was detected at Cy 16.00 and Tm. 80.00. In conclusion, the MSTN gene that was the target in this study was detected, which was characterized by amplification of the target DNA as indicated by the Ct and Tm values in real-time PCR.

Keywords: myostatin, nisi chicken, real-time polymerase chain reaction.

使用實時聚合酶鏈反應檢測尼西雞肌肉生長抑制素基因

摘要：肌肉生長抑制素基因提供了製造肌肉生長抑制素的說明，它有助於控制全身組織的生長和發育並限制肌肉生長。肌肉生長抑制素已在小鼠、牛和其他動物中進行了研究。然

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而，肌肉生長抑制素 基因尚未在尼斯雞中進行過研究。此外，缺乏使用實時聚合酶鏈反應檢測肌肉生長抑制素基因的信息，仍有待探索。因此，本研究旨在使用實時 聚合酶鏈反應 檢測尼西雞（印度尼西亞當地特有雞之一）中的 肌肉生長抑制素 基因。 使用自動 脫氧核糖核酸提取工具提取 25 隻雄性雞的 脫氧核糖核酸，並通過實時 聚合酶鏈反應轉子基 5 丛分析 肌肉生長抑制素 基因。Ct 和 Tm 值是 肌肉生長抑制素 基因成功擴增的指標。結果表明，肌肉生長抑制素基因分別被 Ct 16.00 和 Tm 80.00 檢測到。本研究的創新點是以尼西雞作為印度尼西亞地方特有雞為研究對象，應用實時熒光定量聚合酶鏈反應檢測肌肉生長抑制素基因的方法。 從這項研究中，我們得出結論，實時 聚合酶鏈反應 是檢測尼斯雞 肌肉生長抑制素 基因的有效方法。在尼西的雞中使用實時 聚合酶鏈反應 進行 肌肉生長抑制素 基因檢測是分子生物學研究向前邁出的一步，以確定遺傳多樣性，遺傳多樣性可以成為物種 脫氧核糖核酸 研究中的遺傳標記之一。本研究的創新之處在於使用實時熒光定量 聚合酶鏈反應 方法對尼西雞的脫氧核糖核酸 種類進行檢測。由於缺乏使用該技術的研究領域的信息，因此該研究必須成為類似研究的信息和參考來源，並且是可應用於物種多樣性領域其他研究的遺傳標記之一。本研究使用實時 聚合酶鏈反應 定性檢測方法。結果的解釋基於使用實時 聚合酶鏈反應 擴增期間檢測到的 Ct 和 Tm 值，其中檢測到的目標基因是 肌肉生長抑制素 基因。Ct 和 Tm 值用於查看實時聚合酶鏈反應 在檢測目標基因方面的成功。基於實時聚合酶鏈反應的結果，在半胱氨酸 16.00 和 Tm 處檢測到肌肉生長抑制素基因。80.00。總之，檢測到作為本研究目標的肌肉生長抑制素 基因，其特徵在於目標 脫氧核糖核酸 的擴增，如實時 聚合酶鏈反應 中的 Ct 和 Tm 值所示。

关键词：肌肉生長抑制素，尼斯雞，实时聚合酶链反应。

1. Introduction

Nisi chicken is one of the genetic resources in the province of Gorontalo, Indonesia. This chicken is a small-bodied chicken species, which is a typical chicken with a potential value that needs to be explored and explored further. Much limited information about Gorontalo's local chickens makes this research even more interesting to do because there is much information that can be extracted for research. Several molecular analyses have been carried out on local Gorontalo chickens using the genetic markers IGF-1 [1] gene Co1, BIK-BCL2 [2], PGR, and NGF [3], but not on other genetic markers. The use of MSTN genetic markers was chosen because this genetic has been used in several chicken studies, including Yunnan Wuding chicken conducted by [4] by analyzing its relationship with body weight, Daheng broiler chicken [5] and Bangkok chicken [6] by analyzing the characteristics morphometrics and polymorphisms. Myostatin (MSTN), is also known as a gene that plays a role in growth and differentiation factors in livestock.

The article reviewed by [7] describes natural mutations and associated phenotypes as well as the physiological effects of altering myostatin expression in farm animals (cows, sheep, goats, horses, pigs, rabbits and chickens). Knowledge of the null allele and polymorphisms in the myostatin gene is essential in the field of animal breeding and can be used to increase meat production in livestock. Several studies of MSTN gene polymorphism in chickens have been conducted by [8, 5, 9, and 10]. A study conducted by [11] found SNP in the minimal MSTN promoter associated with body weight in chickens. As there is little useful evidence of MSTN SNPs in chicken growth, it is necessary to study the relationship between MSTN SNPs and the production traits of chickens. So many studies use the MSTN gene as a genetic marker, making this research necessary to conduct root information about the MSTN gene in nisi chickens that can be a source of information that can be explored further for the benefit of the world of science. Research on MSTN gene diversity in chickens in Indonesia has been carried out using PCR RLFP in Bangkok chickens [6].

Many studies on the MSTN gene have been reported. However, the research of the MSTN gene of the local-endemic nisi chicken from Indonesia using real-time PCR is still limited. Therefore, the aim of this

study was to detect the MSTN gene of the nisi chicken using real-time PCR. We hypothesized that the MSTN gene can be detected successfully by real-time PCR.

The aspect that is novel in this study lies in the use of the real-time PCR method in conducting tests to detect DNA species in nisi chickens. The lack of information in the field of research using this technique makes this research important to do so that this research is carried out, to be a source of information and reference in similar research and become one of the genetic markers that can be applied to other research in conducting research in the field of species diversity.

2. Methodology

2.1. Materials

In this study, 25 male nisi chickens used Nuclease-free water, Dneasy mericon Food Kit extraction kit (50) paint 69514 (Qiagen). The tools used in this study were real-time PCR Rotor-Gene 5 Plex (Qiagen), automatic DNA extraction tool Qiacube (Qiagen), NanoDrop (Implen), Refrigerant centrifuge (Eppedorf), micropipette (Eppendorf).

2.2. Sample Preparation

DNA isolation was carried out using a Qiacube (Qiagen) automatic extraction tool. A sample of 0.2 g was then placed in a 2 mL centrifuge tube and added with 700 µL of lysis buffer and 30 µL of proteinase K. Homogenize the sample by vortexing for 10–15 sec. and then incubating at 70℃ for 60 min After this stage is complete, then proceed with centrifuging at a speed of 14000 rpm for 5 min. The sample centrifuged will be divided into 2 phases, remove the top layer or supernatant and placed it in a 2 mL tube and add 500 µL of chloroform. The sample was then homogenized by vortexing for 2 seconds, continuing by centrifugation at a speed of 14000 rpm for 10 min. Take 350 l of the clear layer. Put it in Qiacube, and use the standard method with 100 ul of EB buffer elution. The eluted DNA can be used directly for real-time PCR processing or stored at -20℃ for long storage [12].

2.3. Purity and Concentration Analysis

Analysis of DNA quality to evaluate the quality of isolated DNA used a nanophotometer (IMPLEN) with parameters of the concentration and purity of isolated DNA [2, 3, 13].

2.4. Primers

The primer used in this study was designed from the NCBI site with a sequence length of 152 bp consisting of a forward sequence '5-AAA CGG TCC CGC AGA GAT TT-3' and a reverse primer sequence '5-TCT CCG GAG CAG TAA TTG GC-3.' In conducting 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. Result Interpretation

The interpretation of the results is based on the Ct and Tm values detected during amplification using real-time PCR, where the target gene detected is the MSTN gene. The values of Ct and Tm are used to see the success of real-time PCR in detecting target genes [14, 15].

3. Results and Discussion

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 nanophotometer. From the table, it can be seen that the concentration (conc.) values of the extracted samples were in the range of 442.00–471.95 ng/µL, with an average of 456.23, while the value of purity measured at the wavelength A260/A280 obtained results with a purity range between 1.893 and 1.902, with an average of 1.897. According to [13, 16, and 17], a DNA extract purity value above 2 indicates that the DNA extraction results still contain protein contamination, whereas 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 The results of DNA isolation

Sample	Nano drop result			
	Conc., ng/µl	A260	A280	A260/A280
1	443.65	8.870	4.683	1.894
2	443.25	8.869	4.686	1.893
3	443.10	8.867	4.669	1.900
4	442.60	8.852	4.675	1.893
5	442.70	8.854	4.676	1.893
6	442.25	8.842	4.677	1.890
7	442.50	8.845	4.662	1.896
8	442.00	8.839	4.665	1.895
9	448.45	8.963	4.723	1.897
10	449.05	8.972	4.717	1.900
11	448.40	8.961	4.715	1.899
12	471.95	9.425	4.965	1.896
13	470.15	9.393	4.943	1.898
14	469.40	9.379	4.936	1.898
15	469.20	9.373	4.932	1.898
16	468.80	9.365	4.927	1.899
17	461.70	9.217	4.845	1.899
18	461.45	9.216	4.840	1.902
19	461.35	9.207	4.845	1.897
20	461.10	9.203	4.842	1.897
21	461.55	9.206	4.850	1.894
22	461.50	9.206	4.834	1.900
23	461.30	9.197	4.842	1.894
24	469.60	9.365	4.914	1.901
25	468.70	9.350	4.911	1.899
Average	456.23	9.113	4.799	1.897

DNA isolation was carried out using the centrifuge

column technique. This technique is a common technique that is often used in DNA isolation. The general parameters used to see the quality of the isolated DNA are the parameter values of concentration and purity values [18, 19]. In the DNA extraction process, the proteinase K enzyme plays an important role, where this enzyme works by destroying proteins and then digesting them. The use of this technique is considered more effective compared to methods that use chemicals; this is because enzymes work quite effectively directly targeting amino acid bonds in protein lysis. The active proteinase K enzyme works at a temperature of 65–70℃, so in some studies using this method, it is sometimes necessary to optimize the method before using it. The purpose of this heating process is to activate the proteinase K enzyme so that it can actively work for lysis [20]. In conducting DNA extraction using chloroform, the function of this solvent is to increase the density of the organic phase, preventing phenol from inverting into the aqueous phase, which is possible without the use of chloroform due to its close density to water. Therefore, the function of chloroform can be used to keep DNA from degradation by phenol [21].

3.2. Real-Time PCR

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

Table 2 Real-time PCR results

Targeted gene	**Value**	
	Ct	**Tm**
Negative Control	-	-
MSTN Gene	16.00	80.00

Based on the molecular analysis conducted on 25 DNAs isolated from nisi chickens, it was found that all samples detected the myostatin gene (MSTN). The results of cycling analysis (Fig. 1) using real-time PCR for the myostatin gene (MSTN) detected a Ct value of 16.00. The concentration of the template DNA used during the analysis influenced this value in real-time PCR [22].

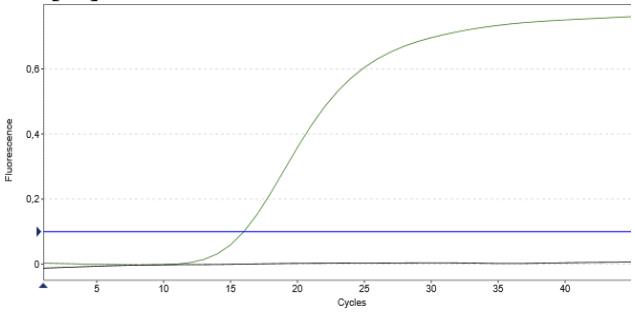

Fig. 1 Results of cycling analysis of targeted MSTN gene

Based on the results of real-time PCR, the average Tm value of the sample was found at a temperature of 80°C. From the melt test temperature range, 72℃ to 95°C. The methyl curve that is formed is shown in Fig. 2. In the melt curve analysis, the formation of a melt curve indicates a positive result or the success of the amplification process. The content of the primary GC used in the study influenced the Tm value in this analysis. According to [23], in performing real-time PCR, the use of appropriate primers is the key to the successful identification of species-specific DNA. The real-time PCR method using the STBR green technique is a quality method that is quite reliable and economical. Another alternative method has better target DNA specificity than the SYBR green technique, namely, the Taqman Probe method. However, this method is more expensive than the SYBR green method.

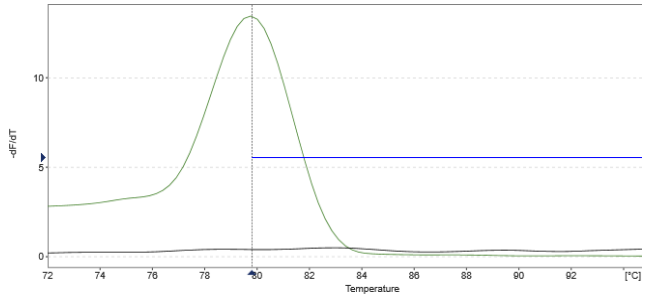

Fig. 2 Results of melt curve analysis of MSTN gene

Based on the results of the study, it was found that the MSTN gene was detected in nisi chickens, which means that this gene has a great opportunity to conduct a comprehensive study in digging up information about nisi chickens. With the detection of this gene, it is hoped that further research needs to be done. The MSTN gene is a gene that plays a significant role in the formation of muscle mass. Previous studies have reported the correlation between MSTN with the morphometric and body weight. Several studies linking species diversity have used MSTN genetic markers to correlate with carcass, body weight, or morphometrics [5, 8, and 24]. The MSTN gene itself has been researched in Bangkok chickens in Indonesia by [6] using the RLFP PCR technique. This technique detects the target DNA and distinguishes it based on the length of the fragment. The other studies was employed the RFLP-PCR method to determine the MSTN gene from Bangkok chicken. In this study, we used real-time PCR to determine MSTN gene from nisi chicken. However, in this study, the technique used is the real-time PCR technique. The application of the real-time PCR to determine the MSTN gene, particularly from the local chicken such as nisi chicken, still limited. However, the limitation of the study could be the strength of the research because we got unique samples and the advance and powerful method. Therefore, we may enrich the data of genetic markers of the chicken in general. The development of this technology is expected to be a source of information for other

researchers to conduct specific gene detection tests using real-time PCR.

4. Conclusion

We concluded that the MSTN gene of the nisi chicken was detected by real-time PCR, as shown by Ct and Tm values, respectively. From this study, we recommended, other genes to be determined by real-time PCR, such as the MMX gene, to enrich the genetic marker data of the chicken. So, in the future we have the complete gene of the nisi chicken. In conclusion, in this study, the MSTN gene was detected as indicated by the Ct and Tm values in real-time PCR. As a suggestion for further research, more other gene references are needed for analysis. Therefore, comprehensive information on the genetic diversity of nisi chickens can serve as an important source for similar research.

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