GHR mRNA Expression in the Liver and Muscles of Local Pigs from The Islands in North Sulawesi, Indonesia

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Abstract. The GHR gene is known as a member of the growth gene's superfamily that function important in controlling the growth. This study was designed to investigate the expression of GHR genes in the liver and muscles of local pig from the islands in North Sulawesi, Indonesia. The parameters measured was GHR mRNA expression by reverse tranriptase RT-PCR. mRNA sample was taken from liver and longisimus dorsi muscle of sacrifice pigs and cDNA was amplified using specific primer for target genes. The GHR primer gene used was 5' TTT TCT GGG AGT GAA GCC AC 3' and R = 5' AGG GCT CTG TAA ACC GTG AC 3' with its housekeeping gene GAPDH. The result showed that GHR is expressed in the liver and muscles of local pig from the islands in North Sulawesi. GHR mRNA expression in muscles was higher than in liver (P>0.05). The GHR gene expression was also regulated by factors such as nutritional intake, GH, ages, temperature and hormones.

1. Introduction

The growth is due to cellular hyperplasia (increase number of cells), cellular hypertrophy (increase in cell size) and apoptosis (cell death). Growth is a process that deals with many complex factors such as genotype, external factors such as nutrition and the environment, and internal signaling systems such as hormones and growth factors. Growth is a complex process. Hormones as one of the factors that influence them are regulated by the pituitary hypothalamus. Growth hormone (GH) is an anabolic hormone synthesized and secreted by somatotropes in the anterior pituitary lobe [1]. The synthesis and secretion of these proteins are influenced by age and sex [2]. GH plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction and metabolism of proteins, lipids and carbohydrates [3,4].

Receptor expression is required for cellular activity against GH. The growth hormone receptor (GHR) is a transmembrane protein that binds GH with high affinity and specificity. The growth hormone is expressed by genes. The growth hormone gene has a high molecular weight, so it is difficult to enter cells that must cross the cell membrane. Therefore, the GH gene requires a growth hormone receptor (GHR) gene as a receptor present in cell membranes, so that the GH gene can have an effect on the target cell without damaging the membrane [5] . This indicates that changes in GHR function may affect the binding capacity of GH and GH activity in the target tissue [6]. The interaction of GH with GHR in the cells causes the intracellular signal of the Janus kinase and activates the transcription sequence [7]. Stimulation of GH affects the synthesis of IGF-1 in almost all tissues. The liver is the organ responsible for the production of IGF-1 serum. The release of GH causes an increase in the synthesis of mRNA and IGF-1 protein in the liver. Animals that lose specific IGF-1 in the liver produce less serum IGF-1 (10 to 25%) than normal animals [8].

Gene expression or emerging characteristics (phenotype) of growth and development are influenced by genetic factors, environmental factors, and environmental interactions with genetics. Gene expression is the process of copying the information contained in the DNA throughout the RNA transcription process and translating it into protein. Gene expression can be measured using the qRT-PCR method in real time (quantitative reverse transcription - polymerase chain reaction). qRT-PCR is a technique of multiplication (amplification) of pieces of DNA in vitro in a specific area limited by two primary oligonucleotide. The primer used as a limit to the zone being propagated is a single-stranded DNA whose sequence is complementary to the template DNA. RT-PCR is part of the normal PCR process. The difference with ordinary PCR, in this process, takes place an additional cycle, namely the modification of the RNA in cDNA (complementary DNA) using the enzyme reverse transcriptase. Reverse

transcriptase is an enzyme capable of synthesizing DNA molecules in vitro using RNA templates. In this study, mRNA was collected from the longisimus dorsi muscle and liver of pigs.

Indonesia has local pigs, including Bali pigs, Batak pigs, Toraja pigs and Minahasa pigs (North Sulawesi, Indonesia) [9]. Many of these local pigs are kept on people's farms, while large farms raise pig varieties that are now officially known as superior pigs. Local pigs are kept by small farmers with traditional farming systems as a side business that is carried out by the family. It was realized that local livestock also played an economic role, as many of them were used as meat producers for food security purposes, although the maintenance was small scale with the ownership of 2 to 6 pigs.

Local pigs are livestock that have undergone old domestication and have high adaptations to the local environment. Local pigs are developed for the purpose of gaining profits from the sale of seedlings, saplings, and pork pigs and further preserving family traditions and participating in national food procurement and fulfillment of good nutrition to produce healthy, strong and intelligent generations [10]. Local pigs from the islands in North Sulawesi Province are maintained by small farmers with traditional systems as part-time businesses and are a buffer for the family economy. Maintenance is generally very easy with the provision of household waste with a simple housing system. When compared to the size of the local pig body of the islands of North Sulawesi with pigs of Landrace and Duroc descent at the same age, this local pig from the Islands in North Sulawesi has a smaller body size.

The GHR gene is located on chromosome 16 in pigs. The expression of GHR in the porcine trophectoderm is responsible for the lengthening of the trophoblast [11]. In chicken, modifications of the GHR gene affect the dwarf (dwarf) phenotype [12]. The reported diversity of the GHR gene in cattle has been found in the promoter region and in exon 10 [13], which influences the quality of the meat [6]. The GH, GHR, and other growth hormone gene groups such as Like Growth Factor-I (IGF-I) and Pituatary Specific Transcription Factor-I (PIT-I) insulin are widely used in the study of candidate genes for the characteristics of livestock production, henceforth used as a genetic marker in selection [14]. Indeed, these hormones are regulators of cattle growth and cattle body development. GH can increase production efficiency, reduce fat deposits, stimulate muscle and bone growth, and increase the efficiency of food use [15]. Several studies have shown that growth hormone genes are significantly related to variations in mean growth in cattle [16] and growth and carcass in pigs [17,18]. This study should therefore be able to study the expression of GHR genes in the muscles and liver of local pigs from islands in North Sulawesi.|

2. Research Methods

A. Location and Time of The Research

This research was conducted in traditional pig farms in several small islands in the province of North Sulawesi, including Mantehage, Bunaken, Bangka, Gangga, Nain, Siau, Lembe and Talaud Islands. Analysis of gene expression with qRT-PCR in real time (quantitative reverse transcriptase polymerase chain reaction) was performed at the Laboratory of Animal Molecular Genetics, Livestock and Genetics, Faculty of Livestock, IPB. This research was conducted from March 2018 to February 2019.

B. Samples

The animals used in these research were the local pigs from the islands in North Sulawesi province, with no special treatment. Local pigs which undergo euthanasia are traditionally kept in the courtyard of people with an average body weight of 24,875 ± 7,954 kg with ages ranging from 2 to 3 months. The study was conducted in three repetitions. These local pigs weres fed in the morning and in the evening, and their drinking water is available at all times. During the daytime, the local pigs were left free to get additional feed from the population's leftovers, and then were caged at night

C. Primary

The primary sequences used in this study were designed with Primary 3 and Primary analysis programs. qRT-PCR requires housekeeping gene as an internal control that is a gene that has 99% homologity with the target of the GHR gene, namely the GAPDH gene.

Table 1. Specific primers of GHR and GAPDH genes

Target genes	Primer sequence	Size (bp)
GHR NM_214254.2	F: 5'- TTT TCT GGG AGT GAA GCC AC - 3'	183
	R: 5'- AGG GCT CTG TAA ACC GTG AC - 3'	
GAPDH NM_001206359.1	F: 5'- GAG TGA ACG GAT TTG GCC G -3'	246
	R: 5'- CAC CCC ATT TGA TGT TGG CG -3'	

D. Extraction of mRNA

MRNA was extracted from longisimus dorsi muscle tissue and liver tissue. The tissue was aseptically removed from about 1 gram and stored in a 1.5 ml eppendorf tube containing RNAshield RNA stabilization solution until the tissue was submerged, then stored at -40 ° C until to the test. MRNA was extracted using the Rneasy Fibrous Mini Kit method (Qiagen, Germantown, USA). The mRNA samples then were ready for use or stored at -20°C. The quality of the mRNA extraction was qualitatively tested to determine the level of purity using a spectrophotometer. The quality of the mRNA would be good if the results obtained at this stage are 260/230>1.80 ng/µl.

E. Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

Reverse transcription occurs when the mRNA is retranscribed to cDNA using the ReverTra RT qPCR kit to mix with the gDNA dissolvent (Toyobo Bio-Technology, Japan). The model RNA used was a sample of RNA and a control of 2 µl each. The results obtained were matrices in the form of standard cDNA and cDNA samples. The next step was a spectrophotometer using blanks and cDNAs. The quality of the cDNA is good if the results obtained at this stage are 260/230> 1.80 ng / µl. The next step then was to develop standards, optimize and leverage qRT-PCR (Analytic Jena, AG qTower 4-channel, Germany). Optimization was performed using a conventional PCR machine with 1.5% agarose gel electrophoresis and using real-time PCR. The optimization aimed to obtain a good standard for RT-PCR results in the sample. Optimization is good if the value of R^2> 0.90. The sample then dispensed into an RT-PCR tube and then centrifuged horizontally at 25,000 rpm for 10 seconds. The material was consisted of DNA templates at 50 ng/µl, 3 µl of nuclease-free water, 5 µl of masterbatch (Toyobo Cybr Green Master Mix, Toyobo, Japan), 0.5 µl of direct priming and 0.5 µl of reverse primer. Next, the operation of the RT-PCR machine with the conditions: at 95 ° C for 1 minute, at 95 ° C for 15 seconds, followed by 58 ° C for 1 minute. The PCR process lasts 40 cycles.

F. Quantification of GHR gene mRNA expression and data analysis

Data on GHR gene expression of mRNA were obtained from local pigs euthanized in liver tissue and longisimus dorsi of muscle. Expression data of the GHR gene obtained were compared between the expression of the GHR gene in the liver and in the muscles. The number or quantification of GHR gene expression was calculated based on the number approach to the target gene (GHR) and the housekeeping genes (GAPDH), with a cycle threshold ratio (C_T). The visualization of the RT-PCR analysis data is in the form of a graph and the quantization value is the number of copies of DNA accurately with a threshold value. C_T (cycle threshold) is the value of the intersection between the fluorescence level of the sample and the average threshold value [19]. C_T data is converted into a linear form and then analyzed statistically through independent samples t-test with a P<0.05 to determine whether C_T values differ significantly

3. Results and Discussion

A. Comparison of the Mean C_T of the Target GHR Gene with the Control of the Mean C_T GAPDH Gene in the qRT-PCR Assay

The visualization of the RT-PCR analysis data is presented in the form of a graph and the quantification value in the form of the DNA copy number after being accredited with the threshold value. C_T (cycle threshold) is the value of the intersection between the fluorescent sample level and the average threshold value. The C_T qRT-PCR mRNA value shows that the gene is expressed in the liver and muscle tissue of local pigs from the islands in North Sulawesi. The target gene in this study was the GHR gene, while the control used the GAPDH gene as a housekeeping gene. The results in Figure 1 show that the mean C_T of the target gene is greater than the average GAPDH C_T. The GAPDH is a gene that is expressed in all types of cell organisms. The GAPDH gene has a relatively constant level of expression in various tissues at all stages of development, so it is used as a reference point for measuring the level of expression of other genes. The nature of the gene of this type makes the GAPDH gene used as an internal control in the analysis of gene expression by the qRT-PCR method [20, 21].

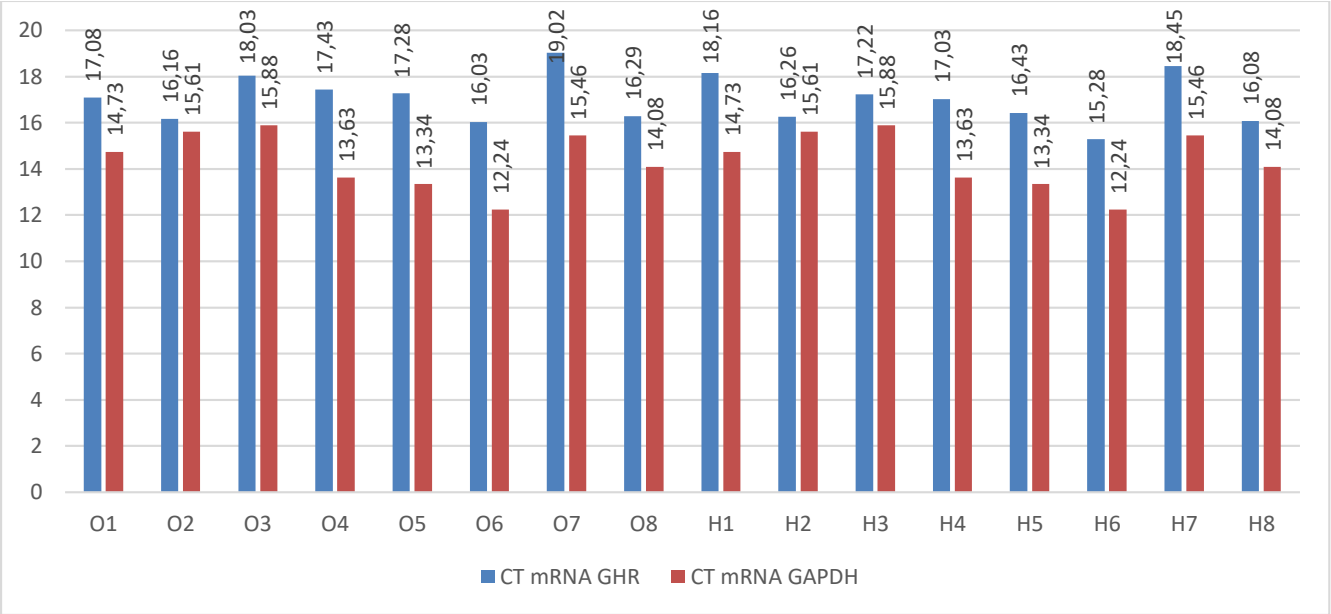

Figure 1. Comparison of C_T GHR genes (targets) and GAPDH genes: O1 to O8 is the C_T muscle, H1 to H8 is the liver

B. Expression of GHR gene mRNA in the liver and muscles of Local pig from the islands in North Sulawesi

The results showed that the GHR mRNA expression level in the longisimus dorsi muscle tissue (2.79 ± 1.19) was higher than that of the GHR gene mRNA expression in the liver (2.49 ± 1.04) (figure 2) but in the statistical analysis there was no significant difference where P> 0.05. GHR gene expression levels are higher in muscle tissue than in liver tissue can be caused by lack of food intake. The maintenance of these local island piglets in North Sulawesi does not use feed that is controlled by its nutritional content, even these local pigs tend to be left to look for their own food or from leftover food from the population. When pigs are under stress that food intake is low, the GHR in the liver will be low and GHR in the muscles will increase activity.

The secretion of growth hormone (GH) is physiologically regulated by the hypothalamus anterior to the pituitary gland. GH plays an important role in growth and development [22]. Growth hormone and its protein product, the GH growth hormone, play an important role in reproduction, embryogenesis and other growths. Growth hormone levels are influenced by many factors, including genetic factors, nutrition, temperature, fasting, sleep patterns and age [23].

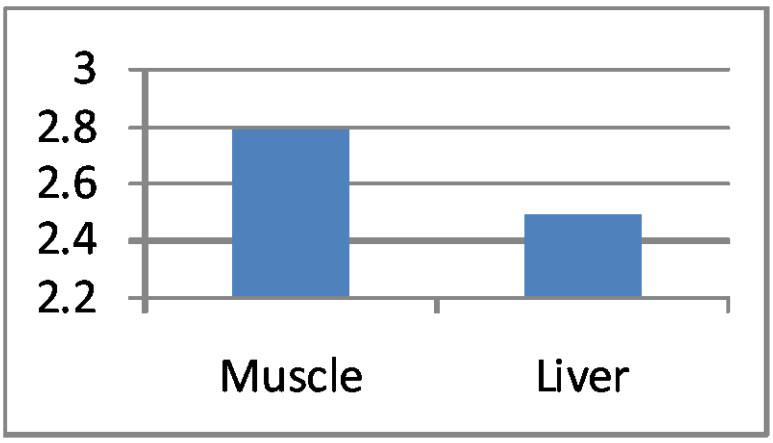

Figure 2. Level of mRNA GHR expression in the liver and muscle of the pigs from the islands in north Sulawesi

The main target of growth hormone (GH) is the liver. The liver will produce IGF-1 (Insulin-like Growth Factor-1) by activating tyrosine kinase which has the potential to regulate metabolism by accelerating the transport of amino acids through cell membranes in the cytoplasm [26]. Increasing the concentration of amino acids in cells will increase the speed of protein synthesis and have an impact on increasing the number of cells so that it accelerates the rate of tissue growth in various parts of the body.

Malnutrition induces tissue-specific changes in GHR mRNA, ie the hepatic level decreases while in the muscle increases [24,25]. Specific regulation of GHR mRNA in response to nutritional status is consistent with the different physiological roles of GHR in these two tissues. In the liver, GHR has a major somatogenic role through the induction of IGF-I gene expression, whereas in muscles it has an important role in metabolism and increases oxidative capacity

4. Conclusion

GHR was expressed in the liver and muscles of local pig from the islands in North Sulawesi. GHR mRNA expression in muscles was higher than in liver (P>0.05). GHR gene expression was also regulated by factors such as nutritional intake, GH, ages, temperature and hormones.

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