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Myostatin mRNA Expression and its Association with Carcass and Body Weight of Local Pigs from the Islands in North Sulawesi, Indonesia

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Abstract: *Myostatin gene is known as a member of the growth gene's superfamily (TGF-β) which works to suppress the muscle growth. This study was designed to investigate the Myostatin mRNA expression and its association with body weight and carcass of local pigs from the islands in North Sulawesi.The parameters measured were Myostatin mRNA expression by reverse tranriptase RT-PCR, body weight, and carcass weight of local pigs from the islands in North Sulawesi. mRNA sample is taken from sceletal muscle of sacrifice pigs. The Myostatin primer gene used is F = 5 'CCA CTC CGG GAA CTG ATT GA 3' and R = 5 'TCT CA 3 AGG AGT CTT GAC GGG' with its housekeeping gene GAPDH.The results showed that myostatin mRNA expression was correlated (P <0.05) with body weight and carcass weight of local pigs from the islands in North Sulawesi. Myostatin regulates the carcass and growth performance. Myostatin mRNA expression was correlated with carcass and body weight of local pigs from the islands in North Sulawesi. The expression of the myostatin gene can be used as a cheap selection model and can be done in a shorter time, especially to select quality livestock breeds.*

Index Terms*: Body Weight, Carcass, Local Pigs, Mrna Expression, Myostatin.Gene Livestock Breeds.*

I. INTRODUCTION

The growth and development of an animal is influenced by many factors such as genetic factors, nutrition, hormonal regulation, efficiency of the body's metabolism, immune response, livestock physiology status, the environment in which the animals are located or maintained, and the presence or absence of diseases or parasites(Cronje et al., 2000).Many genes play a role in controlling the growth and development of the body of animals, character-carrying genes that can provide economic value such as growth hormone (GH), insulin-like growth factor-1 (IGF-1), Pit-1, growth hormone receptor (GHR), myostatin (MSTN) and others.

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One gene that is important for regulating muscle mass growth is the myostatin gene (Ganet al., 2008).Myostatin is encoded by the myostatin gene. The myostatin gene has been widely used as a marker of double muscling phenomena in livestock. Mutations in the myostatin gene can inactivate the expression and produce non-functional proteins that affect muscle growth (Zhang et al., 2013).

Expression of genes or traits that appeared (phenotype) on growth and development is influenced by genetic factors, environmental factors and genetic interaction with the environment.Gene expression is the process of how information in DNA can be copied through the transcription process into RNA and translated into protein. Gene expression can be measured using real time qRT-PCR (quantitative Reverse Transcription - Polymerase Chain Reaction).qRT-PCR is an invitro technique of multiplication (amplification) of DNA pieces in a specific area that is limited by two oligonucleotide primer. The primer used as a boundary to the area being propagated is single-stranded DNA, whose sequence is complementary to the template DNA. RT-PCR is part of the normal PCR process. The difference with the usual PCR, in this process an additional cycle that is the change of RNA into cDNA (complementary DNA) using the Reverse Transcriptase enzyme. Reverse Transcriptase is an enzyme that can synthesize DNA molecules in vitro using RNA templates. In this study, mRNA taken from pig skeletal muscle tissue.

Myostatin is a member of the superfamily transforming growth factor (TGF) -β and plays an important role in regulating muscle growth and meat quality (Zhang et al., 2012). The absence of myostatin in the cells causes enlargement of muscle tissue that exceeds normal hypertrophy and hyperplasia, a condition found in cases of "Double Muscling" Belgian Blue cattle (Oldham et al., 2001). The myostatin gene is composed of one promoter, three exons and two introns in all species including pigs (AY208121).

The livestock raising by the people is intended so that the pigs sold have the right price and the pigs have good meat quality. Indonesia has local pigs, including Bali pigs, Batak pigs, Toraja pigs, and local Minahasa pigs (North

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Sulawesi, Indonesia) (Mege and Mokosuli, 2017). Many local pigs are kept on people's farms, while larger farms raise varieties of pig which are now officially known as superior pigs. Local pigs are kept by small farmers with traditional livestock raising systems as part-time businesses that are carried out by the family.It was realized that local livestock also played an economic role, because many were used as meat producers for food security needs, although maintenance was on a small scale with ownership of 2–6 pigs. Traditional management by feeding from family food leftovers and maintaining animal health is a factor that causes a decrease in productivity as well as a high mortality rate.

Local pigs are livestock that have experienced old domestication and have a high adaptation to the local environment. In addition to easy maintenance, the local pigs have more meat savory taste than the taste of pork descent Landrace, Duroc, and others. Local pigs are developed for the purpose of gaining profits from the sale of seeds, saplings, and meat and then preserving family traditions and participating in national food procurement and fulfillment of good nutrition to produce healthy, strong, and intelligent generations (Sihombing, 2006).

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 ancestry at the same age, this local pig from the Islands in North Sulawesi has a smaller body size.

The Myostatin gene is usually used as a genetic marker in livestock selection programs. The qRT-PCR technique is considered the most accurate and reliable for the validation of data expressions obtained. Research on mRNA expression of the Myostatin gene and its association with body weight and carcass weight of local pigs in islands in the province of North Sulawesi needs to be done to improve the quality of local pigs in terms of local pig breeding.

II. LITERATUREREVIEW

A. Local Pigs from The Islands in North Sulawesi, Indonesia

North Sulawesi is one of the provinces in the Unitary State of the Republic of Indonesia which is located on the northern tip of the island of Sulawesi, with the capital located in the city of Manado or precisely 0°N - 3°N and 123°East - 126°East and is one of the regions north of the equator. North Sulawesi has many island inhabitants whose main livelihoods are farmers, ranchers and fishermen. Pigs are one of the most popular livestock chosen by traditional farmers (Mege*et al*., 2016). Data from the North Sulawesi Central Bureau of Statistics shows that the pig population in North Sulawesi in 2017 was 414,653 (BPS, 2017). Among the pig population, village pigs or local pigs are one of the choices chosen by farmers from the islands in North Sulawesi, because they are easily maintained (Mege*et al*., 2015).

Local pigs are domesticated livestock that have experienced in a long time and has a high adaptability to the local environment. In addition to easy maintenance, local pigs have a more savory meat taste compared to Landrace, Duroc, and others (Soewandi*et al*., 2013). Local piglets are developed with the aim of gaining profits from the sale of seeds, saplings, and meat and then preserving family traditions and participating in national food procurement.

Pigs are one of the livestock that have relatively fast growth and development, have a prolific nature, which in one sow can reach 6-12 per birth and in a year can give birth twice. Pigs are animals that are adaptable to the environment, feed, and resistant to disease. This is evidenced by the pig's livestock still being able to live and reproduce well even in extreme environmental conditions such as environmental conditions with high heat temperatures and relatively cold temperatures. Pigs can directly adjust the condition of the body to the conditions of the surrounding environment.The people of North Sulawesi in general are very fond of raising pigs and are carried out in the lowest descent. Pigsare very important to the people of North Sulawesi because in addition to being used as a daily consumption, the profits from the sale of this pigs can be used to meet the needs of the community. In fact, in terms of productivity and the ability to fulfill food needs, it is undeniable that landrace and duroc race pigs are still higher than local pigs. This choice is based more on how to maintain local pigs that are relatively simpler and cheaper compared to superior types of pigs (Rayer*et al*., 2015).

Research conducted by Mege and Mokosuli (2017) on local Minahasa pigs, one of the districts in North Sulawesi Province shows that local Minahasa pigs are uncertain about the position of species in gene banks, because they do not have a sequence similar to other pig species on gene banks. However, the local North Minahasa pigs show similarities in the closest CO1 sequence to the Pigs DNA sequence from the WTSI_1061-78D9 clone. Similarly, the phylogeny construction shows the closest kinship based on the CO1 gene with the sequence of pig DNA from the WTSI_1061-78D9 clone.

B. Body Weight and Carcass Weight of Pig Livestock

In slaughtering a livestock carcasses and offals (non-carcasses) are produced both edible, and non-edible. According to Jayathilakan*et al*. (2012), edible offal components are the tongue, heart, liver, lungs, brain, digestive tract, and spleen, while horns, nails, bones of the forehead or head bone are included as parts that cannot be eaten (non edible offal). The sum between carcass and non carcass weights is animal body weight. Carcass is a major part of meat-producing livestock. The criteria for carcass value are the basis of the carcass quality needed by consumers, including a high carcass percentage and carcass length.Aberle*et al*. (2001) stated that the main factors that influence the percentage

of carcass are head weight, blood, total internal organs and contents of the digestive tract. The percentage of carcass according to Kariasa and Ilham (2000) is a comparison between carcass weight and live weight multiplied by 100%. So, the percentage of carcass depends on the body weight of the animal. Pigs that are born with uneven or not uniform body weight besides affecting survival, will affect growth performance (Yuan *et al.*, 2015). Thus, birth weight is an important factor that affects the growth of children to adulthood.

C. Myostatin Gene

Genes are characteristic hereditary factors in living things that are passed down from one generation to the next. The expression of various genes is depicted in the outward appearance of the creature (phenotype). The myostatin gene is a gene that regulates the growth of muscle mass in animals such as in pigs. In general, muscle growth is divided into three ways, namely muscle fibers increase in the number, length, size and number of myostatin loops. The mechanism that regulates the multiplication and size of muscle cells is regulated by the myostatin coding gene. The myostatin gene or Growth Differentiations Factor 8 (GDF8) is a member of the superfamily Transforming Growth Factor-β (TGF-β) which secures proteins to control growth and differentiation of body tissues (McNally, 2004; McPherron*et al.*, 1997).

The Myostatin gene plays an important role as a "feed back negative" on muscle mass growth (Ye *etal.*, 2007), where myostatin inhibits myogenin so that the myoblast cannot differentiate into myotubes, which will develop into muscle fibers. In this casemyostatin is synthesized and secreted as an inactive polypeptide. The young Myostatin divides and becomes an adult. Myostatin binds to folistatin and then binds to the receptor, activin receptor IIB in the muscle. These receptors work by giving an intercellular signal to the pathways and protein activity of the regulator genes, thus playing a role in regulating muscle mass (McNally, 2004).

In the process of inhibition or absence of myostatin in the cell causing hypertrophy and hyperplasia, namely enlargement of tissue or muscle parts that exceed normal or better known as "Double Muscling", for example can be seen in Belgian Blue cattle (Cullam *etal.*, 2001). The Myostatin gene (MSTN) is located at the distal end of chromosome 2 and consists of three exons and two introns. According to Grobet*etal.* (1997), and McPherron*et al.* (1997), the same thing also occurs in several other species with DNA sequences found in gene banks with access numbers on pigs (AY208121), buffalo (AH013313), chickens (AF346599), and house mice (AY204900). Mutations that occur in genes that encode the protein myostatin have been widely studied and have an effect on muscle mass increase in rats, dogs, cattle and humans (Grobet*et al.*, 1997; McPherron*et al.*, 1997; Schuelke*et al.*, 2004).

Myostatin expression in skeletal muscles (Naji*et al.* 2014), muscle cardiac (Ma *et al.*, 2014). Ye *et al.* (2007) reported the presence of a non-synonym base thymine to Guanine mutation in exon 2 MSTN gene which caused a change in leucine amino acid to arginine associated with body weight of broiler chickens. Zhang *et al.* (2011) found that mutations in exon 1 in bian chickens could be used as genetic markers for the growth properties of bian chickens. Natural mutations of the MSTN gene in cattle are related to the "double muscling" phenotype that occurs in Belgian Blue cattle (Dunner*et al.*, 2003). So that the Myostatin gene is used as one of the molecular markers in livestock selection.

III. METHODOLOGY/MATERIALS

A. Place and Time of Research

This research was carried out in traditional local pig farms spread across small islands in North Sulawesi Province - Indonesia including Mantehage, Bunaken, Bangka, Gangga, Nain, Siau, Lembe, and Talaud islands. Analysis of gene expression with real time qRT-PCR (quantitative Reverse Transcriptase - Polymerase Chain Reaction), carried out at the Laboratory of Animal Molecular Genetics, Animal Breeding and Genetics Section, Faculty of Animal Science, Bogor Agricultural University (IPB). This research was conducted in March 2018 until November 2018.

B. Research Livestock Samples

The livestock used are local pigs from the islands in North Sulawesi without special treatment. Euthanasia was carried out on nine pigs from the islands in North Sulawesi which were traditionally maintained on the people'syard with an average body weight of 24,875 ± 7,954 kg with ages ranging from 2-3 months. The study was conducted with three replications. This local pig is fed in the morning and evening, drinking water is available at all times. During the daytime the local pigs are left free to get additional food from the population's leftovers, and are grounded at night.

C. Research Procedure

Data on body weight and carcass

$$\text{Carcass weight percentage (\%)} = \frac{\text{(absolute carcass weight)}}{\text{(body weight)}} \times 100$$

D. Primer Gen

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 a homologous 99% with the target of the myostatin gene, namely the GAPDH gene.

Table 1 Specific primers for the Myostatin and GAPDH genes

Target genes	Primer sequence	Size (bp)
Myostatin (NM_214435.2)	F: 5'- CCA CTC CGG GAA CTG ATT GA - 3' R: 5'- AGG AGT CTT GAC GGG TCT CA - 3'	243
GAPDH (NM_001206359.1)	F: 5'- GAG TGA ACG GAT TTG GCC G -3'	246

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R: 5'- CAC CCC ATT
TGA TGT TGG CG -3'

E. Myostatin mRNA gene expression

The myostatin mRNA gene expression data was obtainedfrom the muscle tissue of slaughtered (*euthanasia*) local piglets. Data obtained from the myostatin gene mRNA expression compared between each region and weighting of livestock bodies. The number or quantification of the Myostatin gene expression was calculated based on the number approach relative to the target gene (Myostatin) and housekeeping genes (GAPDH), with a ratio of cycle threshold (C_T). Visualization of data from RT-PCR analysis is in the form of a graph and the quantification value is the number of DNA copy numbers after being accurate with a threshold value. C_T (cycle threshold) is the value of the intersection between the level of fluorescent sample and the average threshold value.

F. mRNAextraction

mRNA is extracted from skeletal muscle tissue. The tissue was taken aseptically 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 -4^{0}C until the time of testing. mRNA was extracted using the Rneasy Fibrous Mini Kit method (Qiagen, Germantown, EU). mRNA samples are ready for use or stored at -20^{0}C.

G. RNA Quality Test for Extraction Results

The quality of mRNA extraction was tested qualitatively to determine the purity level using a spectrophotometer. The quality of mRNA is classified as good if the results obtained are 260/230> 1.80 ng/µl (Sambrook*et al.*, 1989).

H. Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

Reverse transcription is an event in which mRNA is transcribed back into cDNA using the qPCR ReverTra RT kit Master Mix with gDNA Remover (Toyobo Bio-Technology, Japan). The template RNA used was sample template RNA and control 2 µl each. The results obtained are templates in the form of sample cDNA and standard cDNA.

The next stage is a spectrophotometer using blanks and cDNA samples. The quality of cDNA is classified as good if the results obtained at this stage are 260/230> 1.80 ng / µl.

The next stage is making standards and optimizing and operating qRT-PCR (Analytic Jena, AG qTower 4 channels, Germany). Optimization was carried out using a conventional PCR machine with agarose gel electrophoresis 1.5% and also using real time PCR. Optimization aims to get a good standard for RT-PCR results in the sample. Optimization is said to be good if the value of R2> 0.90. The sample is distributed into RT-PCR tube then centrifuged horizontally 25000 rpm for 10 seconds. The material consisted of 50 ng / µl DNA templates, 3 µl nuclease free water, 5 µl mas[19]mix (Toyobo Cybr Green Master Mix, Toyobo, Japan), 0.5 µl forward primer and 0.5 µl reverse primer). Then the RT-PCR machine is operated with the following conditions, 95 ^{0}C for 1 minute, 95^{0}C for 15 seconds, followed by 58 ^{0}C for 1 minute. The PCR process lasts for 40 cycles.

I. Data Analysis

The data obtained were analyzed statistically using variance analysis (ANOVA), which was a completely randomized design. If it is significantly different (P <0.05) then Duncan's further test is conducted (Steel and Torrie, 1993). Correlation coefficients were analyzed using bivariate correlations analysis. Analysis of variance and correlation analysis between gene expression and body weight and carcass were analyzed using theSPSS program.

IV. RESULTS AND FINDINGS

A. Body Weight, Carcass and Level of Expression of Myostatin gene

The visualization of the analysis results data qRT-PCR is presented in the form of a graph and the quantification value in the form of the number of DNA copy numbers after being accredited with the threshold value. C_T (cycle threshold) is the value of the intersection between the level of fluorescent sample and the average threshold value. The C_T mRNA value from qRT-PCR in this study showed that the myostatin gene was expressed in the skeletal muscle skeleton of the local islands of [35]rth Sulawesi, the diverse values showed differences in the level of gene expression. The results showed that the mean Delta Cycle Treshold (ΔC_T) of the Myostatin gene was 1.73 ± 1.45.

Body weight, carcass weight, percentage of pig carcass in this study show differences in values at each sampling location (Table 2). According to Kühn and Männer (2015) and NRC (1998), growth of piglets consists of several [34]ses, namely the frestarter phase (25 to 45 kg), grower (46 to 70 kg), and finisher (71 to 120 kg). According to the growth stage of pigs by Kühn and Männer (2015) and NRC (1998), this study uses frestarter phase piglets with ages ranging from 3 months. In this phase, pigs grow optimally.However, when compared with landrace and duroc pigs, the body weight of local piglets in North Sulawesi can be classified as having a low body weight at this age. The carcass weight shown in this study is directly proportional to the body weight of local island pigs in North Sulawesi, with a carcass weight percentage of 55.07 ± 7.87%. The percentage of carcass weight is lower than the percentage of carcass in pig livestock is 67-77% (Manampiring*et al.*, 2017; Lapian*et al.*, 2013; Forrest *et al.*, 1975; Aritonang, 2011; Silalahi and Sinaga, 2010) According to the USDA, the percentage of class I pig carcasses is at 68-72%.

Table 2: Delta Cycle Treshold (ΔC_T) Myostatin gene, body weight, carcass weight and carcass percentage

Para	Lokasi

meter	N	L	G	K	B	T	S	M	Rerata
ΔC_T	0.77	1.93	1.50	2.83	0.96	4.71	0.07	1.14	1.73 ± 1.45
BB (Kg)	23	24	23	22	25	15	43	24	24.88 ± 7.95
BK (Kg)	14	13	11	10	15	10	25	11	13.70 ± 5.11
PK (%)	60.87	54.17	47.83	45.45	60	66.67	59.3	46.25	55.07 ± 7.87

ΔC_T : Delta Cycle Threshold, BB: Body Weight, BK: Carcass Weight, PK: Percentage of Carcass, N: Nain Island, L: Lembe Island, G: Gangga Island, K: Kalinaun Village, B: Bunaken Island, T: Talaud Island, S: Siau Island, M: Mantehage Island.

B. Correlation Between Body Weight, Carcass Weight and Myostatin Gene mRNA Expression Level

The results of the study on the correlation between Myostatin gene expression and body weight and carcass weight are presented in table 3. The results of this study indicate that the expression of the Myostatin gene correlates with body weight and local carcass weight from islands in North Sulawesi (P> 0.05). The expression of the Myostatin gene was negatively correlated with body weight and carcass weight of local island pigs in North Sulawesi with a value of r = -0.759 on body weight (P> 0.05), and r = -0.898 on carcass weight (P> 0.01). This study shows the trend of increasing myostatin mRNA levels are inversely proportional to body weight and carcass weight.

Table 3: Correlation between body weight and carcass with Myostatin gene mRNA expression level

Parameter	mRNA Myostatin Level
Body Weight	r = -0.759 (P = 0.029) *
Carcass Weight	r = -0.898 (P = 0.002) **

(*) is significantly different <0.05, (**) very significantly different P <0.01

Fast-growing characters in living things are controlled by many factors and are multigenic. One of the growth control factors is myostatin or Growth Differentiations Factor 8 (GDF8) which is a member of the superfamily Transforming Growth Factor-β (TGF-β) which controls the growth and differentiation of body muscle tissue.The absence of myostatin in cells causes enlargement of muscle tissue that exceeds normal hypertrophy and hyperplasia, a condition found in the case of "Double Muscling" Belgian Blue cattle (Oldham *et al*., 2001).

Indonesia's diverse geographical conditions cause the myostatin gene types in local pigs that are kept in Indonesia to vary. The myostatin gene works as a "feed back negative" in the growth of muscle mass, where myostatin inhibits myogenin so that the myoblast cannot differentiate into myotubes, which will develop into muscle fibers (McNally, 2004). In the process of inhibition or absence of myostatin in the cell causing hypertrophy and hyperplasia, namely enlargement of tissue or muscle parts that exceed normal or better known as "Double Muscling", for example can be seen in Belgian Blue cattle (Oldham *et al*., 2001). This phenomenon is found in several other species such as pigs, cattle and mice.

The myostatin gene has been identified in various livestock and plays an important role in regulating muscle growth and meat quality. Until now, six mutations have been identified at MSTN which can provide increased muscle hypertrophy because they are able to deactivate the function of these genes. Previous research from Sadkowski et al (2008) using real time PCR and cDNA microarrays found that the expression of the myostatin gene was lower in cattle that were CC-like than those of the GG and CG genes. This study should be carried out further to determine the genotype of local island pigs in North Sulawesi so that they can be examined in relation to muscle weight and carcass weight. Genotyping or the search for superior traits through the inventory of the myostatin gene in livestock such as in local pigs in Indonesia is an important step to improve the quality of growth and development of subsequent breeds.

The expression of the myostatin gene can be used as a cheap selection model and can be done in a shorter time, especially to select quality livestock breeds. The inherited nature of hypertrophy can be utilized to increase the phenotype of more productive and efficient meat-producing livestock. By studying the level of expression of the myostatin gene in skeletal muscle from local island pigs in North Sulawesi, it is expected to increase the productivity of local pigs in a superior livestock selection program.

V. CONCLUSION

Myostatin mRNA expression was correlated with carcass and body weight of local pigs from the islands in North Sulawesi. The expression of the myostatin gene can be used as a cheap selection model and can be done in a shorter time, especially to select quality livestock breeds.

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