The genetic quality improvement of native and local chickens to increase production and meat quality in order to build the Indonesian chicken industry

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Abstract. Native and local chickens have a great effect in the development of the local chicken industry in rural communities. Local chicken industry significantly contributes to the income value of national agricultural development as well as become an important foundation in building food self-sufficient. Indonesia has a wealth of genetic resources of native and local chicken with abundant genetic diversity, but has not been managed and utilized properly. In Indonesia, local chicken agribusiness is less developed due to lack of genetic improvement and has a direct impact with the very low contribution of meat and eggs. Indonesia has a variety of genetic sources for native and local chickens spread throughout the region. Native and local chickens contribute greatly to the rural economy but their production is still limited. Despite its low productivity, native chicken production is very important for rural communities. However, they face challenges how to increase the productivity of their population, which can benefit them financially and increase food security and reach market potential. This native chicken has the characteristics of slow growth and varied egg production. The development of the potential of native chicken and related issues of food security and food independence, local chicken is suitable for this. Information on the performance and productivity of local chickens is needed for local chicken development. Potential local chickens such as pelung chickens are known as singer chickens with a large body frame. Sentul chickens are another potential local chicken, known as meat-producing chickens with good egg production. Through breeding programs (selection and crossing), increasing the productivity of meat and eggs of native chicken can be done effectively. In addition, improving the quality of local chickens can be improved through conventional feeding management. The availability of selected local chicken in accordance to market demand is still limited. To overcome the lack of selected local chicken, the Faculty of Animal Science, IPB has been developing superior local chicken breed since 2012-2018 namely IPB-D1. The up-to-date Next Generation Sequencing technology through RNA Sequencing can identify candidate genes and potential SNPs quickly, thoroughly and comprehensively, as biomarkers for increasing both productivity (egg production, growth rate, and resistant to diseases) and meat quality (tenderness, flavor, meat fiber and fatty acid composition). During the last 6 years (2012-2018), several studies have been conducted on genes related to various traits: (1) disease resistance: toll receptor-4 (TLR4), mycovirus-1 (Mx-1), natural resistance associated macrophage-1 (NRAMP-1), inducible nitric oxide synthase (iNOS), transforming growth factor-β (TGF-β) genes; (2) meat production: growth hormone
receptor (GH-r), growth hormone secretagogue receptor (GHSR) genes; (3) meat quality: calpastatine (CAST), calpain (CAPN) and myostatin (MSTN) genes; and (4) composition of fatty acids: stearoyl CoA desaturase (SCD) and salutate carrier (SLC) genes. Genetic quality of native and local chickens might be improved to increase their production and meat quality in order to build the Indonesian chicken industry.

1. Introduction

The rapid growth of population, the purchasing ability increment, the economic and social improvement, bring out the consequences of the need for animal protein with ever greater quantity, quality, and variety. In fulfilling the requirement of animal protein of society, the importation is done in the form of live grand parent or parent stock of broiler and layer. The policy of importation with an increasing tendency, bringing the consequences of national food availability and stability becomes vulnerable due to dependence on foreign country. These conditions can become a threat in realizing national food self-sufficiency. Nasional meat demand is fulfilled from 69% of poultry meat consisting of 55% broilers, 9% local chickens, 4% laying hens and 1% ducks. On the other hand the development of the poultry industry, especially both broilers and laying hens in Indonesia is very rapid, result in the population of broilers (1.89 billion) significantly higher compare than local chicken with population (310.9 million) [1].

Molecular genetic biotechnology opens opportunities to improve genetically, manage, and conserve native and local livestock to build sustainable livestock production systems. Molecular technology can be used, among others, to explore genetic diversity at the DNA level, gene mapping, marker assisted selection, gene assisted selection, and molecular-based conservation. If appropriately applied, this technology can be an effective way of managing, genetic improvement, and livestock conservation. The integration of molecular biotechnology in an appropriate selection program will increase productivity, adaptation to the environment, and maintain genetic diversity of native and local livestock [2]. Priority needs to be considered in applying this molecular biotechnology in the order that it may be possible on the basis of convenience, performance, time, cost, and impact factors.

The existence of genetic resources of indigenous and local chicken is a national germplasm that provides abundant genetic diversity, including breed and line breed. The chicken genetic resources live on a wide variety of agroecosystems in Indonesia, makes it a very valuable gene pool for genetic improvement of national chicken industry. The natural interaction process between chiken with specific agroecosystems, making native and local chicken have many advantages, such as adaptive in tough environments, good converters for local feed, disease-resistant and local parasites, and resistant to wet tropical climate stress. The tropical chicken have unique characteristics and many advantages, such as unique production characteristics, adaptive low input production systems, low cost-per-unit production, low-fat meat, wide genetic diversity, heat tolerance and local diseases, potential for biopharmaceutical development, production systems, local conditions integration, and potential to integrate knowledge areas and industrial areas [3]. Therefore, it is essential to be able to identify the existence of various native and local chickens. In developed countries, identification of breeds of chiken is relatively clear.

2. Genetic diversity and chicken breed In Indonesia

Nataamijaya [4] stated that, to date, not less than 32 breeds of native chicken were reported. The list of native and local chicken was shown in table 1. Many of them, such as pelung, sentul, kedu, merawang, gaok, and nusa penida, have special characteristics. The domestication of Gallus gallus hundred years ago resulted most of local chickens found today. The birds might be grouped into many different types such as meat producers, egg layers, dual purposes, and fancy. The development of these native and local chickens is not yet optimum. Even though most local chicken farmers in Indonesia are small farmers, the government's interest regarding the development of native chickens is not remarkable.

Viruses, bacteria, protozoa, and parasites are common causing agents of native chicken infections. However, native chickens have better resistance to avian influenza (AI) due to the higher frequency of the Mx\(^+\) gene compared to the imported hybrid chickens. Selecting native and local chickens for
resistance towards AI and Newcastle disease might be implemented and supported with disease control programs.

Table 1. List of Indonesian chicken breed

| Name of chicken breed | Original place | Reg. No.      |
|-----------------------|----------------|--------------|
| Pelung                | Cianjur regency- West Java | No. 2918/Kpts/OT.140/6/2011 |
| Kokok Balenggek      | Province West Sumantera | No. 2919/Kpts/OT.140/6/2011 |
| Gaga                  | Province South Sulawesi | No. 2920/Kpts/OT.140/6/2011 |
| Merawang              | Bangka regency - Bangka Belitung | No. 2846/Kpts/LB430/8/2012 |
| Kedu                  | Temanggung regency – Central Jawa | No. 2847/Kpts/LB430/8/2012 |
| Nunukan               | Nunukan regency - North Kalimantan | No. 2848/Kpts/LB.430/8/2012 |
| Sentul                | Ciamis - West Jawa | No. 698/Kpts/PD.410/2/2013 |
| Gaok                  | Madura Island | No. 1056/Kpts/SR.120/10/2014 |
| KUB                   | RIAP Ciawi Bogor/ Balitnak | No. 698/Kpts/PD.410/2/2013 |
| Line KUB-1            | RIAP Ciawi Bogor/ Balitnak | No 274/Kpts/SR.120/2/2014 |
| Line SenSi-1 Agrinak  | RIAP Ciawi Bogor/ Balitnak | No 39/Kpts/PK.020/1/2017 |
| Composite Breed IPB-D1| Fact of Anim Sci. IPB University | No. 693/KPTS/PK.230/M/9/2019 |

There are still many other native/local chickens that are very potential to be developed but have not been released as breeds or strains. Therefore, all these chickens must be immediately programmed for the release of breeds or strains in accordance with the regulations of the Minister of Agriculture of the Republik Indonesia No. 117/Permentan/SR.120/10/2014.

3. Marker genetic for meat production and quality improvement in Indonesian chicken

Biotechnology of molecular genetics brings out the great opportunities for verifying genetic diversity at molecular levels. Characterization through the utilization of molecular technologies increases the chance to gain a better understanding of genetic variation, increase genetic variability, genetic improvement, and genetic conservation to DNA polymorphism levels between and within species, and breed. The genetic variation of each individual has a series of DNAs with unique characters, DNA variation occurs due to mutations that include substitution, insertion, or deletion at various sizes of DNA fragments, ranging from one to thousands of nucleotides. There are many molecular markers that can be used for genetic characterization, among others: Microsatellite, Minisatelit, Mitochondria, and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), Amplified Fragment Length Polymorphism (AFLP), and Single Nucleotide Polymorphism (SNP). These molecular markers are often used for identifying genetic diversity and phylogenetic analysis [5]. SNP as a single variation of nucleotides does not alter the overall length of DNA sequences from the genome. SNP mutations are mostly in neutral territory or not codes of the genome and in smaller numbers are in coding or functional sequencing. Functional DNA mutations produce new alleles that can increase or decrease metabolic efficiency compared with wild type alleles. The genetic diversity indicator can be explained against the variation of allele and genotype expressed by observed heterozygosity values and expectations and mean number of alleles (or MNA) for examination in breed; genetic differentiation, fixation index, and variance analysis molecular for examination between breeds; as well as analysis of Bayesian mixes and clusters for examination between populations.
The chicken growth hormone (GH) and its receptor (GHR) together play a key function in chicken growth and its related traits. Khaerunnisa et al. [6] identified the g.2248G>A GH and the g.565G>A GHR polymorphisms in Indonesian Kampung and broiler chicken cross. The GG genotype of the g.565G>A GHR showed a positive effect on chicken carcass and yielded proportional body composition, including live weight, carcass weight, breast weight, thighs weight, breast muscle weight, and thighs muscle weight. While, the g.2248G>A GH gene polymorphisms had no significant effect on carcass components [6].

Myostatin, or growth and differentiation factor-8 (GDF-8), is a negative regulator of skeletal muscle. This protein is classified in the Transforming Growth Factor (TGF)-β superfamily. Khaerunnisa et al. (2016) reported a mutations in exon 2 (T4842G) associated with body weight in kampung chickens. A significant effect was found between genotype and carcass characteristics in the F2 crossbreed kampung x Cobb broiler chickens. Chickens with TT genotype had higher 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 than GG [7].

Ghrelin receptor (GHSR) gene is one of the other growth control genes by modulating growth hormone release from the pituitary by binding to its ligand of ghrelin. Khaerunnisa et al. [8] found that the T1857C GHSR polymorphism was associated with chicken carcass traits. Individuals with the heterozygote genotype were significantly had higher live weight at 26 weeks, carcass weight, commercial cuts weights, and muscles weights than TT genotype in F2 Kampung x broiler cross population.

Insuline-like growth factor binding protein 2 (IGFBP2) is one of the primary binding proteins that is involved in growth, development, and differentiation. Furqon et al. [9] reported that the c.1032C>T IGFBP2 polymorphism had a significantly associated with body, carcass, breast, breast muscle, pectoralis minor, leg, and wings weight in kampung chicken population (P<0.05). The IGFBP2 gene might be a candidate gene that affects growth and body composition traits in chicken [9].

The other gene is the very low density lipoproteins (VLDLs). This gene is a member of major class of lipoprotein particles that is synthesized and secreted by the liver. Furqon et al. [10] reported that a G634A ApoVLDLII polymorphism had a significantly associated with body, carcass, breast, thigh, back and thigh muscle weight in 26 weeks old kampung chicken population (p<0.05). All of genes that have effect on carcass production in chiken is summarized in table 2.

RNA Sequencing technology could be applied to identify control genes in native and local chickens that have fast growth, high percentage of carcasses and good meat conformation through the formation of reference families should be focus of the ongoing research.

4. Marker genetic for meat quality improvement in Indonesian chicken

Over the last decade, there was increase of attention of the link existing between the diet and health and this has made the nutritional quality to be the most critical factor in consumers' meat preference. Fatty acids (FA) compositions in chicken especially unsaturated fatty acids (UFA) have a great importance in chicken meat quality, not only for the nutritional value, but also for the flavor quality and human health. RNA sequencing technology was performed to select chickens which are able to produce meat with high unsaturated fatty acids. Our study revealed the transcriptome profiles of the liver tissue from chicken with high and low unsaturated fatty acids (HUFAs vs LUFAs) content in the breast meat by using RNA-Seq. By using the whole transcriptome sequencing technique, the differentially expressed genes (DEGs) were identified and associated with these genes with UFA levels in chicken. Based on the mapping results, the average number of reads was 28.47 million reads and on an average 90.66% of the reads was classified as mapped reads corresponding to exon reads in Gallus gallus. The digital expression using RNA seq showed 367 genes were differentially regulated in liver tissues from chicken with divergent UFA levels [11]. Among the 367 DEGs screened with stringent criteria in the present study, a large proportion of key genes that actively participate in fatty acid biosynthesis, fat deposition, adipogenesis, fatty acid biosynthesis, and lipid metabolism are
identified, such as ATP5A1W, SLC23A3, COL6A2, SCD, HSD17B4, APOV1, and CYP2J2L4 [11]. Pathway analysis of DEGs resulted into the similar patterns with GO analysis in which the ECM-receptor interaction and focal adhesion were found to be the most dominant pathways in this study. Additionally, peroxisome, Wnt signaling pathway and TGF-beta signaling pathway were found to be enriched in this study. Skeletal muscle extracellular matrix (ECM) is composed of endomysial, perimysial and epimysial connective tissues [12]. This transcriptome analysis using RNA deep sequencing showed potential candidate genes affecting FA composition and metabolism.

**Table 2.** Candidate gene for improving carcass production in local chicken

| Gene and SNP position | Associated traits |
|-----------------------|-------------------|
| Growth Hormone Receptor (GHR); intron 5/ g.565G>A | Live weight, carcass weight, breast weight, breast muscle thighs weight, thighs muscle weight | 26 week old F2 Kampung-Broiler chicken cross [6] |
| Ghrelin Receptor/ Growth Hormone Secretagogue Receptor (GHSR); intron 1/ g.1857 T>C | Live weight, carcass weight, breast weight, breast muscle thighs weight, drum sticks weight, drum sticks muscle weight, wings weight | 26 week old F2 Kampung-Broiler chicken cross [8] |
| Myostatin (MSTN); exon 2/ 4842T>G | Live weight, carcass weight, breast weight, breast muscle thighs weight, thighs muscle weight, drum sticks weight, drum sticks muscle weight, wings weight, and breast meat quality (free water) | 26 week old F2 Kampung-Broiler chicken cross [7] |
| Calpain 1/CAPN 1 g 10568 C> G | Drumstick, Thighs and back weight | 12 weeks old female kampung chicken (Harahap et al 2017, unpublish data) |
| Calpain-1/CAPN-1 g 10541 C> T | Breast and thighs percentage | 12 weeks old female kampung chicken (Harahap et al 2017, unpublish data) |
| Calpain-1/CAPN 1 g 10408 G> C | Breast muscle weight | 12 weeks old male kampung chicken (Harahap et al 2017, unpublish data) |
| Calpain-1/CAPN 1 g 10408 G> C | Thigs muscle weight percentage | 24 weeks old male kampung chicken (Harahap et al 2017, unpublish data) |
| Calpain-1/CAPN 1 g 10551 A> T | Thigs muscle weight percentage | 24 weeks old male kampung chicken (Harahap et al 2017, unpublish data) |
| Calpain-3/CAPN-3 g 12831 C> A | Drumsticks muscle percentage | 12 weeks old male kampung chicken (Harahap et al 2017, unpublish data) |
| Insuline-like growth factor binding protein 2 (IGFBP2); c.1032C>T | body, carcass, breast, breast muscle, pectoralis minor, leg, and wings weight | 12 weeks old female kampung chicken [9] |
| Very low density lipoproteins (VLDLs) g. 634 G>A | body, carcass, breast, thigh, back and thigh muscle weight in 26 weeks old kampung chicken population | 26 weeks old kampung chicken population [10] |

In addition, RNA-Seq technology was used in our study and resulted into a high-resolution map of transcriptional activities in chicken tissue. RNA-Seq platforms were applied in different studies to accomplish and fulfill highly inessential coverage of the genome, a necessity for high quality genome-wide SNP discovery in the complex genomes of animals and plants [13-15]. Genetic variation is responsible for novel variant in phenotypic traits, consequently, the determination of genetic variation in traits of economic importance such as fatty acid composition is considered as one of the main targets of livestock genomic research [16]. Considering this information, we applied this novel approach to identify SNPs in the expressed coding regions of the chicken liver transcriptome with a divergent unsaturated fatty acid. RNA deep sequencing technology was used to obtain differential expression novel variant, and alternative splicing detection. RNA-Seq association study was carried out to identify candidate genes associated with unsaturated fatty acids in chicken. Several of the SNPs (SCD, COL6A2, CYP2J2L4, HSD17B4, and SLC23A3) were explored in our study and might be
included as convenient markers in genotyping platforms to perform association analyses in commercial populations and apply genomic selection protocols in the chicken production [17].

Furthermore characterization of novel candidate genes related to fatty acid composition was explored. The stearoyl-CoA desaturase (SCD) gene encodes an enzyme involved in fatty acid (FA) biosynthesis. Gunawan et al. [17] reported a small nucleotide polymorphism in coding region c.17492542C>G of SCD was significantly associated with FA composition, such as both unsaturated (linoleic (C18:2n6c) and eicosadienoic (C20:2) acids) and saturated (lauric acid (C12:0)). These results will positively impact the understanding of SCD roles in FA composition and will shed light on SCD as a potential candidate in the selection of chickens with higher contents of unsaturated and lower contents of saturated FA. Stearoyl-CoA desaturase (SCD) is an integral membrane protein of endoplasmic reticulum (ER) which catalyzes the rate limiting step in the monounsaturated fatty acids from saturated fatty acids [17]. Furqon et al. [18] reported a significant effect of SCD|AciI SNP g.37284A>G polymorphism on palmitoleic acid (C16:1), fatty acids total and saturated fatty acid in 26 weeks old of F2 kampung-meat type chicken cross (P<0.05). The SCD gene was expressed for polyunsaturated fatty acids in liver tissue in two groups of chickens [18]. SCD gene might be a candidate gene that affects fatty acids traits in F2 kampung-meat type chicken cross. Gunawan et al. [11] reported novel single nucleotide polymorphism from RNA sequencing in coding region c.17492542C>G of SCD and was significantly associated with FA composition, such as unsaturated [(linoleic (C18:2n6c) and eicosadienoic (C20:2) acids) and saturated [lauric acid (C12:0)]] forms. In addition to this, SCD was highly expressed (P<0.05) in tissues collected from high FA chickens than low FA chickens [11].

The SLC23A3 is one of the key genes that are involved in the control the properties of the of fatty acids contents in the meat [17]. Gunawan et al. [17] reported a SNP in coding region c.22385690A>C of the SLC23A3 gene that had a significant effect (P<0.05) on fatty acid composition including stearic acid (C18:0), elaiadic acid (C18:1n9t), and linoleic acid (C18:2n6c). The SLC23A3 was detected in liver from high fatty acids (HFA) and low fatty acid composition (LFA). Though, gene expression of SLC23A3 were not differentially expressed between HFA and LFA (P>0.05). These results will clarify understanding of the great influence of the SLC23A3 in fatty acid traits within the liver and will propose SLC23A3 as a potential genomic candidate gene for selecting chickens with desirable fatty acid traits. All of genes that have effect on meat quality in chiken are summaried in table 3.

Table 3. Gene candidate for fatty acid composition in local chicken

| Name of gene | Association with |
|--------------|-----------------|
| Stearoyl-CoA desaturase (SCD/ c.17492542 C>G) | Fatty acid composition [11] |
| Salute carrier (SLC23A3/ c.22385690 A>C) | Fatty acid composition [17] |

By the change in the paradigm of health issues and the unique taste of meat, the demand for meat with specific qualities such as the composition of polyunsaturated fatty acids and the composition of certain amino acids will increase. Selection using SCD and SLC23A3 gene markers should be the main focus for Indonesian chickens.

5. Genetic marker for heat tolerance and resistent to disease in local chicken

A study of Tamzil et al. [19] reported a relationship between chicken lines and HSP70 genotypes in heat stress resistance. The DD genotype was reported to have the the highest response on panting frequency, rectal temperature, serum corticosterone concentration and expression of HSP70 compared to was AD genotype. It was showed that The AD genotype showed better resistance than the DD. Further, Tamzil et al. [19] concluded that Kampung and Arabic chicken were more resistant to heat stress compared to commercial chicken.

The other candidate gene that plays a role in increasing the immune response in chicken is TLR4 (Toll-like receptor 4). TLR4 is a phagocyte cell surface receptor that has much influence in the recognition of lipopolysaccharide of gram negative bacteria including Salmonella enteritidis. A study
reported by Ulupi et al. [20] showed that Kampung chicken had 3 genotypes of TLR4 gene: AA, AG and GG. There were no significant differences between immune response parameters (expression of TLR4 gene, concentration of leucocytes, differentiation of leucocytes, macrophages activity and capacity) among all genotypes. There was also no S. enteritidis obtained in blood and eggs produced by all genotypes. The IgY specific to S. enteritidis was found in eggs yolk in very high concentration (2.94–3.89 mg/mL). Moreover, a study indicated that Kampung chickens were resistant to S. enteritidis infection in all environmental conditions [21].

The natural resistance-associated macrophage protein-1 gene (NRAMP1) plays major function in immune response against intracellular pathogens. Muhsinin et al. [22] reported that the NRAMP1 genotypes had strong effect to Salmonella pullorum resistant in Sentul chicken. The CC genotype showed higher resistant to Salmonella pullorum than the CT and TT. Even though, the concentrations of leucocytes and differentiation in chickens with all three of NRAMP1 genotypes (CC, CT and TT) were not statistically different, NRAMP1 genotypes were significantly and differently correlated with immune traits [22].

The iNOS (inducible Nitric Oxide Synthase gene) gene is capable of being rapidly expressed in response to proinflammatory stimuli such as cytokines, including inter-feron. Muhsinin et al. (2018) showed that the CC genotypes of the iNOS gene was significantly associated with S. pullorum disease resistance in Sentul chicken compared to the others genotype (TT and TC) [23].

Even though it is not directly related, the TGF-β2 has been reported to have an association with disease resistance in chickens. A study in Sentul chicken showed that the TT genotype of the TGF-β2/RsaI locus was significantly associated with S. pullorum resistance [24]. Although the leucocyte concentration, leucocyte differentiation and H/L ratio in sentul chicken with three of TGF-β2 genotypes (TT, TC, and CC) were not statistically different [24].

| Table 4. Gene candidates for heat resistance and diseases resistance in local chicken |
|------------------------------------------|-----------------|
| Name of gene                            | Association with                                         |
| Heat shock protein /HSP 70              | Chickens that were the most resistant to acute heat stress and had the highest lymphocyte percentage (AD genotype) while those that were the most vulnerable had the lowest lymphocyte percentage (DD) [19] |
| Toll like receptor-4 (TLR-4)            | Kampung chicken is resistant against natural infection S. enteridis in all Genotypes AA, AG and GG [20, 21] |
| Inducible nitric oxide synthase (iNOS)  | CC Genotype more resistant to S. pullorum in sentul chicken [23] |
| Transforming Growth Factor β2 (TGF-β2) | TT Genotype more resistant to more resistant to S. pullorum in sentul chicken [24] |
| NRAMP-1                                 | Much higher resistance for CC genotype than CT and TT genotypes (p<0.05) in Sentul chickens resistant to Salmonella pullorum [22] |
| Mx-1 (Mycovirus)                       | AA and AG genotype are more resistant and show better egg production than GG genotype in Tolaki chicken [25] |

Disease resistance selection in sentul chicken based on immunocompetence indexes such as ND (New castle Diseases) antibody titers, IgY titers and Clearence Test for Salmonella shows strong evidence controlled by many genes. Transcriprome profile between high and low immune resistance in sentul chicken had been studied (Sumantri et al. unpublished). It showed that 875 genes totally had identified 187 gen for low resistance and 688 for high resistance. But only 5 genes that have some specific SNP for the immunocompetence index are BF1, BF2, DMA, TAPBP and LOC101747454. Number of specific SNPs among these top 5 genes is shown in the figure 1.
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Figure 1. Genes and SNPs specific for high and low immunocompetence index

The Chicken Major Histocompatibility Complex (MHC) has strong genetic associations with resistance and susceptibility to infectious diseases [26]. Gene Map for the red Jungle Fowl MHC-B haplotype has described [27] and it has been shown in fig. 2. Future strategies for selection of body resistance to disease resistance in native and local chickens should focus directly on MHC genes and other MHC-related genes as explained in figure 2.

6. The establishment of IPB-D1 breed as a meat type local chicken
The establishment of IPB-D1 chicken aimed to increase the benefits and local chicken as a producer of meat and eggs sources of animal protein. The introduction of fast growth genes from meat type/parent stock Cobb crossed with 3 local chicken breeds so that the genetic composition of IPB-D1 chickens were 75% genetic of local chickens and 25% of meat type/parent stock Cobb. The composition of local chickens were 25% kampung, 25% pelung, and 25% sentul. The ability of IPB-D1 as a meat type local chicken that can be raised intensively or semi-intensive in rural communities. It will benefit the nutritional needs, especially protein from local chicken and can accelerate the speed of the household economy. The specific purpose of developing IPB-D1 chickens was to increase the food security of animal protein sources from local chickens, because all components of agribusiness in the form of breed, feed and breeding technology, product handling to processing can be accessed independently, it does not depend on imports. Production performance, reproduction and disease resistance in chickens IPB-D1 can be seen in tables 5, 6, and 7.

The feed composition given to chicken IPB-D1 as follow: 1) DOC-3 weeks old: 100% commercial feed (19.2% crude protein); 2) 3-4 weeks old: 80% commercial feed and 20% rice bran (17.74% crude protein); 3) 4-5 weeks of age: 70% commercial feed and 30% rice bran (17.00% crude protein); and 4) 5-12 weeks old: 60% commercial feed and 40% rice bran (16.3% crude protein). An increase in feed protein is likely to have a direct impact on improving the final growth in body weight.
The quality of semen of IPB-D1 chickens, in general is very good, the quality of macroscopic and microscopic can be seen in table 6.

Figure 2. Gene Map for the red Jungle Fowl MHC-B haplotype [27]

Table 5. Characteristics of production and reproduction of IPB-D1 chickens

| Parameter observed                              | Value        |
|------------------------------------------------|--------------|
| Age at first lay eggs (weeks)                   | 27±2.80      |
| Body weight at age at 27 weeks (kg)             | 1.86±0.23    |
| Eggs weight at 27 weeks (gr)                    | 33.5±0.97    |
| Hatching Egg Weight (gr)                       | 43.9±0.57    |
| DOC weight (gr)                                 | 32.7±2.32    |
| Eggs production (%)                            | 45.2±10.54   |
| Eggs index                                     | 0.97±0.02    |
| Cock Body weight at 12 weeks (kg)               | 1.18±0.20    |
| Hen Body weight at 12 weeks (kg)                | 1.04±0.12    |
Table 6. The quality of semen of IPB-D1 chickens

| Parameters                        | Value         |
|-----------------------------------|---------------|
| Quality of macroscopic (n = 15)   |               |
| Volume (ml)                       | 0.1 ±0.07     |
| consistency                       | viscous liquid|
| pH                                | 6.97 ± 0.27   |
| colour                            | White milk    |
| Quality of microscopic (n = 15)   |               |
| Mass Motion (+++)                 | 2.73 ± 0.46   |
| Motility (%)                      | 75.0 ± 6.27   |
| concentration (x 10^6)/ml         | 3257.50 ± 1348.88 |
| Abnormality (%)                   | 14.79 ± 4.67  |
| Fertility (%)                     | 84.25 ± 6.79  |

Source: Mulyadi et al. unpublished

7. Hematology profile and ND resistance in IPB-D1 chickens

Chicken blood profile of IPB-D1 is very interesting to have less erythrocytes (1.93 ± 0.28) (106 cells/mm^3) compared to the normal range (2.50-3.20) (106 cells/mm^3) but has a hemoglobin of 8.20 ± 1.29 (g / 100 ml) which is almost the same as the normal standard (7.00 -13.00) (g / 100 ml). Hematology profiles of IPB-D1 chickens can be seen in table 7.

The effect of ND vaccination on chicken IPB-D1 was able to increase the minimum limit from 39.5% to 55.33%, but lower the upper% from 70.17% to 61.66. The effect of vaccination on blood profile can be seen in table 8.

Table 7. Hematology profiles of IPB-D1 chickens

| Traits                        | Value*          | Normal range |
|-------------------------------|-----------------|--------------|
| Leucocyte (10^3 cell/mm^3)    | 11.68±0.88      | 20.00-30.00  |
| Lymphocyte (%)                | 65.2±18.20      | 55.00-60.00  |
| Heterophil (%)                | 30.71±18.13     | 25.00-30.00  |
| Monocyte (%)                  | 4.00±4.48       | 10.00        |
| Eosinophil (%)                | 1-2             | 3.00- 8.00   |
| Basophil (%)                  | -               | -            |
| Heterophil/Lymphocyte         | 0.58 -0.64      | 0.45-0.50    |
| Erythrocytes (10^6 cell/mm^3) | 1.93± 0.28      | 2.50 -3.20 [28] |
| PCV (%)                       | 22.67 ±2.49     | 30.00-33.00 [28] |
| Haemoglobin (g /100 ml)       | 8.20 ±1.29      | 7.00- 13.00 [29] |

*Murtini et al, unpublished

Table 8. Hematology profiles not vaccinated and vaccinated against ND IPB-D1 chickens

| Traits                        | ND vaccinated* | Not vaccinated against ND* | Normal range [28] |
|-------------------------------|----------------|----------------------------|-------------------|
| Leucocyte (10^3 cell/mm^3)    | 14.92 - 22.0   | 14.00-19.6                 | 20.00-30.00       |
| Lymphocyte (%)                | 55.33 – 61.66  | 39.5 – 70.17               | 55.00-60.00       |
| Heterophil (%)                | 32.33- 40.0    | 25.5-55.0                  | 25.00-30.00       |
| Monocyte (%)                  | 2.6 – 5.16     | 3.83 – 4.33                | 10.00             |
| Eosinophil (%)                | 1-2            | 2-3                       | 3.00- 8.00        |
| Basophil (%)                  | -              | -                         | -                 |
| Heterophil/Lymphocyte         | 0.58 -0.64     | 0.64-0.78                 | 0.45-0.50         |

*Murtini et al, unpublished
The results of the IPB-D1 chicken surveillance test against ND were carried out by vaccination and without vaccination then by challenge test. The hematological profile of IPB-D1 chickens is very good (Table 10) and survival rate after the challenge test was very good with vaccination (100%) without vaccination (88.8%). The results of the challenge test can be seen in Table 9.

Table 9. IPB-D1 chicken surveillance test against ND

| Trait                      | Challenge test ND ¹) | Control                                      | Standard [30] |
|----------------------------|----------------------|----------------------------------------------|---------------|
| Number of chicken          | 10                   | 9                                            |               |
| Number of live chicken (head) | 10/10 (100%)        | 8/9 (88.8%)                                  | >80           |
| Antibody titer (log 2)     | 5.1±1.52             | 4.17±1.17                                    | 3             |
| IgY concentration titer (mg/ml) | 3.4-6.6              | 2.4-8.4                                       | 5.7*          |

*Murtini et al, unpublished data

Based on the data above, it is shown that the IPB-D1 chicken has a high capacity of red blood cells in transporting oxygen and nutrients, a high resistance to ND disease so it is more resistant to ND, and good environmental adaptability.

8. Conclusion
Maintaining genetic diversity will remain a target for the future because genetic diversity is essential for genetic improvement in breeding programs, especially selection activities to produce superior breeds or strains of specific agroecosystems. Genome mapping and DNA fingerprint (DNA fingerprint) will be well applied in local and native livestock conservation programs. Epigenetic studies such as nutrigenomics to study the genetic interactions of the environment, especially feeding, are particularly important in native and local animals that have high genetic potential, but their gene expression does not occur because they are fed less optimally. Biotechnology of molecular genetics to be applied needs to be integrated into the breeding scheme in genetic improvement of breeds in certain species in order to provide a faster genetic response and economic value to farmers and businesses.

References
[1] Ditjen Peternakan dan Kesehatan Hewan (DPKH) 2018 *Buku Statistik Peternakan dan Kesehatan Hewan* (Jakarta: DPKH)
[2] Naqvi A N 2007 Applications of molecular genetic technologies in livestock production: Potentials for developing countries *Adv. Biol. Res.* 1 72–84
[3] In-Madan M L 2005 Animal biotechnology: applications and economic implications in developing countries *Rev. Sci. Tech. Off. Int. Epiz.* 24 127–139
[4] Nataamiyaya A G 2000 The native chicken in Indonesia *Bal. Plasma Nutfah* 6 (1) 1-6
[5] Ebegbulem V N and Ozung P O 2013 Application of Molecular Markers in Farm Animal Improvement: Prospects and Challenges *Online J. Anim. Feed Res.* 3 (3) 149-152
[6] Khaerunnisa I, Jakaria, Arief I I, Budiman C, and Sumantri C 2017 The associations of GH and GHR genes with carcass components in Indonesian kampung and broiler chicken cross *Med. Pet (Trop. Anim. Sci. J.)* 40 (2) 78-87
[7] Khaerunnisa I, Pramujo M, Arief I I, Budiman C, Gunawan A, Jakaria, and Sumantri C 2016 Polymorphism of the T4842G myostatin gene is associated with carcass characteristics in Indonesian chickens *Int. J. Poult. Sci.* 15 (8) 316-24
[8] Khaerunnisa I, Jakaria, Arief I I, Budiman C, and Sumantri C 2017 The ghrelin receptor (GHSR) gene polymorphism in Indonesian local chicken and crossbreed is associated with carcass traits Anim. Prod. 19 (2) 71-80

[9] Furqon A, Gunawan A, Ulupi N, Suryati T, and Sumantri C 2018 A polymorphism of Insulin-Like Growth Factor Binding Protein 2 gene associated with growth and body composition traits in kampong chickens J. Vet. 19 (2) 1-9

[10] Furqon A, Gunawan A, Ulupi N, Suryati T, and Sumantri C 2017 Association of apoVLDLII gene polymorphism with body composition traits in Kampung chicken Int. J. Poult. Sci. 16 462-466

[11] Gunawan A, Listyarini K, Furqon A, Jakaria, Sumantri C, Akter S H, and Uddin M J 2019 RNA deep sequencing reveals novel transcripts and pathways involved in the unsaturated fatty acid metabolism in chicken Gene Reports 15 100370

[12] Li Y, Trivedi V, Truong T V, Koos D S, Lansford R, Chuong C M, Warburton D, Moats R A, and Fraser S E 2015 Dynamic imaging of the growth plate cartilage reveals multiple contributors to skeletal morphogenesis Nat. Commun. 6

[13] Gunawan A, Sahadevan S, Neuhoff C, Große-Brinkhaus C, Gad A, Frieden L, Tesfaye D, Tholen E, Looft C, Uddin M J, Schellander K, and Cinar M U 2013 RNA deep sequencing reveals novel candidate genes and polymorphisms in boar testis and liver tissues with divergent androgenone levels PLoS One 8 (5) e63259

[14] Gunawan A, Sahadevan S, Cinar M U, Neuhoff C, Große-Brinkhaus C, Frieden L, Tesfaye D, Tholen E, Looft C, Wondim D S, Hölker M, Schellander K, and Uddin M J 2013 Identification of the novel candidate genes and variants in boar liver tissue with divergent skatole levels using RNA deep sequencing PLoS One 8 (5) e72298

[15] You F M, Hua N, Deal K R, Gu Y Q, Luo M C, McGuire P E, Dvorak J and Anderson O D 2011 Annotation-based genome-wide SNP discovery in the large and complex Aegilops tauschii genome using next-generation sequencing without a reference genome sequence. BMC Genomics 12 59

[16] Lin R, Du X, Peng S, Yang L, Ma X, Gong Y and Li S 2015 Discovering All Transcriptome Single-Nucleotide Polymorphisms and Scanning for Selection Signatures in Ducks (Anas platyrhynchos) Evolutionary Bioinformatics Online 11(Suppl 1), p.6

[17] Gunawan A, Nurajizah E S, Listyarini K, Furqon A, Bilyaro W, Sumantri C, Jakaria, Akter S H, and Uddin M J 2018 Association study and expression analysis of stearoyl Co-A desaturase as a candidate gene for fatty acid composition in Indonesian crossbred chicken Int. J. Poult. Sci. 17 348–55

[18] Furqon A, Gunawan A, Ulupi N, Suryati T, and Sumantri C 2018 Expression and Association of SCD Gene Polymorphisms and Fatty Acid Compositions in Chicken Cross MedPet (Trop. Anim. Sci. J.) 40 (3) 151-57

[19] Tamzil M H, Noor R R, Hardjosutro P S, Manalu W, and Sumantri C 2014 Hematological Response of Chickens with Different Heat Shock Protein 70 Genotypes to Acute Heat Stress Int. J. Poult. Sci. 13 (1) 14-20

[20] Ulupi N, Muladno, Sumantri C, and Wibawan I W T 2013 Association of TLR4 Gene Genotype and resistance against Salmonella enteridis natural infection in kampong chicken Int. J. Poult. Sci. 12 (8) 445-50

[21] Ulupi N, Muladno, Sumantri C, and Wibawan I W T 2014 Identifikasi keragaman gen Toll-Like Receptor-4 ayam lokal dengan Polymerase Chain Reaction-Restiction Fragment Lenght Polymorphism J. Vet. 15 345–52

[22] Muhsin M, Ulupi N, Gunawan A, Wibawan I W T, and Sumantri C 2016 Association of NRAMP1 Polymorphisms with Immune Traits in Indonesian Native Chickens Int. J. Poult. Sci. 15 (10) 401-06

[23] Muhsin M, Ulupi N, Gunawan A, Wayan I W T, and Sumantri C 2018 Influence of iNOS to Salmonella Pullorum Disease Resistance in Sentul Chicken Veterinaria 67 1

12
[24] Muhsinin M, Ulupi N, Gunawan A, Wibawan I W T, and Sumantri C 2017 g.640T>C Polymorphism of the TGF-β2 Gene is Associated with Salmonella pullorum Resistance in Indonesian Chickens *Anim. Prod.* 19 (2) 81-92

[25] Pagala M A, Muladno, Sumantri C, and Murtini S 2013 Association of Mx gene genotype with antiviral and production traits in Tolaki chicken *Int. J. Poult. Sci.* 12 (12) 735–39

[26] Assan N. 2015. Prospects for indigenous chickens genetic improvement and conservation in Zimbabwe *Agricultural Advances* 4 (5) 49-56

[27] Miller M M and Taylor Jr R L 2016 Brief review of the chicken Major Histocompatibility Complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance *Poult. Sci.* 95 375–92

[28] Swenson M J and William O R 1993 *Duke’s Physiology of Domestic Animals*, 11th Ed. (Ithaca and London: Publishing Assocattes a Division of Cornell University)

[29] Jain N C 1993 *Essentials of Veterinary Hematology* (Philadelphia: Lea and Febiger) 76 250

[30] Carlander D, Stålberg J, and Larsson A 1999 Chicken antibodies: a clinical chemistry perspective *Ups. J. Med. Sci.* 104 (3) 179-89