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Polymorphism of the T4842G *Myostatin* Gene is Associated with Carcass Characteristics in Indonesian Chickens

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Abstract: *Myostatin*, or growth and differentiation factor-8 (GDF-8), is a member of the Transforming Growth Factor (TGF)-β superfamily. This family functions as a negative regulator of skeletal muscle. Mutations in exon 2 have been reported to convert Thymine into Guanine (T4842G) that alters the amino acid leucine into arginine, which is associated with body weight in chickens. The objectives of this study were to identify the polymorphism of T4842G mutation in the *myostatin* gene in Indonesian chickens and evaluate their effects on carcass characteristics. The gene polymorphism was identified with the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using the *Bsr*I restriction enzyme. The effect of genotype on carcass and meat quality was analyzed using the SAS General Linear Model (GLM) procedure. Genotyping was performed on 332 chickens from 7 Indonesian chicken populations (*Kampung*, *Merawang*, *Sentul*, *Cobb* broiler, F1 crossbreed of *Kampung* x layer, F1 crossbreed of *Kampung* x *Cobb* broiler and F2 crossbreed of *Kampung* x *Cobb* broiler). The product of amplification was 247 bp. The *myostatin*|*Bsr*I locus was polymorphic in all populations, producing two alleles (G and T) and three genotypes (GG, GT, TT). Results from the analysis of the allele and genotype frequency showed that the T allele had a higher frequency than the G allele in all populations, except for the F1 crossbreed of the *Kampung* x *Cobb* broiler chicken population, which had equal allele frequencies. A significant effect was found between genotype and carcass characteristics in the F2 crossbreed *Kampung* x *Cobb* broiler chickens. A SNP in the coding region of *myostatin* in exon 2 was associated with live weight, carcass weight, breast weight, thighs weight, drum sticks weight, wings weight, breast muscle weight, thighs muscle weight, drum sticks muscle weight and free water. Here, the association of *myostatin*|*Bsr*I gene polymorphism with chicken carcass characteristics in Indonesian chickens has been demonstrated, providing evidence that *myostatin* might be an important candidate gene for chicken carcass characteristics.

Key words: *Myostatin* gene, indonesian chickens, polymorphism, carcass characteristics

INTRODUCTION

Myostatin is a member of Transforming Growth Factor-beta superfamily (TGF-β), which works as a negative regulator of skeletal muscle growth and plays an important role in skeletal muscle homeostasis (Burks and Cohn, 2011; Ye *et al*., 2007). *Myostatin* is a factor that contributes to protein balance in muscle fibers (Baldwin *et al*., 2013). *Myostatin*, also known as the growth and development factor-8 (GDF-8), is predominantly expressed in the skeletal muscle of young and adult animals, both in the prenatal and postnatal phases (Dayton and White, 2008; Kambadur *et al*., 2004; Sundaresan *et al*., 2008). *Myostatin* has been reported as a negative regulator of satellite cell activation and postnatal cell regeneration (McCroskery *et al*., 2003). Kambadur *et al*. (2004) reported that *myostatin* supports the status of satellite cells to remain inactive but also plays a role in regulating activation of satellite cells in postnatal growth. *Myostatin*-null mice have a significantly greater muscle mass compared to normal mice due to an increase in the number of muscle fibers (hyperplasia) and in muscle fiber size (hypertrophy; Dayton and White 2008; Lee and McPherron, 2001). Moreover, it has been shown that *myostatin* is a single gene that regulates muscle hypertrophy in cattle (Kambadur *et al*., 2004). Deletions of 11 base pairs in the nucleotide position 821 in the coding

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region of the *myostatin* gene of the Belgian Blue causes double muscling characterized by abnormal muscle growth that is inherited through an autosomal recessive gene (Dayton and White, 2008; Kambadur *et al.*, 2004).

Exploration of *myostatin* in chickens was initiated by Baron *et al.* (2002) who explored the structure of the chicken *myostatin* gene. The chicken *myostatin* gene is conserved with the *myostatin* gene of other vertebrates. In the chicken, *myostatin* gene is located on chromosome 7 and consists of three exons and two introns, for a total length of 6693 bp and produces 375-376 amino acids (Baron *et al.*, 2002; Bhattacharya *et al.*, 2015). The molecular weight of the proprotein is 42.68-42.75 kDa while mature protein is 13 kDa (Bhattacharya *et al.*, 2015). Until 2012, it was believed that 20 SNPs in chicken *myostatin* gene were found between 5'UTR to 3'UTR (Genxi *et al.*, 2014; Scheuermann *et al.*, 2004; Ye *et al.*, 2007; Zhiliang *et al.*, 2004). Ye *et al.* (2007) suggested that five SNPs have pleiotropic effects with growth traits showed a genotype by environmental interaction; one of these SNPs involved a non-synonymous mutation, T4842G, which is located in exon 2.

A study of genetic diversity in chickens discovered *myostatin* in several breeds, including the broiler chicken (Ye *et al.*, 2007), White Leghorn chicken (Mott and Ivarie, 2002), China silky chicken (Zhiliang *et al.*, 2004), Bian, Jinghai, Arbor Acre and Youxi chickens in China (Genxi *et al.*, 2012), local Western Azerbaijan chickens (Zandi *et al.*, 2012) and India native chickens (Bhattacharya and Chatterjee, 2013; Kumar *et al.*, 2007). However, there are no studies which have examined the characteristics of the *myostatin* gene in Indonesian chickens. The availability of genetic information of the *myostatin* gene in chicken can be used to recommend Single Nucleotide Polymorphisms (SNPs) as candidates for Marker Assisted Selection (MAS) to select chickens with desired carcass characteristics. The objectives of this study were to identify the T4842G polymorphism in the exon 2 *myostatin* gene in Indonesian chickens using PCR-RFLP and evaluate their effects on carcass characteristics.

MATERIALS AND METHODS

Animal and data collection: Blood samples were used as a source of total DNA and were obtained from 332 chickens from 7 different populations: *Kampung* (97), *Merawang* (17), *Sentul* (36), *Cobb* broiler (39), F1 crossbreed of *Kampung* x layer (18), F1 crossbreed of *Kampung* x *Cobb* broiler (43) and F2 crossbreed of *Kampung* x *Cobb* broiler (92) chickens. All animals bred in the Animal Breeding and Genetics Division, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB), Indonesia. All animals used in the current study were maintained according to the principle of animal welfare. Chickens were maintained and fed under the same conditions to minimize the effect of environment. Carcass characteristics, both quantitative and physicochemical characteristics, of an F2 crossbreed of *Kampung* x *Cobb* broiler (26 weeks; 36 chickens) were measured. Quantitative characteristics of the carcass included live weight, carcass weight, breast weight, thigh weight, drum sticks weight, wing weight, breast muscle weight, thigh muscle weight, drum sticks muscle weight and percentage of each carcass part and muscle. Physicochemical characteristics of the carcass included pH, free water (mg H_2O and % mg H_20) by using filter paper press method, meat tenderness (Warner Bratzler Shear Force) and the cooking loss of breast muscle. All meat samples were frozen until evaluation procedures were held.

DNA extraction: The DNA extraction protocol was modified from Sambrook and Russel (2001) with the following procedures: (1) Sample preparation. Each 20 µL of chicken blood was added to 1000 µL of 0.2% NaCl and then homogenized and centrifuged (8000 rpm) for 5 minutes and the supernathan part was removed.

(2) Protein degradation. The precipitate was added to 40 µL of 10% SDS, 10 µL proteinase-K (5 mg/mL) and 1 x STE up to 400 µL. Then, the mixture was slowly shaken in an incubator at 55°C for 2 h.

(3) Organic material degradation. Phenol solution (400 µL), CIAA (400 µL) and NaCl 5M (40 µL) were added into the mixture and slowly shaken in room temperature for 1 h. 4) DNA precipitation. DNA molecules were separated from phenol using a centrifuge (12000 rpm) for 5 min. DNA phases was moved and added to 800 µL EtOH absolute and 40 µL NaCl 5M. Then, samples were frozen overnight. DNA molecules were centrifuged (12000 rpm) for 5 min and supernathan part was removed. DNA precipitate was air dried and dissolved into 100 µL 80% TE.

Amplification and genotyping of *myostatin* gene: Primers used to amplify the T4842G *myostatin* fragment target in exon 2 were designed using Primer Designing Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) according to GenBank (GenBank access number: AF346599.2). The T4842G SNP was based on Ye *et al.* (2007). Primers used were forward primer 5'-AAC GGT GTT TGT GCA GAT CC-3' and reverse primer 5'-CAA TCC ATC TTC ACC CGG TCC-3'. These primers generated 247 bp DNA fragments located in exon 2 (Fig. 1).

PCR was performed with a total volume 25 mL for each reaction containing DNA samples (50 ng/µl), primer (0.5 pmol), GoTaq Green Master Mix (Promega, 0.5 unit) and water. This mixture was incubated in a thermocycler machine (GeneAmp® PCR System 9700, Applied Bio Systems^{TM}). The amplification process was run with 30 cycles consisting of denaturation at 95°C for 10 sec, annealing at 60°C for 20 sec and elongation at 72°C for 30 sec.

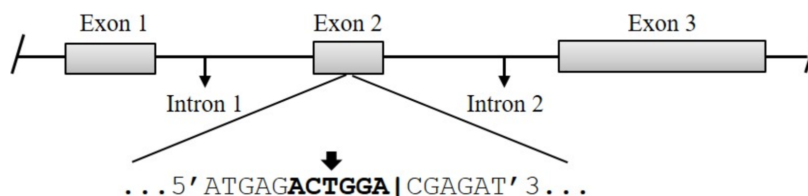

Fig. 1: Fragment target *myostatin|BsrI* locus. Arrow shows T4842G SNP position; bold shows *BsrI* restriction site (ACTGGN|, GenBank accession number: AF346599.2)

Fig. 2: Forward and reverse primer annealing positions (underline), bold shows BrsI restriction sites, and box shows the T4842G SNP position (GeneBank accession number: AF346599.2)

Genotyping was performed using a Restriction Fragment Length Polymorphism (RFLP); the restriction enzyme used was *BsrI* (Thermo Fisher Scientific, EU, Lithuania). PCR product was incubated at 65°C for 12 h. Primers annealing position and *BsrI* restriction site are shown in Fig. 2. Allele and genotype identification were conducted through electrophoresis analysis on 2.5% agarose gel (v/w), which was stained with FluoroSafe DNA Stain (1st Base, Singapore) above UV Transilluminator (Alpha Imager, Gel Documentation, Alpha Innotech). Sequencing was performed on an ABI-PRISM3730 sequencer to identify the mutation site.

Data analysis: Genotype frequency, allele frequency, heterozigosity and the Hardy-Weinberg Equilibrium were used to analyze polymorphism information according to Nei and Kumar (2000). The association of myostation|*BsrI* genotype with carcass characteristics was analyzed using SAS General Linear Model (GLM) procedure (SAS, 2008). Duncan's multiple range test was used to identify significant differences between means. The genetic effects were analyzed using the following model:

$$y_{ijk} = \mu + S_i + G_j + \varepsilon_{ijk}$$

where:

y_{ijk} = observed phenotypic value (carcass characteristics) for k_{th} individual with i_{th} sex and j_{th} genotype

μ = overall mean

S_i = genetic effect of i_{th} sex

G_j = genetic effect of j_{th} genotype

ε_{ijk} = residual effect

RESULTS AND DISCUSSION

Polymorphism of the T4842G *myostatin* gene: A total of 332 samples were successfully amplified and resulted in a 247 bp fragment of the *myostatin* gene partial exon 2 through PCR (Fig. 3). This fragment contained three *BsrI* restriction sites (4707, 4746 and 4845 bp) and the SNP target was in the third restriction site (position T4842G). Genotyping using *BsrI* restriction enzyme successfully identified two alleles. The T allele was indicated by 4 bands (99, 64, 45 and 39 bp) and the G allele was indicated by 3 bands (144, 64 and 39 bands). The two alleles derived three genotypes, TT, GG and GT (Fig. 4 and 5). This SNP was reported to replace Thymine into Guanine nucleotide and alter the amino acid Leucine into Arginine (Ye *et al*., 2007).

The PCR-RFLP analysis showed that primers and restriction enzymes could be used to identify a non-synonymous mutation (T4842G) in exon 2 of the *myostatin|BsrI* locus, as was reported previously by Ye *et al*. (2007). All chicken populations were polymorphic with two alleles found (T and G). Nei and Kumar (2000) and Allendorf *et al*. (2013) proposed that a population could be classified as polymorphic if there is more than one allele in a locus and if the frequency of the most common allele is less than 0.99. The frequency of allele T was found to be higher in the *Kampung*, *Merawang*, *Sentul*, *Cobb* broiler, F1 crossbreed of *Kampung* x layer, F2 crossbreed of *Kampung* x *Cobb* broiler and overall population. The frequency of the T allele was found to be highest in the broiler chicken population (0.879). In F1 crossbreed of *Kampung* x *Cobb*, the T allele had an equal frequency with the G allele (0.50), which is consistent with Ye *et al*. (2007) that reported a higher T allele frequency (0.54) in a

Table 1: Genotype and allele frequency of *myostatin|Bsr*I locus in Indonesian chickens

		Genotype frequency			Allele frequency	
Population	n	GG	GT	TT	G	T
Kampung	97	0.196 (19)	0.433 (42)	0.371 (36)	0.412	0.588
Merawang	17	0.176 (3)	0.176 (3)	0.647 (11)	0.265	0.735
Sentul	36	0.028 (1)	0.583 (21)	0.389 (14)	0.319	0.681
Cobb broiler	39	0.034 (1)	0.172 (5)	0.793 (23)	0.121	0.879
F1 Crossbreed of *Kampung* x Layer	18	0.167 (3)	0.556 (10)	0.278 (5)	0.444	0.556
F1 Crossbreed of *Kampung* x *Cobb* broiler	43	0.163 (7)	0.674 (29)	0.163 (7)	0.500	0.500
F2 Crossbreed of *Kampung* x *Cobb* broiler	92	0.207 (19)	0.543 (50)	0.250 (23)	0.478	0.522
Overall population	332				0.401	0.599

n: Number of samples

Table 2: Hardy-Weinberg equilibrium and heterozygosity of *myostatin|Bsr*I locus in Indonesian chickens

Population	n	χ^2	Ho	He
Kampung	97	1.102	0.433	0.485
Merawang	17	5.080*	0.176	0.389
Sentul	36	4.201*	0.583	0.435
Cobb Broiler	39	1.021	0.172	0.212
F1 Crossbreed of *Kampung* x Layer	18	0.281	0.556	0.494
F1 Crossbreed of *Kampung* x *Cobb* Broiler	43	5.233*	0.674	0.500
F2 Crossbreed of *Kampung* x *Cobb* Broiler	92	0.729	0.543	0.499
Overall population	332	0.004	0.482	0.480

n: Number of sample, *Significantly different ($\chi^{2}_{0.05}$ = 3.84)

commercial broiler chicken line. Allele and genotype frequencies of *myostatin|Bsr*I locus are described in Table 1.

Polymorphisms can be analyzed by using heterozigosity number (Table 2). High polymorphisms were found in the *Sentul*, F1 crossbreed of *Kampung* x layer, F1 crossbreed of *Kampung* x *Cobb* broiler and F2 crossbreed of *Kampung* x *Cobb* broiler chickens. Allendorf *et al.* (2013) proposed that a population can be classified as highly polymorphic if the population has a theterozogosity number greater than 0.5. The observed heterozigosity (Ho) numbers in the *Kampung*, *Merawang* and *Cobb* broiler chicken populations were lower than the expected heterozigosity (He) number. According to Allendorf *et al.* (2013), these results indicated that the occurrence of inbreeding is a result of intensive selection of *Myostatin* gene in *Kampung*, *Merawang* and *Cobb* broiler chicken populations. The Ho number in the *Sentul*, F1 crossbreed of *Kampung* x layer, F1 crossbreed of *Kampung* x *Cobb* broiler and F2 crossbreed of *Kampung* x *Cobb* broiler chicken populations were higher than He, demonstrating that there was no intensive in those populations. The χ^2 analysis showed that all chicken populations were in equilibrium with the Hardy-Weinberg's equation, with the exception of the *Merawang*, *Sentul* and F1 crossbreed of *Kampung* x *Cobb* populations. Allendorf *et al.* (2013) explained that χ^2 is in Hardy-Weinberg equilibrium if the allele and genotype frequencies remain constant from generation to generation. Heterozigosity and χ^2 of the *myostatin|Bsr*I locus are shown in Table 2.

Several studies have evaluated chicken *myostatin* polymorphisms. Genxi *et al.* (2014) analyzed 5'UTR polymorphisms and found four novel SNPs (G673A, G985C, G1085A and A1278T). Only the A1278T mutation

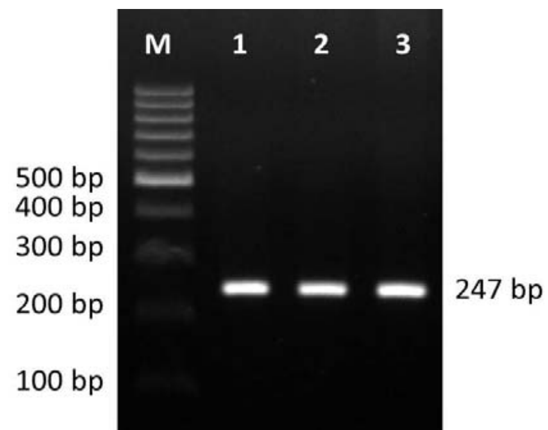

Fig. 3: Visualization of *myostatin* gene amplification in 1.5% agarose gel (M: marker; 1-3: samples)

has been reported to be polymorphic and showed significant association with growth traits in the Bian chicken (Genxi *et al.*, 2014). Paswan *et al.* (2014) reported a SNP at A241T in the 5'UTR that was significantly associated with higher body weight at hatching. In exon 1, Zhang *et al.* (2011) reported a c.234G>A mutation which is also significantly associated with body weight in Bian chicken from 6 to 18 weeks. Ye *et al.* (2007) reported one SNP in exon 1 (g.2244G>C), which is strongly associated with growth and mortality in elite commercial broiler chicken lines under low hygiene conditions. Zhiliang *et al.* (2004) found a SNP in 3'UTR (A7263T) that is associated with breast muscle weight and breast muscle percentage of the F2 chicken line derived from broilers crossing to silky chicken.

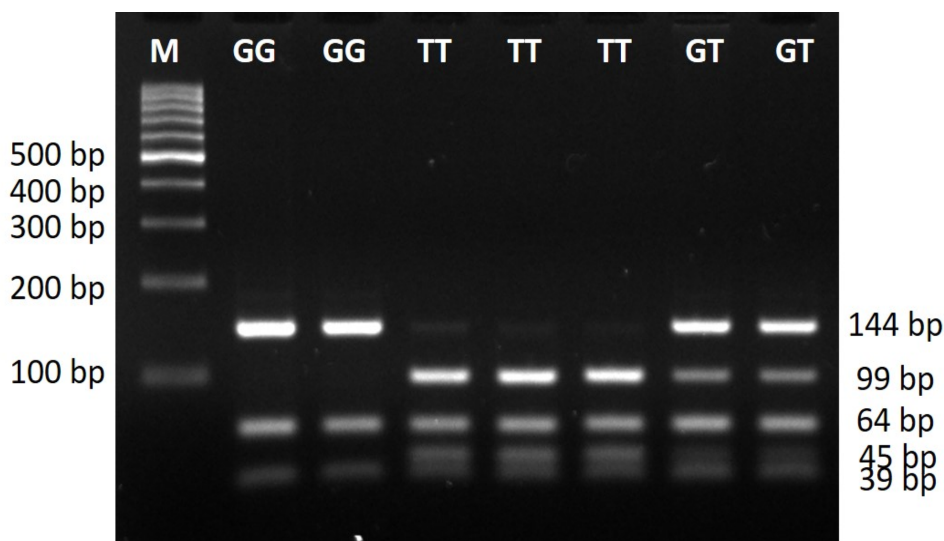

Fig. 4: Visualization of *myostatin|Bsr*I genotyping in 2.5% agarose gel (M: marker; GG, TT, GT: genotype)

Effects of gene polymorphism on carcass characteristics: Association analyses showed that the *myostatin|Bsr*I polymorphism had a significant effect on both quantitative (Table 3) and physicochemical (Table 4) carcass characteristics in the F2 crossbreed of *Kampung* x *Cobb* broiler chickens. Genotypes had a significant effect on live weight, carcass weight, breast weight, thighs weight, drum sticks weight, wings weight, breast muscle weight, thighs muscle weight and drum sticks muscle weight (Table 3). Animals with TT genotype had higher values than GG across all categories ($p<0.05$).

The effect of T allele on the live weight were in agreement with Ye *et al.* (2007). Ye *et al.* (2007) described that T allele had positive effect on growth in elite broiler commercial chicken lines. Nucleotide mutation occurred in the T4842G bp exon 2 of *myostatin* and this mutation replaced amino acid Leusine into Arginine. This non-synonymous mutation was predicted to have different effect on chicken growth related to carcass and meat quality in chicken populations used in this study, although the functional studies of this mutation has not been discovered. However, this is the first time our result provided first evidence on the effect of the relationship between the T allele and carcass weight and its percentages. It is interesting to be noted that carcass percentage, commercial cuts percentages and muscle percentages were not significantly affected by the T allele (Table 3). This suggested that despite T allele promotes the growth rate yet it yielded proportional body composition.

Myostatin mRNA and protein in chicken embryonic tissue are highly expressed in the muscle, gizzard, large intestine, testes and ovaries (Kubota *et al.*, 2007). In postnatal chicken, *myostatin* mRNA and protein are found in breast muscle (Bhattacharya *et al.*, 2015). Higher expression of *myostatin* in layer type chicken than meat type chicken is expected to contribute in lower muscle growth in layer (Bhattacharya *et al.*, 2015).

Myostatin interacts with TGF-β1 and decorin in the formation of skeletal muscle. Zhu *et al.* (2007) found that TGF-β1 stimulates *myostatin* expression and *myostatin* regulates TGF-β1 secretion in C2C12 myoblasts. Decorin upregulates follistatin regulation, which is a *myostatin* inhibitor (Zhu *et al.*, 2007). Kubota *et al.* (2007) explained that follistatin (Lee and McPherron, 2001), follistatin-related genes and the *myostatin* propeptide (Kim *et al.*, 2007) can inhibit the binding of *myostatin* to its receptor, activin receptor type IIB (ActRIIB). *Myostatin* binding to ActRIIB inhibits myoblast proliferation (Kubota *et al.*, 2007; Thomas *et al.*, 2000).

In the broiler chicken, injecting a monoclonal antibody against *myostatin* into the yolk resulted in higher body and muscle mass in both male and females (Kim *et al.*, 2006). This study suggested that immunological neutralization of *myostatin* during embryonic development can improve growth of broilers. A similar result was shown by Kim *et al.* (2007) in which injecting rabbit polyclonal anti-*myostatin* antibody into yolk reduced the muscle weight of broilers, indicating that *myostatin* activity is increased by the binding of the polyclonal antibody to the propeptide; this also shows that the *myostatin* propeptide most likely inhibits the function of *myostatin*. *Myostatin* has also been reported to inhibit activation, proliferation and differentiation of myogenic satellite cells (McCroskery *et al.*, 2003; McFarland *et al.*, 2007). McFarland *et al.* (2007) showed that *myostatin* inhibits satellite cell differentiation of the chicken pectoralis major muscle but has no effect on satellite cells in the biceps femoris. In the embryo,

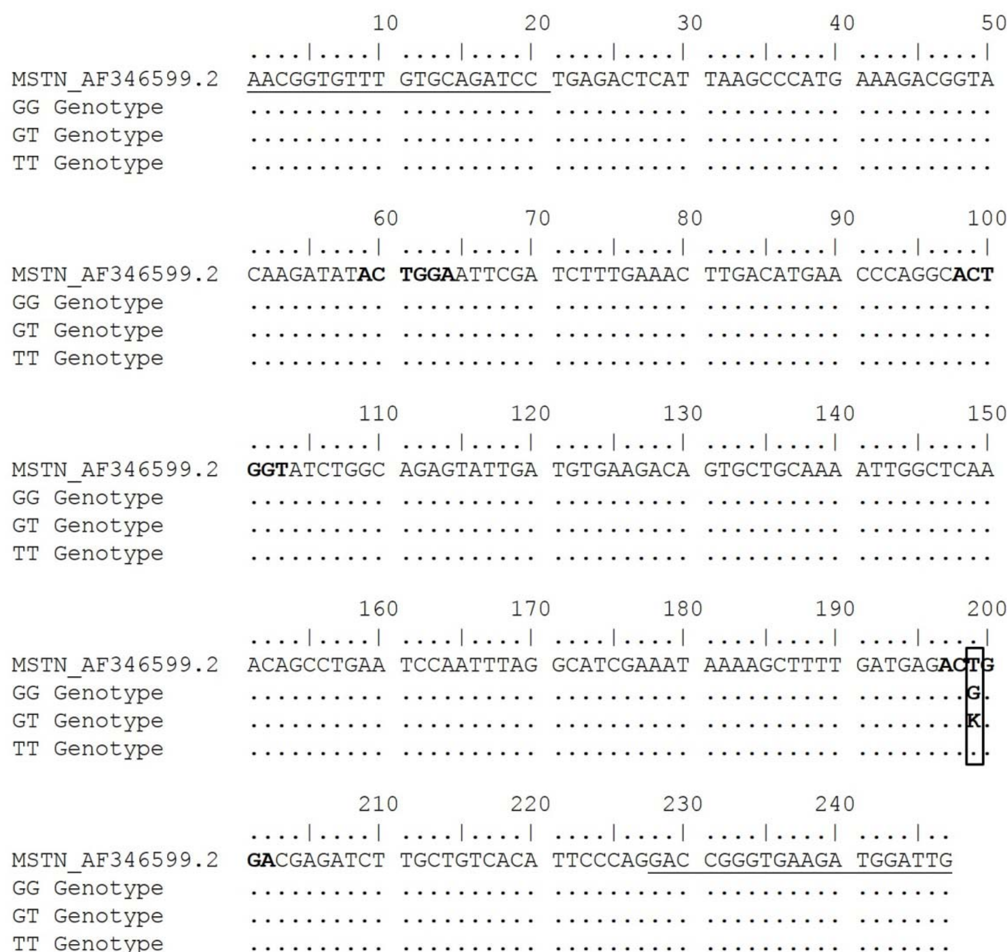

Fig. 5: Nucleotide sequence alignment of *myostatin* gene partial exon 2. Underline shows forward and reverse primer annealing positions; bold shows *Bsr*I restriction sites; boxes show T4842G SNP target (GenBank accession number: AF346599.2)

satellite cells promote terminal differentiation; however, in the adult, satellite cell maintains progenitor-like characteristics despite *myostatin* expression (Manceau *et al*., 2008).

Moreover, *myostatin*|*Bsr*I polymorphism had significant effect in free water (mg H_20 and % mg H_20) in breast muscle (Table 4). This result is in conflicting with Duclos *et al*. (2007) who proposed that there was no effect of the growth rate or muscle development to free water. The significant effect of T allele on living weight and free water (Table 4) implied that it was indeed growth rate has relation to the free water. This relationship is might be due to involvement of T allele in myogenesis. T allele might increase myogenesis processes hence yielded muscle proteins accumulation that further retain the water molecules in the tissue. Yet, this assumption remain to be experimentally evidenced.

Free water properties refer to the water-holding capacity (WHC) of the meat. WHC is the ability of meat to hold all or part of its own and/or added water (Honikel, 2004). Table 4 shows that chickens with TT and GT genotype had lower free water (mg H_20 and % mg H_20) than those with GG or GT ($p<0.05$). This result indicated that animals with TT and GT genotype had a higher WHC than GG genotype. WHC is one of the most important quality characteristics of raw products (Huff-Lonergan and Lonergan, 2005; Lawrie and Ledward, 2006). Improving the WHC has been widely shown to eliminate unfavorable traits of pork and poultry meat, the pale, soft and exudative (PSE) problem (Barbut *et al*., 2008; Petracci and Cavani, 2012). The amount of free water could be used to determine the WHC of meat, as the more free water that comes out of meat, the lower ability of the meat to hold the water. In pre-rigor meat, free water is not found since free water is generated from entrapped (immobilized) water (Huff-Lonergan and Lonergan, 2005). The free water appears at the muscle surface as the result of pH

Table 3: Effect of *myostatin|Bsr*I polymorphism on quantitative carcass characteristics in F2 crossbreedd of *Kampung* x *Cobb* broiler chickens

	Genotype		
Trait	TT (n = 5)	GT (n = 24)	GG (n = 7)
Live weight (g)	2586.60±488.34[a]	2271.52±469.33[ab]	1875.32±558.17[b]
Carcass weight (g)	1738.20±266.92[a]	1518.33±339.06[ab]	1248.00±388.90[b]
Breast weight (g)	436.80±46.31[a]	385.58±100.03[ab]	321.57±119.65[b]
Thighs weight (g)	339.80±60.56[a]	275.79±64.38[ab]	226.29±85.46[b]
Drum sticks weight (g)	319.00±66.38[a]	269.92±65.39[ab]	219.29±77.72[b]
Wings weight (g)	235.80±37.04[a]	216.83±43.50[a]	171.00±46.52[b]
Breast muscle weight (g)	321.40±55.53[a]	271.00±79.99[ab]	230.29±97.73[b]
Thighs muscle weight (g)	257.40±59.86[a]	196.33±42.64[b]	168.57±73.59[b]
Drum sticks muscle weight (g)	211.40±58.80[a]	171.58±43.10[ab]	144.71±53.38[b]
Carcass percentage (%)	67.60±3.94	66.82±4.45	66.34±2.30
Breast percentage (%)	25.34±2.44	25.34±2.41	25.58±3.06
Thighs percentage (%)	19.49±0.82	18.19±1.76	17.94±1.99
Drum sticks percentage (%)	18.24±1.30	17.77±2.22	17.45±1.38
Wings percentage (%)	13.57±0.55	14.44±1.57	13.89±0.95
Breast muscle percentage (%)	18.55±2.51	17.80±2.62	18.05±2.67
Thighs muscle percentage (%)	14.67±1.36	13.09±1.87	13.22±2.29
Drum sticks muscle percentage (%)	11.98±1.63	11.28±1.32	11.48±1.35

Different superscript indicates difference at $p<0.05$

Table 4: Effect of *myostatin|Bsr*I polymorphism on physicochemical carcass characteristics in the breast muscles of F2 crossbreeds of *Kampung* x *Cobb* broiler chickens

	Genotype		
Trait	TT (n = 5)	GT (n = 24)	GG (n = 7)
pH	5.58±0.17	5.53±0.17	5.50±0.54
Free water (mg H_2O)	67.93±10.86[a]	75.09±17.58[a]	94.35±15.16[b]
Free water (% mg H_2O)	22.64±3.62[a]	25.03±5.86[a]	31.45±5.05[b]
Tenderness (kg/cm^2)	1.88±0.74	2.24±0.72	1.46±0.63
Cooking loss (%)	41.06±5.08	41.50±7.18	41.10±6.21

Different superscript indicates difference at $p<0.05$

decreasing post mortem. This pH reduction approaches the isoelectric point (pH 5.3) of the muscle, which causes myofilament shrinkage, decreases the interfilamental space and slowly translocates water into the extracellular space (Honikel, 2004).

In conclusion, Indonesian chickens are polymorphic in the *myostatin|Bsr*I locus (T4842G). The T allele frequency is higher in all populations exception for in the F1 crossbreed of *Kampung* x *Cobb* broiler chicken population. A significant effect was found between genotype and carcass characteristics (live weight, carcass weight, breast weight, thighs weight, drum sticks weight, wings weight, breast muscle weight, thighs muscle weight, drum sticks muscle weight and free water) in the F2 crossbreed of the *Kampung* x *Cobb* broiler chicken population. Favorable carcass characteristics in chickens were mostly found in genotype TT.

Noteworthy, the study on T allele were so far limited to its relationship to the growth rate. Our findings, to our knowledge, emphasized the relationship to the live weight, carcass weight and its percentages, for the first time. The association of T4842G *myostation* gene polymorphism with carcass characteristics has been described in Indonesian chickens for the first time, providing evidence for *myostatin* as an important candidate gene for carcass characteristics in chicken. Interestingly, the previous study were performed on meat type chicken. Indonesian chicken used in this study was dual-purpose chicken. Thus, we assumed that TT genotype is promising to be used as a potential marker for chicken carcass not only for meat type but also for the other chicken type. However, further study of different type of chicken remains to be performed to confirm this proposal.

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