International Journal of Scientific Research in Science, Engineering and Technology (www.ijsrset.com)
© 2020 IJSRSET | Volume 7 | Issue 1 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
DOI : https://doi.org/10.32628/IJSRSET20716

Association of TGF-β2 Gene Polymorphism with Salmonella pullorum Bacterial Infection Resistance in Tolaki Chickens

Muh Akramullah[1], Cece Sumantri[2], Niken Ulupi[2], Muh Amrullah Pagala[3]

[1] Master's Student of Animal Production and Technology Study Program, Postgraduate School, Bogor Agricultural University, Bogor, Indonesia.

[2]Teaching Staff of the Department of Animal Husbandry Production and Technology, Bogor Agricultural University, Bogor, Indonesia.

[3] Teaching Staff of the Faculty of Animal Husbandry, Halu Oleo University, Kendari, Indonesia.

Corresponding email: muhakramullah44@gmail.com

ABSTRACT

Transformation growth factor-beta 2 gene (TGF-β2) is a gene which belongs to the cytokine gene group. The present study aimed to identify Single Nucleotide Polymorphism (SNP) g. 640 T> C of Transformation growth factor-beta 2 gene (TGF-β2) and associate its genotypes with Salmonella pullorum Bacterial Infection Resistance in Tolaki Chickens. This study used blood samples collected from 70 chickens. Discussion was conducted on leucocyte concentration, leucocyte differentiation (heterophils, monocytes, lymphocytes, eosinophils, basophils and H/L ratio) and clearance test. Identification of Single Nucleotide Polymorphism of Transformation growth factor-beta 2 gene (TGF-β2) was performed by PCR-RFLP method. Association of (SNP) g. 640 T> C of Transformation growth factor-beta 2 gene (TGF-β2) with leukocyte profiles and their differentiation and resistance to bacterial infections was carried out using analysis of variance with GLM (General Linear Model). SNP g.640 T> C of Transformation growth factor-beta 2 gene (TGF-β2) was polymorphic and was in Hardy-Weinberg equilibrium. Based on molecular and biological testing, Tolaki chickens in genotypes (TT, TC and CC) have normal leucocyte concentration and differenciation and resistant to Salmonella pullorum.

Keywords : Tolaki chicken, TGF-β2 gene, SNP (640) T>C, Salmonella pullorum

I. PENDAHULUAN

Tolaki chicken is one of Indonesian's local chicken and is a germplasm native to Indonesian's Southeast Sulawesi province. Tolaki chicken has the potential to produce eggs, for both consumption and hatching as egg production is quite high (Rusdin et al. 2011). Tolaki chickens during one period were able to produce as many as 25 eggs / head, higher than native chickens which were 10 eggs / head (Nafiu et al. 2012). Adult male Tolaki chicken body weight reaches 1.4-1.8 kg / head while adult female chickens 0.6-1.6 kg / head (Sulandari et al. 2007). Tolaki chicken behavior is included in the aggressive chicken category. In addition to this, Tolaki chicken has resistance to virus and bacterial attack (Pagala et al. 2018).

Chicken body endurance in addition to being influenced by the environment and feed, is also controlled by genes. There are several genes which control the resistance of chickens, one of which is the Transforming Growth Factor Beta 2 (TGF-β2) gene. TGF-β2 gene is a gene that controls the nature of growth while controlling the nature of endurance in chickens (Tohidi et al., 2012). Tang et al., (2011)

stated that single nucleotide polymoprism (SNP) has been identified in the TGF-β2 promoter region which has a positive effect on chicken endurance. TGF β2 gene identification has also been carried out on exon 1 with a product length of 284 bp PCR using the enzyme Rsal, in broiler chickens and leghorns. The results obtained that the TGF β2 gene is polymorphic with SNP (640) T> C as reported by Li at al., (2003).
Based on the results of research conducted by Tohidi et al. (2012), the TGF-β2 gene is one of the genes that can be potential candidate to used in the selection program to increase genetic resistance to Salmonella enteritidis in local Malaysian chickens. The TGF-β2 gene is associated with the immune properties of sentul chickens (local Indonesian chickens) against Salmonella pullorum (Muhsinin et al. 2017). The TGF-β2 gene has high expression in broiler chickens (Carlos et al. 2014).

This TGF-β2 gene can control the nature of resistance to bacterial infections. Therefore, the present research was conducted to examine the TGF-β2 gene polymorphism and its association with resistance to Salmonella pullorum bacterial infection. The results of this study are expected to add scientific information on the polymorphism of the TGF-β2 gene in Tolaki chicken.

II. RESEARCH METHODS

Research Location and Time

This research was carried out in the Poultry Cage Unit, Faculty of Animal Science at Halu Oleo University, Kendari and in the Molecular Genetics Laboratory of the Breeding and Genetics Section, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University. This research took place from October 2018 to August 2019.

Research Materials

Tolaki chicken used in this study amounted to 70 tails obtained from the hatchery. Hatched eggs were obtained from Tolaki chicken broodstock taken in the Konawe Selatan District community. Tolaki chickens were maintained from 0 days to 12 weeks. The type of feed given was commercial feed PAR-G with a crude protein content of 17% and metabolic energy of 2950 kcal kg-1. Feed was given 2 times a day at 06.00 WITA and 16.00 WITA.

Procedure

This research consists of two stages. The first step is genotyping of the TGF-β2 gene. The second step is to associate the TGF-β2 gene genotype with resistance to bacterial infections.

Research Phase I: TGF-β2 Genotyping

Research at this stage, aimed to identify the TGF-β2 gene polymorphism. It started with blood sampling, DNA extraction, Polymerase Chain Reaction (PCR) Amplification, Restriction Fragment Lenght Polymorphism (RFLP) analysis and PCR-RFLP Electrophoresis of products.

1) Blood Sampling
2) At the end of the 12th week, blood was taken from the brachial vein in the wing area. This blood sampling was used as a sample for genotyping. Blood sampling was conducted using 1 mL syringe 0.5-1 mL.

DNA extraction and TGF-β2 gene amplification

DNA extraction was carried out using the phenol-chloroform method (Sambrook and Russel, 2001). Amplification of the TGF-β2 gene fragment was carried out by the Polymerase Chain Reaction (PCR) Amplification method. The primer was designed at the SNP mutation point (640) T> C in exon 1 with the target sequence length of 284 bp with the help of the

primer designing tools program (http://www.ncbi.nlm.nih.gov/tools/primer-blast). A pair of primers was used, forward (F: 5 'GGTTCAGTGCAAGGCATTTC 3') and reverse (R: 5'CTTCTGTCAAGTGCAGTGAG 3 ') (Muhsinin et al. 2018).

DNA amplification was carried out at a total volume of 15 µL, consisting of 1 µL DNA, 10.85 µL DW, 0.30 µL primer, 0.05 µL taq polymerase, 1.50 µL buffer, 0.30 µL dNTP and 1.00 µL MgCl2. The reactant mixture was put into a 1.50 µL tube to be homogenized, then distributed to each tube containing DNA samples and then put into a PCR machine. DNA amplification took place in the Applied Biosystems PCR machine with predenaturation temperature conditions of 95 °C for 5 minutes, cycles for the stages of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 20 seconds and elongation at 72 °C for 30 seconds, then with the final elongation stage at 72 °C for 5 minutes in one cycle. PCR products were electrophoresed using 1.5% agarose gel.

3) Analisis Restriction Fragment Lenght Polymorphism (RFLP) TGF-β2 gene

4) Determination of TGF-β2 gene genotype in Tolaki chickens was performed by using the RFLP method. Amplification products from the TGF-β2 gene were cut using restriction enzymes. Restriction enzyme was designed based on the SNP that has been found in the TGF-β2 gene with the NEBcutter V2.0 program. RsaI was a restriction enzyme used to bypass the SNP gene TGF-β2 at the GT | AC site. A total of 5 µL of the PCR product was transferred into a 0.5 mL tube added 0.9 µL DW, 0.4 µL of RsaI enzyme and 0.7 µL buffer. The mixture was incubated at 37 °C for approximately 16 hours (overnight).

Electrophoresis of PCR-RFLP TGF-β2 gene products

5) PCR-RFLP products were electrophoresed using agarose gel with a concentration of 2%. 0.6 gram agarose was added with 30 mL 0.5 x TBE (Tris Borat EDTA). The mixture was heated in the microwave for 5 minutes, shaken with a magnetic stirrer, added 1 µL fluorosafe, and molded to form wells in the gel. 2 µL of 100 bp DNA marker was put into the far left well as a marker. Each 5 mL PCR DNA sample was put into a gel well. The gel has 100V electricity for 30 minutes. After electrophoresis was complete, the gel is photographed using a UV-Transilluminator machine.

6) Analisis Data

Using genotyping data obtained from PCR-RFLP, the allele frequency, genotype frequency, Hardy-Weinberg equilibrium value were calculated using the Popgene32 application program (Yeh et al., 1999).

Research phase II: Association of TGF-β2 Genotypes with Resistance to Bacterial Infections

Maintenance

Tolaki chicken used in this study amounted to 70 tails. Maintenance of DOC from 0 days to 2 weeks. Before being put into a cage, they were weighed and give the leg number. Weighing was done after the hair was dry.

At the age of 3-12 weeks chickens were placed in individual plots (cages), which have been numbered in each plot. The sizes of the cage around were 35 x 35 x 40 cm. The cage was placed in a cage measuring 5 x 15 m2.

The type of feed given was commercial feed PAR-G with a crude protein content of 17% and metabolic energy of 2950 kcal kg-1. Feed is given 2 times a day at 06.00 WITA and 16.00 WITA. Chickens were weighed every week to find out the weight gain produced during maintenance.

At the end of the 12th week, blood was taken from the brachial vein in the wing area. This blood sampling was used as a sample for genotyping, leococyte concentration testing and its differentiation and clerence test. Blood sampling was done using 1 mL syringe 0.5-1 mL.

Observed variables

a. Testing leukocyte profile and its differentiation

Leukocyte testing and its differentiation were carried out according to Sastradipadja et al. (1989) with a total sample of 34 samples. Leukocyte counts were measured by taking blood using an erythrocyte pipette with the help of a suction device (aspirator) to the limit of 1.0. The tip of the pipette was cleaned with a tissue. The Rees and Ecker diluent solution is sucked until the mark 101 is printed on the erythrocyte pipette, then the aspirator pipe was removed. Ends of the pipette were closed with the thumb and index finger of the right hand, the contents of the pipette are shaken by forming the number 8 movement, and the liquid which was not shaken is removed. A drop of liquid was put into the arithmetic chamber and the items in the arithmetic chamber are settled. White blood grains were counted under a microscope at magnification 400 times. Leukocytes were counted on a neubauer hemocytometer using 5 leukocytes from 9 main boxes by taking the following parts: one upper right corner box, one upper left corner box, one middle box, one lower right corner box and one lower left corner box . The number of leukocytes obtained from the results of calculations with a microscope (b) multiplied by 200 to determine the number of leukocytes every 1 mm3 of blood.

Leukocyte differentiation was measured by making a blood sample preparation preparation about 2 cm from the tip of the object glass. The preparation was reviewed and fixed using 75% methanol for 5 minutes. After that, lift it to dry in the air. Reviews of the blood with Giemsa solution for 30 minutes, removed and washed using running water to remove excessive dyes, then dried with blotting paper. Preparations of cereals were placed under a 1000 times magnification microscope and added with emersion oil then lymphocytes, heterophils and monocytes were counted 1000 times magnification to a total of 100 leukocytes.

b. Clearance test

This clearance test aimed to determine the ability of native chickens to kill bacteria. This study foused on Salmonella pullorum bacteria. The Clearance test was based on George (1998). The testing procedure consisted of 3 stages which include preparation of bacterial culture, examination and interpretation of results.

Data analysis

The association of gene diversity with leukocyte profiles and their differentiation and resistance to bacterial infections was carried out using analysis of variance with GLM (General Linear Model) procedure (SAS Inst. Inc., Cary, NC). Significant differences between general averages and genotypes were performed by Duncan's test. Significant differences are indicated by the value of $P < 0.05$. The model used is as follows:

$Yijk = \mu + \alpha i + \varepsilon ijk$

Information:

$Yijk$ = Observation value
μ = Common midpoint
αi = Effect of genotype
εijk = Effect of trial error

III. RESULTS AND DISCUSSION

TGF-β2 Genotype in Tolaki Chickens

The TGF-β2 gene in chickens is located on chromosome 3. Based on data in GenBank (with access number: NC_006090.5) the TGF-β2 gene has 64065 bp. Based on its structure, the TGF-β2 gene consists of promoter, exon, intron and flanking regions. The structure of the TGF-β2 gene, initiated by a promoter region measuring 600 bp. The TGF-β2 gene has seven exons and six introns. The seven exons are 1, 2, 3, 4, 5, 6, and 7, each measuring 391 bp, 163 bp, 132 bp, 110 bp, 174 bp, 153 bp, 3443 bp. Intron 1, 2, 3, 4, 5, and 6, each measuring 38631 bp, 14775 bp, 117 bp, 2184 bp, 559 bp, and 2033 bp. The TGF-β2

gene structure is terminated by a 600 pb flanking region.

TGF-β2 genotayping was carried out on exon one, with a PCR product measuring 284 bp. The results of the visualization of the results of electrophoresis from this study are presented in Figure 1.

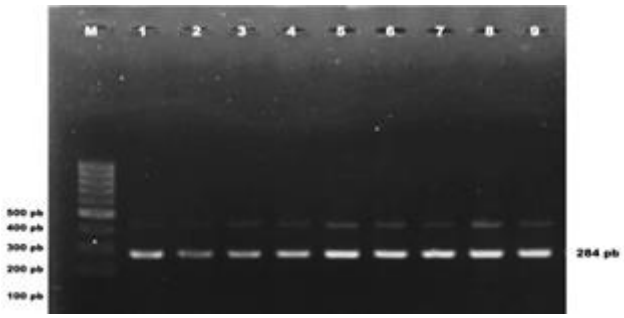

Figure 1. Visualization of the results of the amplification of the TGF-β2 gene fragment in Tolaki chickens using 1.5% agarose gel (M = 100 bp DNA marker; 1-8 = Tolaki Chicken DNA sample)

The results of cutting the TGF-β2 gene with the restriction enzyme RsaI succeeded in identifying 2 alleles, T and C. These two alleles produced 3 genotypes, namely TT, TC, and CC (Figure 2). T allele has 1 band which is 284 bp. Alel C has 2 bands namely 184 bp and 100 bp. The combination of T and C alleles has 3 bands namely 284 bp, 184 bp and 100 bp.

Mutations in g.640 T> C cause the RsaI restriction enzyme site, GT | AC to GC | AC. This is consistent with the results of research conducted by Tohidi et al. (2012) stated that SNP in exon 1 TGF-β2 gene is a substitution between thymine base to cytosine (T→C) at position 640. The mutation is a synonymous mutation because both codons (GTA and GCA) produce the same amino acid, alanine. Synonymous mutations are mutations that do not cause changes in the amino acid squad of a protein, so it cannot be detected at the amino acid level. However, synonymous mutations are not always silent mutations, for example they can create new splicing sites or eliminate existing splicing sites so that an exon sequence turns into an intron and causes the production of different polypeptides (Li and Graur 1991).

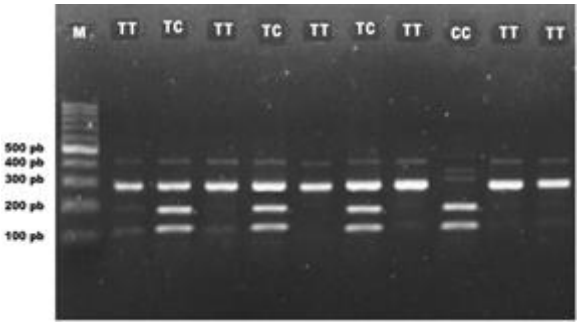

Figure 2. Visualization of PCR-RFLP results of the TGF-β2 gene fragment using 2% agarose gel (M = 100 bp DNA marker)

Allele frequency and TGF-β2 gene genotype

Values of allele frequencies and TGF-β2 genotypes at the RsaI locus in tolaki chickens are presented in Table 1.

Table 1 Allele frequency, genotype frequency and Hardy-Weinberg balance TGF -β2 gene

Locus	n	Allele Frequency		Genotype Frequency			χ^2
		T	C	TT (n)	TC (n)	CC (n)	
RsaI	70	0.80	0.20	0.64(45)	0.31(22)	0.04(3)	0.02

n = number of individuals

Alleles are alternative forms of genes that have the same locus (position on chromosomes), but have different effects. The RFLP results using the RsaI enzyme in the TGF-β2 gene fragment successfully identified two alleles, namely the T allele and C. The

T allele dominates the frequency of the TGF-β2 gene allele. The value of T allele frequency in Tolaki chickens is 0.80. The allele C frequency value is 0.20. The results of this study can be interpreted that the TGF-β2 gene at the RsaI locus is polymorphic. This is in accordance with Allendroft et al. (2013) who stated that a population can be said to have polymorphic properties if the allele frequency is less than 0.99.

Genotype is the genetic makeup of an individual trait or character. In this study, the TGF-β2 gene in Tolaki chicken identified three types of genotypes, namely TT, TC, and CC. The highest genotype frequency was found in the TT genotype and the lowest was the CC genotype. This shows that the TT genotype in the three populations has the highest chance of appearance compared to the CC genotype. Similar results were reported by Tang et al. (2011), Niarami et al. (2014) and Muhsinin et al. (2017) who obtained three genotypes (TT, TC, and CC) from the cutting of the TGF-β2 RsaI locus gene. Furthermore Muhsinin (2017) reported that the frequency of TT, TC, and CC genotypes were 0.48, 0.25, and 0.27, respectively.

Genotype balance of TGF-β2 gene in the population

The TGF-β2 gene balance in the population (Hardy-Weinberg balance) was tested by chi-square (χ2). Chi-square test is used to determine the population is in Hardy-Weinberg balance. Chi-square value which is not significant at the 5% level indicates a match between the observed value and the expected value. The population is said to be in balance if the calculated value χ2 is smaller than the χ2 table (Allendorf et al. 2013). The results obtained in the Tolaki chicken population indicate that it is in Hardy-Weinberg balance.

Allendorf et al. (2013), a livestock population is stated to be in equilibrium if the allele frequency and genotypic frequency are constant from the generation of generations generated by randomly combined gametes that occur in large populations. Large populations will not change from one generation to another if there is no selection, migration, mutation and genetic drift (Noor 2008).

The balance of the TGF-β2 gene genotype at the RsaI locus in this Tolaki chicken shows that the intersection of mutations, intensive selection and migration can be said to be low. Tolaki chicken has been maintained by the community extensively (diumbar), this allows the random marriage of generations of generation.Asosiasi Gen TGF-β2 dengan Sifat Ketahanan

Association of the TGF-β2 gene with resistance to bacterial infections

Leukocyte Concentration and Differentiation

Leukocytes are cells that can be used to estimate the potential for endurance. Leukocytes differentiate into several cells, namely heterophils, lymphocytes, monocytes, eucinophils and basophils. Heterophyll is the body's main line of defense when the body is attacked by disease agents. Lymphocytes are cells that function in antibody formation. Monocytes are precursors of macrophages that are in the blood. Eucinophils are cells produced during parasitic infections and allergic reactions, while basophils are leukocyte cells that have a role in allergic reactions and release heparin into the blood which inhibits blood clots (Guyton and Hall 2006). The association of the TGF-β2 gene with leukocyte concentration and leukocyte differentiation in tolaki chickens is presented in Table 2.

Table 2 Association of the TGF-β2 gene with leukocytes and leukocyte differentiation in Tolaki chicken

Variable	Genotype			Standard
	TT (n=3)	TC (n=3)	CC (n=3)	
Leukocytes (10^3 cell mm^{-3})	25.39 ± 1.92	25.62 ± 2.57	28.08 ± 4.32	[1]22.30 - 30.10
Heterophil (%)	29.33 ± 1.15	28.67 ± 1.58	31.00 ± 1.00	[1]23.00 - 30.00
Lymphocytes (%)	64.33 ± 2.08	64.67 ± 2.52	61.67 ± 2.08	[1]63.00 - 73.00
Monocyte (%)	1.67 ± 0.58	2.67 ± 1.15	3.00 ± 1.00	[1]0.00 - 3.00
Eosinophils (%)	4.67 ± 1.15	4.00 ± 1.00	4.33 ± 1.53	[1]1.00 - 6.00
Basophils (%)	-	-	-	-
Ratio H/L	0.46 ± 0.03	0.44 ± 0.04	0.50 ± 0.02	[2]0.45 - 0.5

n = number of individuals; H/L = Heterophil/ Lymphocytes; [1]Turcul et al. (2011); [2]Swenson dan William (1993)

The concentration of leukocytes and the percentage of heterophils, lymphocytes, monocytes, eucinophils and the H/L ratio, between the genotypes of TT, TC, and CC were not statistically different. From this table it can be seen that the concentration of leukocytes and the percentage of their differentiation in the three TGF-β2 genotypes of the Tolaki chicken (TT, TC, CC) are in the physiologically normal range.

The H / L ratio is an indicator of heat stress. The stress causes the H / L ratio to increase (Altan et al. 2000), this is due to hormones that increase stress secreted by the adrenal glands will increase the H / L ratio (Gudev et al. 2011). This study shows that toalki chickens have a percentage of H / L in each genotype within the normal range (TT = 0.46, TC = 0.44, and CC = 0.50). According to Swenson (1993), in chickens, the ideal range of heterophile and lymphocyte percentage ratio is 0.45-0.50. The percentage ratio of H / L that is outside this range, indicates that the cattle are under stress.

Clearence Test

Tolaki chicken endurance testing in this study used the clearence test method. The results of the chicken blood test in this study are presented in Table 3.

Table 3. Association of TGFβ2 genotypes with resistance to Salmonella pullorum bacterial infection in tolaki chickens

Genotype (n)	Initial concentration (cfu/mL^{-1})	Final concentration (cfu/mL^{-1})	Bacterial mortality rate (%)
TT (3)	2.7 x 10^{14}	7.2 x 10^{7}	99.99
TC (3)	2.7 x 10^{14}	4.0 x 10^{8}	99.99
CC (3)	2.7 x 10^{14}	1.2 x 10^{11}	99.95

n = number of individuals

The observations statistically show the number of bacteria that can be killed is no different. The ability of each genotype to kill Salmonella pullorum bacteria reaches 99% (Table 3). Thereby clearance test treatment on Tolaki chicken blood with Salmonella

pullorum at infection dose (2.7 x 1014 cfu / mL^{-1}), did not cause physiological disturbance. This means that although the tolaki chicken is infected with Salmonella pullorum, it does not clinically show any disturbance.

IV. CONCLUSION

The TGF-β2 gene in tolaki chickens is polymorphic and three genotypes (TT, TC, and CC) were found. The TGF-β2 gene might be used as a genetic marker for resistance to Salmonella pullorum bacterial infection in tolaki chickens. Based on molecular and biological testing, tolaki chickens in all genotypes (TT, TC, and CC) are resistant to Salmonella pullorum.

V. REFERENCES

[1]. Allendroft FW, Luikart G, Aitken SN. 2013. Conservation and The Genetics of Populations. Ed ke-2. Oxford (UK): Blackwell Publishing.

[2]. Altan O, Altan A, Cabuk M, Bayraktar H. 2000. Effects of heat stress on some

[3]. blood parameters in broiler. J Vet Anim Sci. 24: 145-148.

[4]. Carlos I, Diez L, García-Porrero JA, Hurlé JM, Montero JA. 2014. Decorin gene expression in the differentiation of the skeletal connective tissues of the developing limb. Gene Expression Patterns, 15: 52-60.

[5]. George JJ. 1998. Bacteriogical Analytical Manual. Maryland (US): AOAC International.

[6]. Guyton AC, Hall JE. 2006. Texbook of Medical Physiology Ed 11. Philadelphia: Elsevier Inc.

[7]. Li WH, Deep N, Zhou H, Mitchell AD, Ashwell CM, Lamont SJ. 2003. Chicken quantitative trait loci for growth and body composition associated with transforming growth factor- beta genes. Poultry Science, 82:347-356.

[8]. Li WH, Graur D. 1991. Fundamentals of Molecular Evolution. Sunderland (GB): Sinauer.

[9]. Muhsinin M, Ulupi N, Gunawan A, Wibawan I, Sumantri C. 2017. g. 640T> C Polymorphism of the TGF-β2 gene is associated with Salmonella pullorum resistance in Indonesian chickens. Animal Production. 19(2):81-92.

[10]. Nafiu LO, Rusdin M, Aku AS. 2012. Produksi dan Karakteristik Telur Ayam Tolaki pada Pemeliharaan Intensif. Agriplus. 22 (3): 207-214.

[11]. Niarami D, Mojtaba, Masoudi AA, Torshizi RV. 2014. Association of single nucleotide polymorphism of GHSR and TGF-β2 genes with growth and body composition traits in sire and dam lines of a broiler chicken. Animal biotechnology, 25(1): 13-22.

[12]. Noor RR. 2008. Genetika Ternak. Jakarta (ID): Penebar Swadaya.

[13]. Pagala MA, Aku AS, Badaruddin R, Has H. 2018. Karakteristik fenotip dan genotip gen cGH (growth hormon) pada ayam tolaki. JITRO, 5(3): 1-5.

[14]. Rusdin M, Nafiu LO, Saili T, Aku A.S. 2011. Karakteristik fenotipe sifat kualitatif ayam tolaki di Kabupaten Konawe Sulawesi Tenggara. Agriplus, 21(3): 248-256.

[15]. Sambrook J, Russell D. 2001. Molecular Cloning: a Laboratory Manual. 3rd ed. New York (US): Cold Spring Harbor Laboratory Press.

[16]. Sastradipraja D, Sikar SHS, Wijayakusuma R, Ungerer T, Maad A, Nasution H,

[17]. Suriawinata R, Hamzah R. 1989. Penuntun Praktikum Fisiologi Veteriner.

[18]. Pusat Antar Universitas Ilmu Hayati. Bogor (ID): Institut Pertanian Bogor.

[19]. Sulandari S, Zein MSA, Paryanti S, Sartika T. 2007. Taksonomi dan asal usul ayam domestikasi. Dalam buku Keragaman Sumber Daya Hayati Ayam Lokal Indonesia:Manfaat

dan Potensi. Editor: Kusumo Diwyanto dan Siti Nuramaliati Prijono. Pusat Penelitian Biologi, LIPI.

[20]. Swenson MJ, William OR. 1993. Duke`s Physiology of Domestic Animals. Ed ke-11. Publishing Assocattesa Division of Cornell University, Ithaca and London.

[21]. Tang S, Ou J, Sun D, Zhang Y, Xu G, Zhang Y. 2011. A novel 62 bp indel mutation in the promoter region of transforming growth factor-beta 2 (TGF-β2) gene is associated with body weight in chickens. Animal genetics, 42(1): 108-112.

[22]. Tohidi R, Idris I, Panandam JM, Bejo MH. 2012. The effects of polymorphisms in IL-2, IFN-γ, TGF-ß2, IgL, TLR-4, MD-2, and iNOS genes on resistance to Salmonella Enteritidis in indigenous chickens. Avian Pathol. 41(6), 605-612.

[23]. Turcul D, Oporanu1 M, Grigorescu1 P, Roman M. 2011. Studies on hematological parameters in broiler chicken treated with amoxidem 50%. Medicamentul Veteriner. 5(1):93.

[24]. Yeh FC, Yang RC, Boyle T. 1999. POPGENE version 1.31 : Microsoft Window-based Freeware for Population Genetic Analysis. Edmonton, AB. Canada : University of Alberta Canada.

Cite this article as :

Muh Akramullah, Cece Sumantri, Niken Ulupi, Muh Amrullah Pagala, "Association of TGF-β2 Gene Polymorphism with Salmonella pullorum Bacterial Infection Resistance in Tolaki Chickens", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 7 Issue 7, pp. 46-54, January-February 2020. Available at doi : https://doi.org/10.32628/IJSRSET20716
Journal URL : http://ijsrset.com/IJSRSET20716