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Identification of Myostatin Gene Polymorphism (MSTN|Taq1) Exon-2 in Pitalah Duck Using The PCR-RFLP Method

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ABSTRACT

This study aimed to identify the polymorphism of the exon-2 myostatin gene (MSTN) in Pitalah ducks using the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method. 28 blood samples of Pitalah ducks were used in this study. Blood samples were extracted using Intron Biotechnology's Intron G-SpinTM Total DNA Extraction Kit protocol. The DNA extraction results were then amplified using a pair of primers, namely the Forward: 5' TCCACTTGTTACTGATGCTGT-3' primer and the Reverse: 5'-TAGGGAAATGGAGGCACAGG-3' primer, with a fragment target of 700 bp. Furthermore, the amplified product is restricted using the *Taq*1 enzyme, which recognizes the cutting site on T|CGA. Based on the results of the study, two genotypes were found, namely 89.29% truncated homozygous (+/+) and heterozygous (+/-), as well as 10.71% of the total sample used, while untruncated homozygous genotypes (-/-) were not found in this study. Meanwhile, the allele frequency (+) was 0.946, the allele frequency (-) was 0.054, and the observed heterozygosity value was greater than the expected heterozygosity value (Ho>He). This study concludes that the myostatin exon-2 gene in Pitalah ducks is polymorphic and is in Hardy-Weinberg equilibrium.

Keywords: *Genotyping, Myostatin gene, PCR-RFLP, Pitalah duck, Polymorphism, Taq1 enzyme*

Introduction

West Sumatra has four types of local ducks with their advantages and characteristics, including the Pitalah duck. In the Decree of the Minister of Agriculture Number 2923/Kpts/OT.140/6/2011, which regulates the recognition of the Pitalah duck family, it is explained that the Pitalah duck is one of the types of local Indonesian ducks that comes from the West Sumatra region, precisely from Nagari Pitalah, Tanah Datar Regency. The visualization of the pitalah duck is shown in Figure 1.

Suhaemi et al. (2018) stated that Pitalah ducks have a higher economic value than Bayang ducks as meat. Suhaemi (2017) also reported that overall, the production potential of Pitalah ducks is superior compared to Kamang ducks and Bayang ducks. Pitalah ducks have the potential to be broiler ducks and laying ducks, while Kamang ducks have the potential to be broilers, and Bayang ducks excel in terms of the average weight of eggs.

One way to increase duck meat production is to improve genetic quality through a selection program. Advances in biotechnology in the field of molecular genetics can be used as an effective, accurate, and efficient alternative through genetic characterization based on functional genes that control meat growth and production. Molecular selection aims to identify precisely the desired character based on the genes that control it.

One of genes that has a major role and plays a significant role in regulating growth and meat production is the myostatin gene (MSTN). The myostatin gene structure consists of one promoter, three exons, and two introns (Zhao *et al*., 2016). The MSTN gene is also known as Transforming Growth Factor-β (TGF-β), which acts as a negative regulator in skeletal muscle growth. (Ye *et al*., 2007). This phenomenon was found in the “Double Muscling” situation in Belgian Blue cattle (Oldham, 2001). Studies conducted in cattle (Distasio *et al*., 2005) and chicken (Gu *et al*., 2003) show that the myostatin gene plays a crucial role in carcass quality and meat traits. Identification of myostatin gene diversity is needed to obtain preliminary information on gene traits that impact livestock productivity (Hartatik *et al*., 2018).

Figure 1. Pitalah duck (Subekti, 2019)

One of the methods that can be used to identify genetic polymorphism is Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Genetic diversity recognition is carried out by replicating DNA sequences and then cutting them using enzymes as restriction sites to identify possible genetic mutations (Viljoen *et al*., 2005). The high degree of precision in the RFLP process allows for accurate identification of nucleotide bases, making this method suitable for obtaining a picture of genetic populations encoding specific amino acids (Montaldo and Herrera, 1998).

Research related to myostatin in poultry has been conducted by Khaerunnisa et al. (2016), who examined the polymorphism of the myostatin T4842G gene in exon-2 related to carcass characteristics in Indonesian chickens. The results showed that the myostatin locus was polymorphic in all populations. In addition, Shidiq et al. (2023) have also conducted research related to polymorphism of myostatin genes in free-range chickens using the PCR-RFLP method and showed polymorphic results. Al-Sobri et al. (2022) also examined the identification of myostatin gene polymorphism in kerinci ducks and found polymorphisms. Meanwhile, in Pitalah ducks, the polymorphism of myostatin genes has never been identified.

Based on the description above, the author is interested in researching the myostatin gene entitled "Identification of Myostatin Gene Polymorphism (MSTN|Taq1) Exon-2 in Pitalah Ducks Using the PCR-RFLP Method". This is important as an effort to complete genetic information in local Indonesian ducks.

Materials and Methods

Time and Location of Research

The research was conducted at the Livestock Biotechnology Laboratory, Faculty of Animal Husbandry, and the Biomedical Laboratory, Faculty of Medicine, University of Andalas, in March-May 2024.

Sampling

A total of 28 Pitalah duck blood samples were obtained by taking blood using a syringe (disposable syringe) in the area under the wing, precisely the brachial vein. The blood was taken from Pitalah ducks that were intensively reared at the UPT Faculty of Animal Husbandry, Andalas University. A total of ± 1 ml of duck blood was taken and then accommodated in a vacutainer tube containing EDTA, which was then stored at -20 °C.

DNA Isolation

DNA isolation was performed using a DNA Extraction Kit (Intron G-SpinTM Total DNA Extraction Kit). A total of 100 µL of Pitalah duck blood sample, along with 100 µL of CL buffer solution, was put into a 2 ml tube. Then 20 µL proteinase K and 5 µL RNAse were added and homogenized. Then 200 µL of BL buffer was added and homogenized. After that, it was incubated for 2 min at room temperature and 10 min at 56°C. Then, it was centrifuged to remove dew. Next, 200 µL ethanol absolute was added and then homogenized and centrifuged. Then put the mixture into the spin column and centrifuged for 1 min and transferred into a new 2 ml tube. After that, 200 µL of WA buffer was added to the spin column, followed by a centrifugation process for 1 min at

13000 rpm, then the liquid was discarded. Then 200 µL of WB buffer was added, and then centrifuged, the liquid was discarded again and transferred to a new tube, and then centrifuged for 3 min at 13000 rpm to remove the ethanol completely. Next, the spin column was transferred into a 1.5 ml tube and 30 µL of CE buffer was added and incubated for 1 min. The process continued with centrifugation at 1300 rpm for 1 min, and 20 µL CE buffer was added again, incubated for 1 min, and centrifuged for 1 min at 13000 rpm.

DNA Amplification

The isolated DNA sample was put into the PCR tube as much as 1-2 µL, and 18-19 µL premix solution was added. The premix solution is composed of 10 µL I-Max II, 2 µL forward-reverse primer mixture, and 6-7 µL nuclease-free water. The primer design is presented in Table 1. This mixture was incubated in a PCR machine for amplification. The amplification process begins with an initial denaturation step at 94˚C for 2 min. The second stage consists of 35 cycles, with each cycle consisting of denaturation at 94˚C for 30 s, annealing at 56°C for 30 s, and extension at 72˚C for 1 min. The last stage is final elongation at 72˚C for 5 min. The primers and cut points of the *Taq*1 enzyme are presented in Figure 2.

Table 1. MSTN Gene Primers

Gen	Primer	Tm^0	Produk PCR
MSTN-L	5'- TCCACTTGTTACTGATGCTGT -3'	57,23	700 bp
MSTN-R	5'- TAGGGAAATGGAGGCACAGG -3'.	59,08	

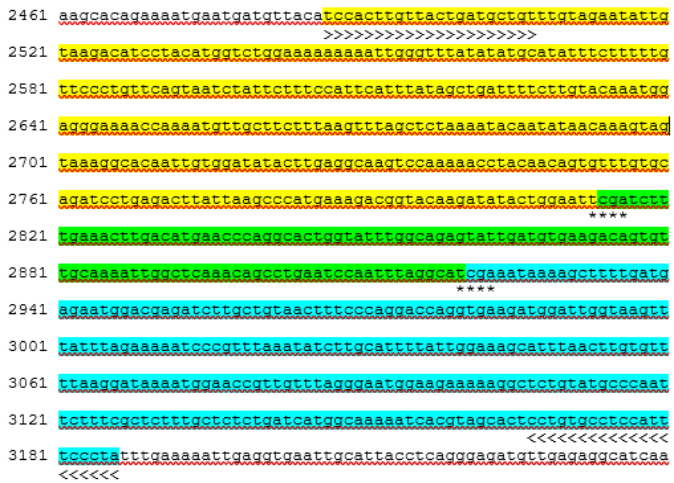

Figure 2. Position of primer attachment (>>>> = forward and <<< = reverse) on MSTN sequence with *Taq*1 cutting site on T|CGA sequence

Electrophoresis

DNA amplification results were visualized through electrophoresis with 1.5% agarose gel. The gel was made of 1.5 g agarose and 100 ml of 0.5x TBE, heated in a microwave at medium-high temperature for 2 min. The gel was printed on a printing tray and left to set. A total of 5 µL of amplification product was taken and then electrophoresed at a voltage of 100 V for 60 min, namely until the DNA fragments finished migrating in the gel. Electrophoresis uses a 50 bp ladder. The results of DNA electrophoresis can be seen with the help of UV light.

Genotyping

Genotyping was carried out using the RFLP technique. The materials used in this genotyping process were 5 µL amplification product, 1 µL *Taq*1 enzyme, 2 µL buffer R, and 12 µL nuclease-free water. The *Taq*1 enzyme, used to cut the MSTN exon-2 gene, was incubated at 65°C for 60 min and 80°C for 20 min. The electrophoresed DNA samples were observed under UV light. DNA fragments that appeared from the electrophoresis results were compared with the marker to determine the length of the fragment and its genotype.

Data Analysis

Genotype frequency was calculated with the following formula (Nei and Kumar, 2000):

$$x_{ii=}\frac{n_{ii}}{N}$$

Allele frequency was calculated using the formula according to (Nei and Kumar, 2000) as follows:

$$xi = \frac{(2nii + \sum j \neq i\ nij)}{2N}$$

Description:
xi = i-th allele frequency
xii = i-th genotype frequency
nii = number of individuals with genotype ii
nij = number of individuals with genotype ij
N = total number of individuals sampled

Heterozygosity can be calculated using the formula of Yeh et al. (1999) as follows:

$$Ho = \sum_{k}^{s} Wk \sum_{i \neq j}^{q} Xkij \; ; He = 1 - \sum_{k}^{s} |Wk \sum_{l}^{q} X_{ki}^{2}$$

Description:
Ho = observed heterozygosity
He = expected heterozygosity
Wk = effective population size
Xki j= genotype frequency ij, k population

Based on the Hartl formula (1988) to determine whether the allele frequency and genotype frequency of the myostatin gene are in balance p2 + 2pq + q2 tested by chi-square, the Hardy-Weinberg law balance test is carried out as follows:

$$X^2 = \sum_{i=1}^{k} \frac{(Oi - Ei)^2}{Ei}$$

Description:
X² = chi-square
O = number of observations of the i-th genotype
E = expected number of i-th genotypes

Results and Discussion

DNA Isolation

The success of the DNA isolation process can be assessed through several ways, including by checking the presence of DNA bands by electrophoresis method or by measuring DNA concentration using spectrophotometry (Phillips *et al*., 2012). DNA quality was measured using spectrophotometry at wavelengths (λ) of 260 nm and 280 nm. DNA absorbs UV light optimally at a wavelength of 260 nm, while proteins achieve maximum absorption at 280 nm (Muladno, 2002 in Saili, 2010). Meanwhile, the purity of DNA was evaluated by calculating the absorbance ratio between A260 and A280 (ratio A260:A280) (Darmono, 2011). The results of DNA quality testing from 28 Pitalah duck samples showed a variation in purity in the range of 1.18-1.87.

DNA molecules are considered pure if their absorption ratio is in the range of 1.8-2.0 (Novita, 2013). According to Amanda and Cartealy (2015), information about the concentration and purity of DNA is crucial for assessing the level of contamination of a sample. Therefore, measurements are made of quality, both concentration and purity of genomic DNA.

Amplification *Myostatin* (MSTN) Gene

The amplification results are visualized on 1.5% agarose media as shown in Figure 3. The amplification of the exon-2 myostatin gene (MSTN) in Pitalah ducks resulted in a single band measuring 700 bp. The appearance of this one band suggests that the DNA primer used in this study is specific. MSTN gene amplification is declared successful if the DNA primer attachment is by the target gene (Shidiq, 2023). Factors such as the purity of DNA after isolation, the selection of the right primer, and the accuracy of the PCR cycle conditions greatly affect the PCR results. Primers are an essential part of PCR because primers are useful in initiating the formation of DNA targets (Ye *et al*, 2007).

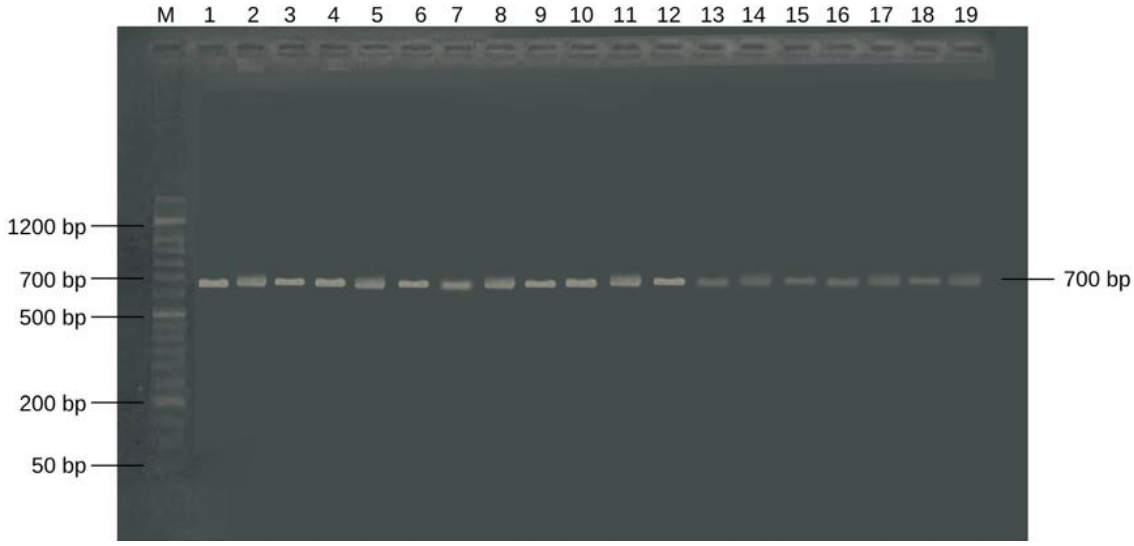

Figure 3. Amplification result of MSTN gene
Notes: 1-19 = Sample Number, M = Marker (50 bp)

The accuracy of PCR conditions also affects the results of PCR reactions, which are based on the accuracy of mixing reactions and temperature conditions (Bauzen *et al*. 2000). Annealing temperature has a crucial role in PCR reactions (Muladno, 2010). The process of attaching the primer to the DNA strand that has been exposed requires an optimal temperature, so

it is necessary to optimize the temperature before performing PCR (Rahmadhan *et al*., 2019). If the temperature chosen is too high, it can cause amplification failure because there is no primer attachment; on the other hand, if the temperature is too low, it will cause the primer to stick to the other side, resulting in the DNA formed becoming non-specific. Specific PCR products formed by the presence of a single DNA band that appears to indicate the accuracy of the PCR reaction in tandem with the accuracy of the primer used (Hill *et al*., 2010).

Genotyping of *Myostatin* (MSTN) Gene

Polymorphism in a population can be known through the PCR-RFLP method, which is one of the advanced analysis methods of amplified PCR products. In this study, this method was carried out by cutting MSTN gene fragments on exon-2 using the Taq1 restriction enzyme, which has a T↓CGA cutting site in PCR products which are at bases to 327 bp and 434 bp. The incubation temperature of this enzyme is 65°C for 1 h and 80°C for 20 min. The results of the cutting of MSTN fragments by the Taq1 enzyme on a 1.5% agarose gel and a 50 bp ladder are presented in Figure 4.

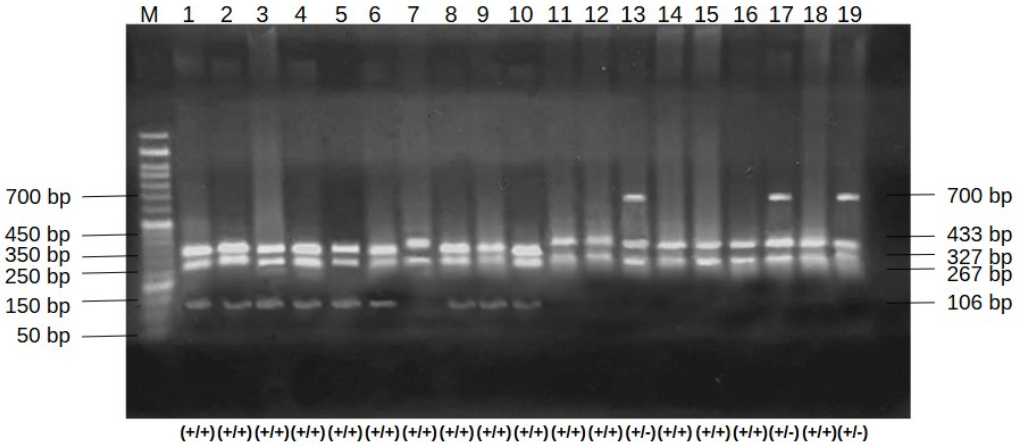

Figure 4. Restriction result of MSTN|HaeIII gene
Notes: 1-19 = Sample Number, M = Marker (50 bp)

The results of cutting MSTN exon-2 fragments in Pitalah ducks by the Taq1 enzyme produced two types of cutting, namely heterozygous (+/-) of 10.71% with band sizes of 700 bp, 433 bp, and 267 bp. This ribbon pattern results in one band exactly where there is an amplification fragment and two bands below the amplification fragment. The second cutting pattern was homozygous cut (+/+) by 89.29% which resulted in two bands of 433 bp and 267 bp, and three bands of 327 bp, 267 bp, and 106 bp under the amplification fragment.

The part of DNA that is subjected to the cutting action of restriction enzymes is called the recognition sequence (Nova *et al*., 2016). Changes in the sequence of base pairs in the D-loop mtDNA analyzed can result in different haplotype patterns within a population or at the individual level. These changes include the addition, substitution, or loss of certain bases that result in the enzyme being unable to cut at the same site, resulting in a shift in the cutting site (Irmawati, 2003). This is by the opinion of Shidiq *et al*. (2023) which states that restriction enzymes will not recognize the cutting site if there is a mutation in the gene sequence, namely a change in the base pair.

Genotype Frequency and Allele Frequency

The results of genotypic frequency and frequency of the MSTN exon-2 gene allele in Pitalah ducks are presented in Table 2 and Figure 5. Allele frequency is the relative frequency of an allele in a population or the number of alleles relative to the total alleles in a population. Based on the data above, the results of allele frequency + were 0.946, and allele frequency – was 0.054. This shows that the MSTN exon-2 gene in Pitalah ducks is polymorphic. This is following the opinion of Nei and Kumar (2000) who stated that a gene is said to be polymorphic if one of its alleles has a frequency of less than 99%. Polymorphism can be indicated by the presence of two or more alleles in a population.

Table 2. Genotype and allele frequencies of MSTN|*Taq*1 in Pitalah ducks

Genotype Type	Number of Individuals	Genotype Frequency	Number of Allel		Allel Frequency	
			+	-	+	-
(+/+)	25	0,9	50	0		
(+/-)	3	0,1	3	3	0,946	0,054
(-/-)	0	0	0	0		
Total	28	1	53	3		

Description: (+/+) = truncated homozygous genotyped individuals, (+/-) = heterozygous genotyped individuals, and (-/-) = untruncated homozygous genotyped individuals.

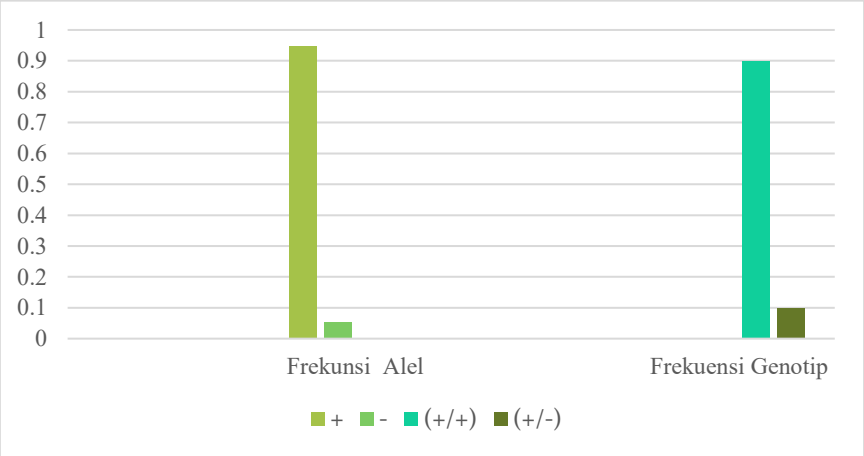

Figure 5: Graph of genotype frequency and allele frequency

Based on the data in Table 2, it can be seen that 2 genotypes were found, namely truncated homozygous genotypes (+/+) and heterozygous genotypes (+/-). The frequency of the truncated homozygous genotype (+/+) was found to be 0.9, and the heterozygous genotype (+/-) was 0.1. Meanwhile, the frequency of allele + was found to be 0.946, and allele – was 0.054. Cutting of PCR products using the Taq1 enzyme produces fragments with diverse cut patterns (polymorphic). The genotypic frequency and frequency of the MSTN exon-2 gene allele were determined in Pitalah ducks based on the Taq1 enzyme cleavage site visualized from the results of electrophoresis on a 1.5% agarose gel. Polymorphism can be shown by the presence of two or more alleles in a population (Nova *et al*., 2016). Based on the results of the above data analysis, the myostatin gene in Pitalah ducks observed in this study is diverse or polymorphic because the frequency of one of the alleles < 99%. This is in line with the opinion of Nei and Kumar (2000), who stated that a gene is said to be polymorphic if one of its alleles has a frequency of less than 99%.

Heterozygosity

Heterozygosity is one of the indicators that can be used to measure the level of genetic variation in a population. The heterozygosity value is the average percentage of heterozygous individuals in a population. The higher the heterozygosity value, the greater the genetic diversity in the population, and vice versa (Nei and Kumar, 2000). The heterozygosity value of the exon-2 MSTN gene in Pitalah ducks is presented in Table 3.

The results of heterozygosity analysis showed that the MSTN exon-2 gene in Pitalah ducks had an observed heterozygosity value (Ho) higher than the expected heterozygosity value (He). The values of observation heterozygosity and expected heterozygosity can be used as one way to estimate the value of the inbreeding coefficient in a group of livestock (Hartl and Clark, 1997). Machado *et al*. (2003) stated that if the value of Ho>He, it can indicate the absence of endogamy or marriage in the group. This is in line with the opinion of Noor (2008), who stated that a high heterozygosity value in a population indicates a high outbreeding that can affect the proportion of heterozygous genotypes.

Tabel 3. Heterozygosity Value

Heterozigosity	Value
Ho	0,107
He	0,102

Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium is stated to be valid in a population if the genotypic frequency p2+2pq+q2=1 and the frequency of the p and q alleles are constant from generation to generation (Vasconcellos *et al*., 2003). A chi-square test is needed to determine whether the data obtained in this study are in Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium if the value of X2 is calculated to be smaller than X2 table. Hardjosubroto (1998) stated that genotype frequency and allele frequency are constant from generation to generation if there is no random selection, mutation, migration, and intermarriage in a population. The results of observations regarding the Hardy-Weinberg balance in the Pitalah duck population are presented in Table 4.

Tabel 4. Hardy-Weinberg equilibrium chi-square test

HW Equilibrium	Genotype Frequency			Total	X^2h	$X^2t_{(0,05)}$
	(+/+)	(+/-)	(-/-)			
O	25	3	0	28		
E	25,05	2,87	0,08	28	0,086	5,99
$(O-E)^2/E$	0,0000998	0,0058885	0,08	0,085		

Description : $X^2t_{(0,05)}$ = 5,99, $X^2h < X^2t_{(0,05)}$ = not significantly different

Based on Table 4, the value of X2 is smaller than the X2 table, indicating that the observed genotype is not significantly different from the expected genotype frequency. From the above test data, it can be seen that the polymorphism of the MSTN exon-2 gene in Pitalah ducks is in Hardy-Weinberg equilibrium. This is in line with the opinion of Allendorf and Luikat (2007), who stated that a population is said to be in Hardy-Weinberg equilibrium if genotype frequency and allele frequency are constant from generation to generation due to random gamete incorporation.

Conclusion

There are two types of genotypes in MSTN gene, cutting|Taq1 exon-2 in Pitalah ducks, namely homozygous truncated (+/+) as much as 89.29% and heterozygous (+/-) as much as 10.71% of the total sample used. Identification of MSTN|Taq1 exon-2 in Pitalah ducks using the PCR-RFLP method is polymorphic, and MSTN gene genotypic frequency|Taq1 exon-2 in Pitalah ducks is in Hardy-Weinberg equilibrium.

Conflict of interest

No potential conflict of interest relevant to this article was reported. All authors have agreed with the contents of the manuscript.

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Author's contribution

The authors confirm their contribution to the paper as follows: study conception and design: KS, FA; data collection: WS, NIS; analysis and interpretation of results: KS, WS, NIS, FA; draft manuscript preparation: KS, WS, NIS, FA.

Ethics approval

Animal experiments were conducted following the Republic of Indonesia Law No. 18 of 2009 (section 66), which addressed animal keeping, raising, killing, and proper treatment and care.

References

Allendorf, F. W., and G. H. Luikat. 2007. Conservations and the Genetiks of Populations. Blackwell Publishing. UK.

Al-Sobri, S. N., D. Depison, dan E. Wiyanto. 2022. Polimorfisme gen *myostatin* (MSTN) dengan menggunakan metode PCR-RFLP pada itik Kerinci (*Doctoral dissertation*, Universitas Jambi).

Amanda, U. D., dan I. C. Cartealy . 2015. Isolasi RNA total dari mesokarp buah kelapa sawit (*Elaeis guineenssis Jacq. var. Tenera*).1(2):171–176.

Bauzen, N. D., M. J. Stear, and K. C. Chang. 2000. Molecular markers and their use in animal breeding. The Veterinary Journal, 160(1), 42-52.

Darmono, T. W. 2011. Analisis keragaman genetik *Ganoderma sp* yang berasosiasi dengan tanaman kakao dan tanaman pelindungnya Random Amplified Polymorphic DNA (RAPD). 2011;79(1):6–14.

Distasio, L., A. Brugiapaglia, G. Destefanis, A. Brugiapaglia, A. Albera and A. Rolando. 2005. Polymorphism of the ghr genes with milk production traits for italian holstein-friesian bulls. J. Dairy Sci. 79: 1446-1453.

Gu, Z. L., D. H. Zhu, and C. X. Wu, 2003. Studi on relationship between of single nucleotide polymorphism of *myostatin* gene with skeletal muscle and growth fat in chicken. Science in China. 33: 173-180.

Hardjosubroto, W. 1998. Genetika Hewan. Fakultas Peternakan. Universitas Gajah Mada, Yogyakarta.

Hartatik, T., D. E. Putra, S. D. Volkandari, T. Kanazawa, and Sumadi. 2018. Genotype analysis of partial growth hormone gene (GH891|MspI) in pesisir cattle and simmental-pesisir crossbred cattle. J. Indonesian Trop. Anim. Agric. 43(1): I-8.

Hartl, D. L. and Clark.1997. Principle of Population Genetik. sinauer associates. Sunderland: Inc. Publisher

Hill, E. W., B. A. McGivney, J. Gu, R.Whiston, and D. E. MacHugh. 2010. A genome-wide SNP-association study confirms a sequence variant (g. 66493737C> T) in the equine *myostatin* (MSTN) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses. BMC genomics, 11(1),1-10.

Irmawati. 2003. Perubahan keragaman genetik ikan kerapu tikus generasi pertama pada stok hatchery. Thesis. IPB: Bogor. PP. 35.

Kepmentan. 2011. Keputusan Menteri Pertanian No. 2923/Kpts/OT.140/ 6/2011 tentang penetapan rumpun itik pitalah. Kementrian Pertanian RI. Jakarta.

Khaerunnisa, I., M. Pramujo, and I. Arief. 2016. Polymorphism of the t4842g *myostatin* gene is associated with. International Journal of Poultry Science. 15(8): 316-324.

Machado, M. B. B., M. M. Alencar, A. P. Pereira, and H. N. Oliveira. 2003. QTL affecting body weight in a candidate region of cattle Chromosome 5. Genetic Moleculer Biology. 26:259-265.

Maghfiroh, K., I. Mangisah dan V. D. Y. B. Ismadi. 2012. Pengaruh penambahan sari jeruk nipis (*citrus aurantifolia*) dalam ransum terhadap kecernaan protein kasar dan retensi nitrogen pada itik magelang jantan. Animal Agriculture Journal. 1(1): 669-683.

Montaldo, H. H. and C. A. M. Herrera. 1998. Use of molecular markers and major genes in the genetik improvement of livestock. Journal Biotechnology 1(2): 83-89.

Muladno, 2002. Seputar Teknologi Rekayasa Genetika. Pustaka Wira Usaha Muda, Bogor.

Muladno. 2010. Teknologi Rekayasa Genetika. Edisi Kedua, Bogor.

Nei, M. and S. Kumar. 2000. Molecular Evolution and Genetiks. Oxfor University Press, NewYork.

Noor, R.R. 2008. Genetika Ternak. Penebar swadaya, Jakarta.

Nova, T. D., Yurnalis dan A. K. Sari. 2016. Keragaman genetik gen hormon pertumbuhan (GH|*MboII*) pada itik Sikumbang Janti menggunakan penciri PCR-RFLP. Jurnal Peternakan Indonesia. 18(1): 44-52.

Novita L. 2013. Analisis genetik karakter morfoagronomi jarak pagar hasil pemuliaan berbasis pendekatan kuantitatif dan molekuler. *Tesis*. 2013:1–50.

Oldham. 2001. Molecular expression of *myostatin* and myod is greaterin double-muscled than normal-muscled cattle fetuses. Am J Physiol Regulatory Integrative Comp Physiol. 280: R1488-R1493.

Phillips, K., McCallum, and Welch. 2012. A comparison of methods for forensic DNA extraction: Chelex-100 and the QIAGEN DNA investigator kit (manual and automated). Forensic Science International: Genetics. 6(2) 282-285.

Rahmadhan, D., R. Sari, dan P. Apridamayanti, 2019. Pengaruh suhu annealing terhadap amplifikasi gen tem menggunakan primer dengan persen GC rendah. Jurnal Mahasiswa Farmasi Fakultas Kedokteran Untan. Vol. 4 No. 1.

Shidiq, F., Yulianto, A. Gunawan, C. Sumantri. 2023. Identifikasi keragaman gen myostatin (MSTN|*Bsr*I) pada ayam kampung dengan metode PCR-RFLP. Jurnal Ternak Tropika. 24(1):59-68.

Suhaemi, Z. 2017. Potensi keragaman genetik itik lokal Sumatera Barat. Padang: Disertasi, Universitas Andalas.

Suhaemi, Z. dan Febriani. 2018. Perbandingan nilai ekonomis itik pitalah dan bayang sebagai itik pedaging. 20(18): 451-455.

Vasconcellos L. P. M. K., D. T. Talhari, A. P. Pereira, L. L. Coutinho and L. C.A. Regitano. 2003. Genetik characterization of aberdeen angus cattle using molecular markers. Genetik Moleculer Biology. 26: 133-137.

Viljoen, G. J., H. N. Louisand and R. C. John. 2005. Molecular Diagnostic PCR Hand Book. Springer : IAEA-FAO (Fiat-Panis).

Ye, X., S. R. Brown, K. Nones, L. L. Coutinho, J. C. Dekkers, and S. J. Lamont. 2007. Associations of *myostatin* gene polymorphisms with performance and mortality traits in broiler chickens. Genetiks Selection Evolution. 39(1): 73-89.

Yeh, F. C., R. C. Yang, and T. Boyle. 1999. POPGENE Versi 1.31: Microsoft Window Based Freeware for Population Genetik Analysis. Canada: University Of Alberta.

Zhao, Z. H., H. Li., H. J. Yi, and B. X. Peng. 2016. The correlation between polymorphism of the mstn gene and slaugterntraits in sansui ducks. Pakistan J. Zool. 48 (5): 1283-1290.