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Original article

Three-dimension Glyceraldehyde-3-Phosphate Dehydrogenase protein structure of substitution and insertion sequences of GAPDH gene of chicken drumstick meat (*Gallus gallus*)

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Abstract

The study aimed to observed the 3-D structure of GAPDH protein and identify the GAPDH gene sequences mutation of chicken drumstick meat (*Gallus gallus*). The sample of chicken meat was randomly taken in four districts in Malang city. In this study, the DNA was isolated from drumstick meat chicken samples, amplified using proper primers, and then sequenced using ABI 3730xl DNA Sequencer. The DNA sequences alignments analyzed by BioEdit software and the control sequence of GAPDH gene was obtained from NCBI GenBank (sequence Gene ID: 374193). Then, the amino acid sequence and 3D structure of GAPDH protein were determined based on the change of nucleotide sequences using Swiss model and PyMol software. The nucleotide sequence of a partially GAPDH gene of drumstick meat chicken from districts two is completely different with a 97% similarity level, which found twelve nucleotides' substitutions mutation between nucleotide base number 354 until 777 and three nucleotides inserted between T_{753} and G_{754} nucleotide base. These mutations changed the amino acid sequence and 3D structure of GAPDH protein. This result suggests that the differential drumstick chicken meat GAPDH sequences and 3D structure may induce the change of protein-protein interaction and induction.

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Keywords: 3D structure, chicken, drumstick meat, GADPH, nucleotide mutation

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Introduction

The GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) gene of the chicken (*Gallus gallus*) gene is located on chromosome 1 between CNP1 (C-type Natriuretic Peptide 1) and IFFO1 (Intermediate Filament Family Orphan1) genes. The GAPDH gene (Gene ID: 374193) has 12 exons and 1001 bp of CDS (coding DNA sequence) from 1288 bp of gene source (GenBank). The GAPDH gene CDS is nucleotide sequence of mRNA mature which is translated into GPDH protein that may perform specific modifications of nucleic acid complexes and other functions at the cellular level (Sirover, 2020; Habenicht, 1997; Lorentzen et al., 2004).

GAPDH has a biological function as a glycolytic enzyme and it has certain position to regulate the signaling of PI3K/AKT and MEK/ERK pathway, and stimulates a nuclear function due to the GAPDH-dependent cell death (Sirover, 2020; Ping et al., 2021; Zhu et al., 2021). Recently studies using SH-SY5Y cells proved that silencing of GAPDH gene expression can decrease the intracellular ROS levels. Furthermore, that silence expression also upregulates the autophagy activity which decline the cell apoptosis and necrosis (Ping et al., 2021). Hwang et al. (2009) in a study using HEK-293T cells [human embryonic kidney-293 cells expressing the large T-antigen of SV40 (simian virus 40)] reported that the GAPDH molecule had a function not only provides in glycolysis metabolism, but also the oxidatively modified GAPDH regulat es its cellular function by altering interacting proteins on its catalytic site. In particular, cysteine residues in oxidative GAPDH cause a change in their surface structure and result in a binding shift to p54nrb.

The selectable of conventional housekeeping genes are HPRT1 (hypoxanthine phosphoribosyl transferase 1), HMBS (hydroxymethylbilane synthase), TBP, ß-actin ACTB, RPL13 (ribosomal protein L13) and GAPDH genes which were showing stable expression in all tissues of chicken (Zhang et al., 2017). According to Genome-wide association studies (GWAS) analysis, there are sixty candidate genes susceptibility with intramuscular fat (IMF) for lipid in muscle, dry matter (DM) for thigh muscle, abdominal fat (AbF) and meat color for meat quality traits (Sun et al., 2013).

Broiler chicken meat is one of the sources of nutrition that has been consumed by the people of Malang city for decades. Broiler chicken farms have developed rapidly, however, the identification of genotyping related to meat quality has not been widely studied. This study is a small part of the research on genotyping of broiler chicken genes, we choose the GAPDH gene because it has other functions related to cellular alternation that may be affected by any nucleotide change in GAPDH gene sequence of broiler chicken meat. Also, we construct the three-dimensional structure of the chicken GAPDH protein in each district. This study explored the three-dimension structure of Glyceraldehyde-3-Phosphate

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Dehydrogenase protein based on differential nucleotide sequences GAPDH gene of drumstick chicken meat from four different districts in Malang city.

Methods

DNA isolation and amplification

The twelve samples of drumstick broiler chicken meat were obtained from 4 districts in Malang city and three samples were randomly taken for each district. The samples code is D means district and M means meat that DM1 from district 1, DM2 from district 2, DM3 from district 3 and DM4 from district 4. The broiler meat for DNA isolation were weighed 2.5 g and isolated with double analysis of each sample by using a standard method that referred to Fatchiyah et al. (2011) with some modifications. The DNA concentration and purity were quantified by using NanoDrop spectrophotometer. The quality of the DNA was analyzed using agarose gel 1%. The amplification mix solution composition was referred to Fatchiyah et. al., 2011 [8]. The GAPDH gene primer for DNA amplification were F: 5'-CCAGAACATCATCCCAGCGTC-3' and R: 5'-ACGGCAGGTCAGGTCAACAA-3'. The PCR program was cycled for the hot start at 95 C for 5 minutes, and the 35 cycles for denaturation at 95 C for 30 seconds, annealing at 56.2 C for 30 seconds, and extension at 72 C for 40 seconds, and then, one cycle for post-extension at 72 C for 10 min. The amplification results were tested by agarose gel 1.5% and visualized using with ChemiDoc gel imaging (BioRad) and measured the quality of DNA-amplified using ChemiDoc MP imaging systems (BioRad).

DNA sequencing and in silico analysis

The sequencing of purified PCR products was carried out by using ABI-Prims 3730xl DNA Sequencer (Köln, Germany). All of DNA sequencing samples were aligned using BioEdit software. GAPDH gene ID 374193, GAPDH DM1, DM2, DM3, and DM4 sequences were translated to amino acid using MEGA 6.0 and were checked by Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The three-dimensional structures of GAPDH proteins were constructed using Swiss model (https://swissmodel.expasy.org/) and were analyzed by PyMol v2.2. (https://pymol.org/2/) and Discovery Studio ver. 21.1.1 (BIOVIA Discovery Studio Visualizer; https://discover.3ds.com/).

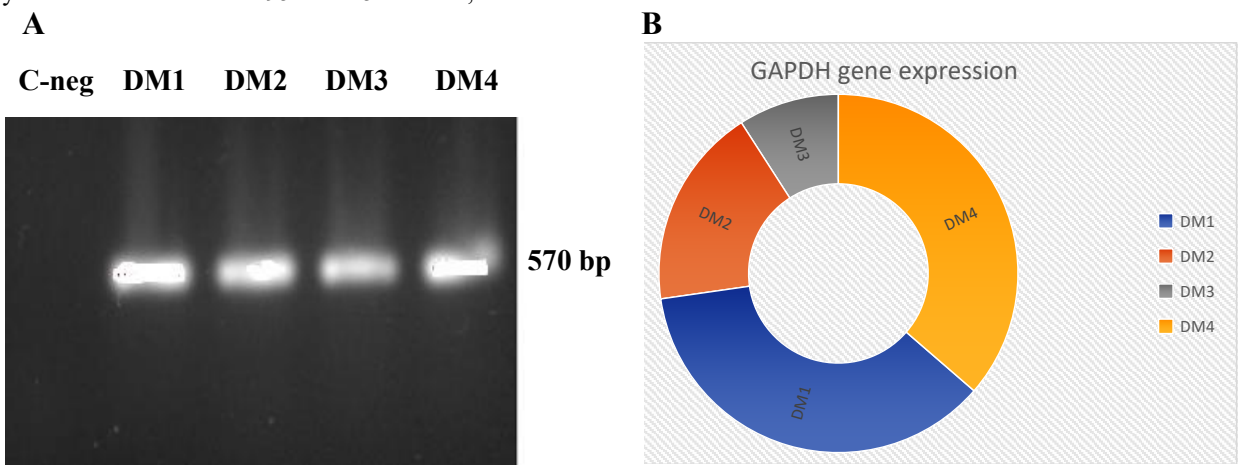

Figure 1. The amplification result of drumstick chicken meat GAPDH gene and percentage of gene expression. A. DNA amplification is run ~~by~~ in 1,5% agarose. B. Percentage of GAPDH gene expression.

Results

The result of GAPH gene amplification for all drumstick chicken meat was 570 bp, in Figure 1A. The percentage of GAPDH gene expression level showed the DM1 and DM4 group are 36.36%; DM2 group is 18.18% and DM3 is 9.09%, at Figure 1B.

This study was identified GAPDH gene sequences alignments that showed substitution and insertion mutations. In all drumstick chicken meat, we found the T_{488} changed to G_{488}. Interestingly, the GAPDH gene sequence of DM2 showed a lot of substitutions and one insertion mutation compared with all samples, in Figure 2. The substitution mutations were found at C_{354}-A_{354}, T_{355}-G_{355}, C_{357}-G_{357}, C_{622}-T_{622}, C_{623}-T_{623}, C_{624}-T, $_{624}$ T_{625}-G_{625}, A_{724}-C_{724}, A_{725}-C_{725}, T_{747}-A_{747}, T_{748}-G_{748}, and T_{777}-C_{777}. And insertion mutation of three nucleotide bases of GGT was found between T_{753} and G_{754} base. The similarity of the nucleotide sequence of drumstick meat chicken in district 1, 3 and 4 identified 99.7% compare to GADPH gene sequence of *Gallus gallus* with Gen ID 374193. However, DM2 chicken meat showed 97% nucleotide similarity with a lot of nucleotide substitutions and insertion which are not found in other samples.

According to the differential nucleotide sequences of GAPDH gene in each district (Fig. 2), we analyzed the protein sequence using BLAST and an alignment software proved that the substitution and insertion nucleotides in GAPDH gene caused mutation in amino acid sequence (Fig. 3A). Moreover, the amino acids sequence of GAPDH protein was constructed the three-dimensional structures using Swiss model and PyMol software. The three-dimensional structure of the chicken GAPDH protein in from district 2 shows a different catalytic region compared to 3D structures of GAPDH

proteins from other samples, shown as purple color of GAPDH DM2 with the red arrow in Figure 3B.

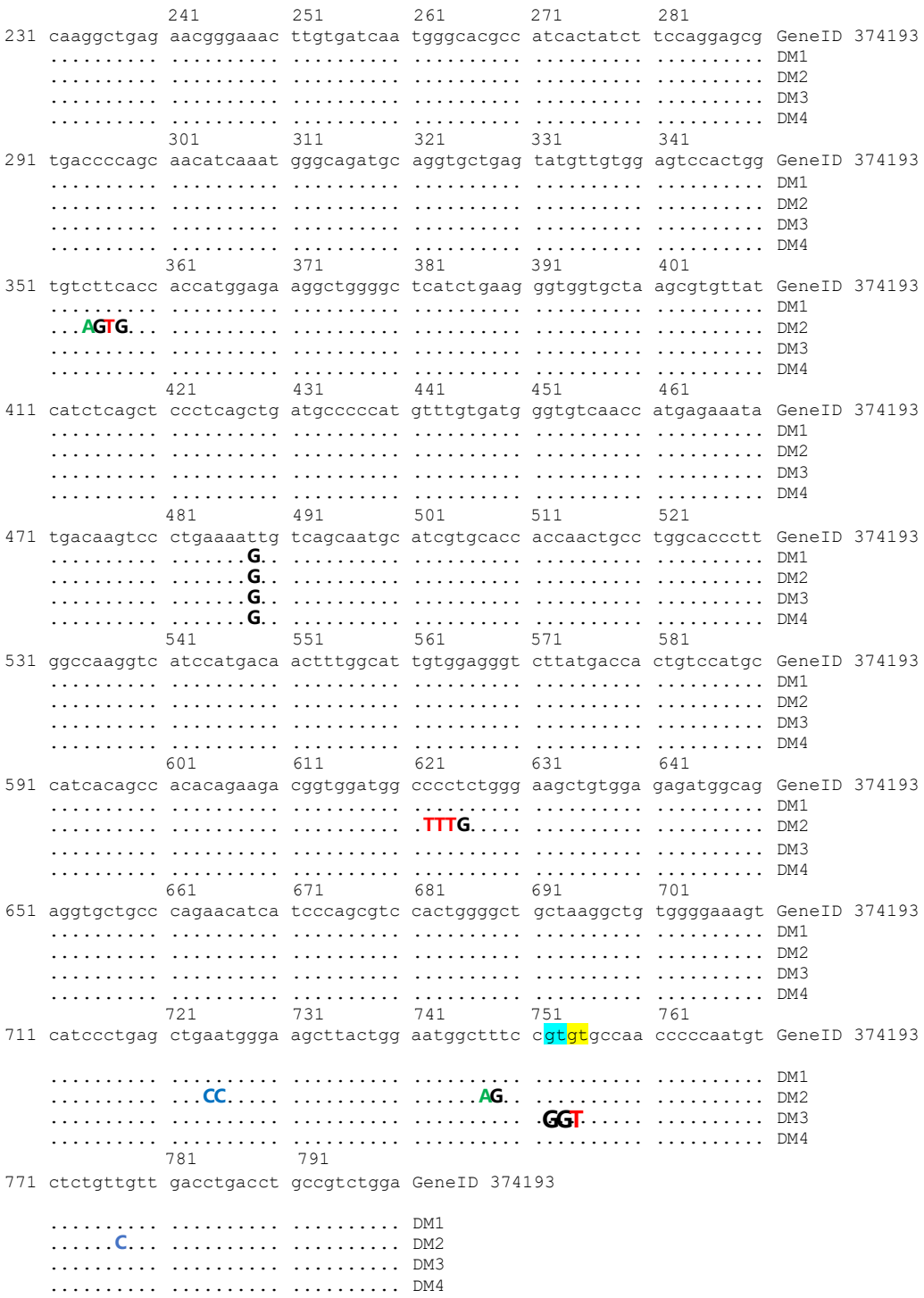

Figure 2. The sequences alignment of GAPDH gene of drumstick meat chicken. Uppercase with color-bold letters are substitution mutations. The box showed an insertion mutation of GGT nucleotide sequences.

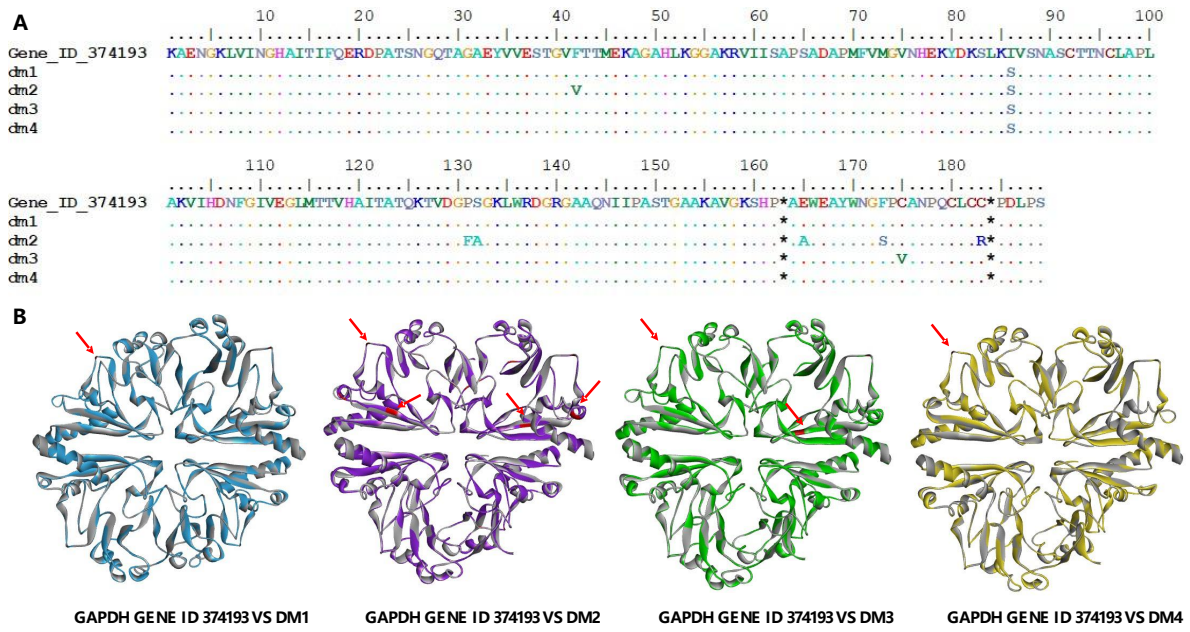

Figure 3. Amino acid alignment and 3D model of superimposed GAPDH gene ID 374193 protein with GAPDH DM1, DM2, DM3, and DM4. The grey color represented GAPDH gene ID 374193, blue color is GAPDH DM1, purple is GAPDH DM2, green color is GAPDH DM3, and yellow color is GAPDH DM4, red arrows and red color performed mutation, A. Alignment of GAPDH gene ID 374193 protein with GAPDH DM1, DM2, DM3, and DM4, B. Superimposed of 3D model GAPDH protein.

Discussion

The ancestor of commercial broiler chicken was dominant from domestic chickens (Gallus gallus) with a lot selection and breeding to get different phenotypes and to perform the good qualities of meat and eggs (Li et al., 2019). Breeding selection triggers nucleotide changes in broilers that differ from the nucleotide sequence of their ancestral chickens. The GAPDH gene in chicken meat reported that its expression could each induce twenty-fold muscle fiber development. Stone's study (1985) also reported that the structural gene GAPDH has twelve exons with eleven introns. Intron I to V is structurally the domain of the NAD binding site (Ini maksudnya Intron I to V di transkripsi dan ditranslasi untuk jadi NAD binding site? Kalimat diatas sulit untuk dipahami.). Exons located between introns VII and VII (sepertinya mistyping untuk nomor exonnya) can function as catalytic domains. This study found differences in the catalytic region of GAPDH protein 3D structures among chickens from different districts. These changes may affect the function of GAPDH as a housekeeping gene or apoptosis regulator and protein-protein interaction of specific metabolism. A study done by Hwang and colleagues has reported that GAPDH is highly sensitive to modification of the cysteine residue (Cys152) located at its active site. The active site cysteine residue of GAPDH can be thiolated by hydrogen peroxide, which caused GAPDH as the main target of oxidative stress (Hwang et al., 2009). Recent study also reported that GAPDH can interact with Siah1 protein as nuclear localization signal protein, and this binding mediated the nuclear translocation and apoptosis (Chuang et al., 2005). In neuron cells induced 1-methyl-4-phenylpyridinium expressed high accumulation of GAPDH in nucleus (Fukuhara et al., 2001).

As a housekeeping gene, GAPDH has multiple functions at the cellular level. GAPDH, distributed throughout the cell, including the nucleus, cytosol and membranes, and it plays a role not only in glycolytic function, but also in membrane fusion, phosphotransferase activity, ribosome interactions, nuclear RNA export, DNA replication and repairing (Hwang et al., 2009). It is well-known that the oxidative phosphorylation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate catalyzed by GAPDH with converting NAD+ into the high-energy electron carrier NADH (Hara et al., 2006; Batthyany et al., 2006).

The quality of gene expression is depends on genetic and environmental factors. Environmental factors such as nutrients composition, temperature, humidity and physic-chemical mutagenic factors are affecting the prevalence of gene expression (Ralston & Shaw, 2008). Molecular genotyping of cells or tissues can cause a cellular environment induction (Hunter, 2005). The promotor region of the GAPDH gene had an eighty percent of GC-rich sequence between -58 to -93 from the first nucleotide (+1) of the transcription site (Stone et al., 1985). GAPDH is overexpressed and accumulated in the nucleus during various perturbation-induced apoptosis in different cell types (Chuang et al., 2005) due to differences in nucleotide sequences or gene expression. These results suggest that the chicken meat from district two may derived from different chicken variants or ancestor chicken breeds. This nucleotide sequence change factor is influenced by inter-species breeding, food composition, availability of hormones and enzymes

in cells, continuous environment exposure and other important factors (Ralston & Shaw, 2008; Hunter, 2005).

In conclusion, the nucleotide sequence of a partially GAPDH gene of drumstick meat chicken from districts two is markedly different with 97% similarity level and twelve nucleotides' substitutions mutation and three nucleotides inserted between T_{753} and G_{754} nucleotide base. These nucleotide mutations affected amino acid sequences and the three-dimension structure of GAPDH protein.

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