Carcass and fatness traits of Central Javanese local ducks based on Lipoprotein Lipase (LPL) and Perilipin (PLIN) genes

R Susanti*

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

*Corresponding author: r.susanti@mail.unnes.ac.id

Abstract. This research is an exploratory study that determines the genotypes of carcass and fatness traits in Central Javanese local ducks based on the lipoprotein lipase (LPL) and perilipin (PLIN) genes. A 35 ducks were sampled from seven local ducks in Central Java. The duck's DNA was isolated from its feathers. PLP and PLIN genes were amplified by PCR method using specific primers. In the RFLP analysis, PCR products were digested with the restriction enzymes MvaI (BstNI) (5’CC/WGG) and Bsp1286I (SduI) (GDGCH/C), for LPL and PLIN gene, respectively. The results of PCR-RFLP analysis on local ducks in this study showed a monomorphic in exon 2 of the PLIN gene and polymorphic in exon 5 of the LPL gene. Based on the LPL gene, as many as 29 ducks (82.85%) had GG genotypes, 3 ducks (8.57%) had AG types and 3 ducks (8.47%) had AA types. Both PLIN and LPL genotypes were clustered the Central Javanese local ducks into 3 haplotypes (A-C). The duck samples were dominated by haplotype A (82.85%) followed by haplotype B (8.57%) and haplotype C (8.57%). Results showed that 82.85% of Central Javanese local ducks genetically had a good of fatness traits but a low of carcass traits.

1. Introduction

In the duck (Anas platyrhynchos) farming industry, the quantity and quality of production is one of the main focuses. Various efforts are needed to obtain ducks with a lower slaughter age, high feed efficiency, and high meat production and growth. Intensive selection on the aspect of growth speed, will trigger an increase in body fat deposition. Excess body fat deposition can affect the performance of the carcass and the quality of the meat. Several studies were conducted to identify the factors that cause fat deposition and fat loss. The selection of individuals with a genetic approach is more accurate and has a permanent impact on the offspring. Fatness and carcass trait will determine the quality of meat production.

Animal breeding programs can be carried out on a molecular basis. Many quantitative trait loci (QTL) affect carcass characteristics in various animals. In cattle, carcass trait has been reported to be associated with several activation pathways, including peroxisome proliferator-activated receptor signaling, glucagon and leptin [1-3]. In pigs, the SSC1 and SSC8 loci were associated with carcass traits. The TBC1D1, BAAT, and PHLPP1 genes were also candidate genes for trait of growth and obesity swine [4,5]. Some candidate genes, including TBC1D1, LCORL, LAP3, LDB2, and TAPT1, were associated with the carcass traits of chickens [6-9]. Previous studies suggested that the carcass and meat quality traits of duck influenced by the fat mass and obesity-associated gene (FTO) [10] and mutations in intron
2 of the growth hormone gene (GH) [11]. The carcass and fat traits were also affected by the perilin gene (PLIN) [12], and lipoprotein lipase (LPL) [13].

Identification and utilization of potential gene candidates related to reproduction and economic traits is very important in poultry breeding programs [14]. Molecular identification using genetic markers associated with economic traits can be used to enhance genetic improvement in domestic animals. Molecular analysis is a high-reliability approach, combined with quantitative approaches and traditional breeding strategies for design efficiency of preservation strategy. The molecular marker cannot be affected by the environment and is stable. Genetic studies on Indonesian domestic ducks are still lacking. This study aims to analyze the genotype of carcass and fatness traits in Central Javanes local ducks based on the exon-5 lipoprotein lipase (LPL) and exon-2 perilipin (PLIN) genes.

2. Methods

An exploratory study to determine the genotype of local ducks based on LPL and PLIN genes was carried out using the PCR-RFLP method. In this study, 35 ducks were sampled from seven type of local ducks in Central Java (namely Tegal Blorong duck (TB), Tegal Branjangan duck (Tbr), Tegal Lemahan duck (TL), Tegal Jarakan duck (TI), Magelang duck (M), Peking duck (PK), and Pengging duck (PG)). The duck's DNA was isolated from its feathers. The feathers of each sample duck in this study were taken from the inside of the right and left wings. DNA isolation was carried out using the gSYNCTM DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan). The isolated DNA was then amplified using the GeneAmpR PCR thermocycler system 2400 (Perkin Elmer, Massachusetts, USA). The PCR program for LPL and PLIN genes amplification is shown in Table 1. The Primer sequences, amplification products and references for each gene are shown in Table 2. The LPL and PLIN gene amplification was conducted using Dream Taq polymerase master mix (Thermo Fisher Scientific) with the composition of 1.2 µL of the primer, 2 µL of DNA template (50 ng), 12.5 µL of PCR mix and 8.1 µL of ddH2O. The amplification results were visualized by 3% agarose electrophoresis. Agarose powder used was A9539 SIGMA Agarose BioReagent (Sigma-Aldrich, USA).

| Gene | PCR Program |
|------|-------------|
| Exon 5 LPL | 1) 1 cycle at 94 °C for 7 minute (predenaturation)  
(2) 32 cycles of 94 °C for 40 sec (denaturation), 58 °C for 40 sec (annealing)*, and 72 °C for 40 sec (extension)  
(3) 1 cycle at 72 °C for 7 minute (post extension) |
| Exon 2 PLIN | 1) 1 cycle at 94 °C for 4 minute (predenaturation)  
(2) 38 cycles of 94 °C for 30 sec (denaturation), 57 °C for 30 sec (annealing)*, and 72 °C for 30 sec (extension)  
(3) 1 cycle at 72 °C for 10 minute (post extension) |

* The annealing temperature from the optimization results

| Gene | Primers Sequence | Size of PCR product (bp) | References |
|------|------------------|-------------------------|------------|
| Exon 5 LPL | Lexon5F: 5'-GGG CCC ACC TTT GAG TAC-3'  
Lexon5R: 5'-TGC AAG GCC TTT TTC AGC-3' | 219 | Yang et al. (2012) |

| Exon 2 PLIN | PLIN-F: 5'-ATCTCGACCTGCGAAAGCC-3'  
PLIN-R: 5'-TTGGAGGAATCACACTGTGGC-3' | 173 | Zhang et al. (2013) |

In the RFLP analysis, PCR products (amplicons) were digested with the restriction enzymes MvaI (BstNI) (5'CC/WGG) and Bsp1286I (Sdul) (GDGCH/C) (Thermo Fisher Scientific), for LPL and PLIN gene, respectively. The RFLP reaction was performed using a reaction mixture containing 5 µL of amplicons, 4U of restriction enzyme, 1 µL of buffer and ddH2O to a total volume up to 10 µL. The
solution was then incubated at 37 °C for overnight. The PCR-RFLP results were visualized in 3% agarose electrophoresis. DNA band in agar gel was analyzed for the genotype according to Table 3. The genotype frequency, allele frequency and expected heterozygosities (HE) analyze with the PopGene32

| Table 3. Restriction enzymes and genotypes for the LPL and PLIN genes |
|-----------------------------|-----------------------------|-----------------------------|
| **Gene** | **Restriction enzyme** | **SNP mutation** | **Genotype product** |
| Exon 5 LPL | MvaI (BstNI) (5’CC/WGG) | G/A mutation at 726 bp position | AA: 168 and 51 bp |
| | | | AG: 219, 168 and 51 bp |
| | | | GG: 219 bp |
| Exon 2 PLIN | Bsp1286I (SduI) (GDGCH/C) | C/T mutation at 135 bp position | CC: 134 and 39 bp |
| | | | TT: 173 bp |
| | | | CT: 173, 134 and 39 bp |

3. Result and Discussion

All duck DNA samples in this study were successfully amplified using 2 pairs of specific primers for 2 target genes (LPL and PLIN). Amplification was carried out with annealing temperature optimization results, i.e. 58.0 °C and 57.0 °C for LPL and PLIN, respectively. The LPL and PLIN gene fragments which digested by MvaI and SduI restriction enzymes, were shown in Figures 1A and 1B, respectively. With the RFLP PCR technique, differences in homologous DNA sequences can be detected based on differences in fragment lengths after being digested by restriction enzymes.

![Figure 1](image)

The results of PCR-RFLP analysis on Central Javanese local ducks in this study showed a monomorphic in exon 2 of the PLIN gene. The RFLP product of all duck samples showed one band with 173bp length (TT genotype) (Table 4). The results of this study differ from previous research on duck populations in China, i.e. Peking ducks (Z2, Z4, Z2xZ4 series) and Cherry valley ducks. These ducks in China showed genotypic polymorphisms at the SduI locus of the PLIN exon 5 gene. In the four duck populations in china, there were more heterozygous individuals than homozygous ones [12]. Meanwhile, all of the local ducks in this study were homozygote genotype TT. The homozygote genotype TT in the ducks of this study was probably a marker of the highly carcass weight. This was consistent with zhang's ([12] research that the Peking duck series Z4 with TT genotype had a significantly higher carcass net weight than the CT and CC genotypes. The PLIN gene was a potential major gene, which affects the quality of meat traits. It is necessary to analyze more than one SNP on the
PLIN gene, as a molecular marker of the carcass traits and fatness traits in ducks. Some diplotypes of PLIN exon 1 gene could be positive molecular markers to enhance the percentage of eviscerated weight and the percentage of breast muscle in chickens [15].

Based on the LPL gene, there were 29 ducks (82.85%) with GG genotype, 3 ducks (8.57%) with AG genotype and 3 ducks (8.47%) with AA genotype. The allele frequency results showed that G was the dominant allele and the GG was the dominant genotype (Table 4). Analysis of the diversity of LPL locus showed that Shannon’s information index was 0.3837 and the expected heterozygosity (He) scores was 0.2273 (Table 5). The SNP G726A was synonymous and caused restriction enzyme site change (MvaI) and did not result in an amino acid change [13]. The homozygote genotype GG was significantly associated with the lower abdominal fat weight and the subcutaneous fat (plus skin) than the CT and TT genotypes [13]. These shown that 82.85% of local ducks in Central Java have a good of fatness traits because of their low levels of abdominal and subcutaneous fat. Central Java local duck meat that is genotypically a good of the fatness can be used as an alternative source of healthy animal protein, especially for patients who have a low-fat diet. The results of this study also showed that 82.85% of Central Javanese local ducks genetically had a low carcass trait. The carcass traits and body weight of ducks with the heterozygote genotype GG were significantly lower than the genotypes GA and AA. The parameters of the carcass traits are carcass weight, eviscerated with giblet weight, eviscerated weight, leg muscle weight, breast weight, neck weight, wing weight, shank plus palma weight, heart weight, liver weight and muscular stomach weight [13].

Based on the LPL gene, there were 29 ducks (82.85%) with GG genotype, 3 ducks (8.57%) with AG genotype and 3 ducks (8.47%) with AA genotype. The allele frequency results showed that G was the dominant allele and the GG was the dominant genotype (Table 4). Analysis of the diversity of LPL locus showed that Shannon’s information index was 0.3837 and the expected heterozygosity (He) scores was 0.2273 (Table 5). The SNP G726A was synonymous and caused restriction enzyme site change (MvaI) and did not result in an amino acid change [13]. The homozygote genotype GG was significantly associated with the lower abdominal fat weight and the subcutaneous fat (plus skin) than the CT and TT genotypes [13]. These shown that 82.85% of local ducks in Central Java have a good of fatness traits because of their low levels of abdominal and subcutaneous fat. Central Java local duck meat that is genotypically a good of the fatness can be used as an alternative source of healthy animal protein, especially for patients who have a low-fat diet. The results of this study also showed that 82.85% of Central Javanese local ducks genetically had a low carcass trait. The carcass traits and body weight of ducks with the heterozygote genotype GG were significantly lower than the genotypes GA and AA. The parameters of the carcass traits are carcass weight, eviscerated with giblet weight, eviscerated weight, leg muscle weight, breast weight, neck weight, wing weight, shank plus palma weight, heart weight, liver weight and muscular stomach weight [13].

The non-synonymous substitution mutation of the LPL gene may influence LPL activity. The results of the Sun et al. [16] research revealed that the Leu452His mutation caused LPL dysfunction, produced from resistance to the AMPK/PGC-1α signaling pathway. LPL activity in tissues is affected by many factors, including strain, age, nutrition, and environment. Research by Ding et al. [17] revealed that the LPL mRNA level in adult longissimus dorsi yaks is much lower than in adult cattle. The growth pattern of subcutaneous adipose tissue varies between regions. From the research of Kou et al. [18], it was known that the average number of cells decreases drastically along with the fattening process, but the average cell volume and triglyceride content per cell increases gradually during the initial growth of ducks.

| Tabel 4. Genotype and allele frequency of ducks based on LPL and PLIN genes |
|---------------------------|----------------|----------------|
| Gene  | Genotype Frequency | Alleles Frequency |
| Exon 5 LPL | GG 0.8285  (29)  | G 0.8714 |
|      | AG 0.0857  (3)  | A 0.1286 |
|      | AA 0.0857  (3)  |         |
| Exon 2 PLIN | CC 0.0000  (35)  | C 0.0000 |
|      | TC 1.0000  | T 1.0000 |
|      | TT 1.0000  |         |

| Tabel 5. The genetic diversity of local ducks in Central Java based on the LPL exon 5 gene |
|---------------------------|----------------|
| Statistics parameters | Value    |
| $N_A$ (observed number of alleles) | 2.0000 |
| $N_E$ (effective number of alleles) | 1.2888 |
| $I$ (Shannon’s information index) | 0.3837 |
| Observed homozygosity | 0.9143 |
| Observed heterozygosity | 0.0857 |
| Expected homozygosity | 0.7727 |
| Expected heterozygosity | 0.2273 |
| Average heterozygosity | 0.1800 |

Both PLIN and LPL genotypes were clustered the Central Javanese local ducks into 3 haplotypes (A-C). The duck samples were dominated by haplotype A (82.85%) followed by haplotype B (8.57%)
and haplotype C (8.57%) (Table 6). The high allele frequency has belonged to G allele (87.14%) of the LPL gene and T allele (100%) of the PLIN gene (Table 4). The allele frequencies in a single locus change from generation to generation. Its due to the population increased and migration decreased. This condition can result in a high chance of panmixia (random mating) in this population [19].

| Haplotype | Genotype PLIN | Genotype LPL | Duck sample | Amount (%) |
|-----------|--------------|--------------|-------------|------------|
| Haplotype A | TT | GG | TB1, TB2, TB3, TB4, TB5, TJ1, TJ2, TJ4, TJ5, TL1, TL2, TL3, TL4, TL5, M1, M2, M3, M4, M5, PK1, PK2, PK3, PG2, PG3, PG4 | 29 (82.85%) |
| Haplotype B | TT | GA | TB5, TJ3, PG1 | 3 (8.57%) |
| Haplotype C | TT | AA | PK4, PK5, PG5 | 3 (8.57%) |
| Total | | | | 35 (100%) |

Central Javanese local ducks with GG genotype (82.85%) indicated that genetically these ducks had a good of fatness traits but a low of carcass traits. Likewise, haplotype A ducks (GG and TT genotypes) may also had a good of fatness traits but a low of carcass traits. The reason of it is the TT genotype was only related to the carcass weight but not related to the other carcass traits parameter. It is necessary to analyze the association of GG and TT genotypes with the carcass and fatness traits using the bigger population of duck. The further research is needed to provide a genetic basis for carcass traits. The previous reasearch reveal that the 36 candidate genes of Pekin ducks were related to the body size and carcass traits [20]. The identification of potential gene candidates for a particular trait can be used to understand the mechanism of the trait. The TCF21 gene on chromosome 3 was reported as a candidate gene for testicular growth and development, while the TCF12 gene was associated with testicular weight of chicken. Six genes on chicken chromosome 21, i.e TNFRSF1B, PLOD1, NPPC, MTHFR, EPHB2, and SLC35A3, play an important role in bone development [21]. Studies on genetic markers in Central Java ducks have been carried out. From this study, it was known that ducks a genetically had a high hatchability (genotype +/+ ) based on the ovalbumin gene [22]. Central Java local ducks are also reported to had a high egg production and quality, based on the PRL and COLX genes [23].

In recent decades, one of the important factors affecting consumer preferences is the quality of meat. The nutritional value and quality of meat is determined by the intramuscular fat content and fatty acid composition. Related to this, it is necessary to make a great effort to identify genes and their networks that are connected with the quality of duck meat. Fan et al. (2020) [24] revealed that the accumulation capacity of SFA (especially C16: 0) and MUFA (especially C16: 1n-7 and C18: 1n-9) in Pekin ducks mainly occurred at 6-8 weeks of age. Expression of CEBPA (transcription factor) and PPARGC1A (peroxisome proliferatoractivated receptor gamma coactivator 1-alpha) genes may affect the deposition of fatty acids in duck breast muscle. The results of Zhang et al 2016's [25] study also showed that the C1251G polymorphism in the LPL exon 8 gene was a useful genetic marker in duck breeding to increase the content of beneficial fatty acids.

4. Conclusion
The results of PCR-RFLP analysis on Central Javanese local ducks in this study showed a monomorphic in exon 2 of the PLIN gene, but polymorphic at the LPL exon 5 gene. Based on the LPL gene, as many as 29 ducks (82.85%) had GG genotypes, 3 ducks (8.57%) had AG types and 3 ducks (8.47%) had AA types. Local ducks with GG genotype (82.85%) indicated that genetically these ducks had a good of fatness traits but a low of carcass traits. Both PLIN and LPL genotypes were clustered the Central Javanese local ducks into 3 haplotypes (A-C). The duck samples were dominated by haplotype A (82.85%) followed by haplotype B (8.57%) and haplotype C (8.57%).
Acknowledgements
This work is supported by the Institution of Research and Community Service, Universitas Negeri Semarang through Fundamental Research Fund 2019. We greatly thanks for research financial support.

References
[1] Sanchez M, Govignon-Gion A, Croiseau P, Fritz S, Hozé C, Miranda G, Martin P, Barbat-Leterrier A, Letäief R, Rocha D, Brochard M, Boussaha M and Boichard D 2017 Genet. Sel. Evol. 49 68
[2] Inoue K, Honda T and Oyama K 2015 J. Anim. Sci. 93 2714
[3] Doran AG, Berry DP and Creevey CJ 2014 BMC Genomics. 15 837
[4] Guo Y, Qiu H, Xiao S, Wu Z, Yang M, Yang J, Ren J and Huang L 2017 J. Appl. Genet. 58 499
[5] Sanchez M, Tribout T, Iannuccelli N, Bouffaud M, Servin B, Tenghe A, Dehais P, Muller N, Del Schneider M, Mercat M, Rogel-Gaillard C, Milan D, Bidanel J and Gilbert H 2014 Genet. Sel. Evol. 46 12
[6] Shahjahan M, Liu RR, Zhao GP, Zhang JJ, Zheng MQ, Li QH and Wen J 2015 Genet. Mol. Res. 14 18839
[7] Gu X, Feng C, Ma L, Song C, Wang Y, Da Y, Li H, Chen K, Ye S, Ge C, Hu X and Li N 2011 PLoS One. 6 e21872
[8] Wang Y, Xu HY, Gilbert ER, Peng X, Zhao XL, Liu YP, Zhu Q 2014 Gene. 547 288
[9] Venturini GC, Stafuzza NB, Cardoso DF, Baldi F, Ledur MC, Peixoto JO, El FL and Munari DP 2015 Poult. Sci. 94 2863
[10] Gan W, Song Q, Zhang NN, Xiong XP, Wang DM and Li L 2015 Genet. Mol. Res.14 6699
[11] Wu Y, Pan AL, Pi JS, Pu YJ, Du JP, Liang ZH and Shen J 2012 Mol. Biol. Rep. 39 8027
[12] Zhang HL, Fan HJ, Liu XL, Wu Y and Hou SS 2013 Genet. Mol. Res. 12 1582
[13] Yang Y, Gong P, Li S, Peng X, Feng Y and Gong Y 2012 J. Anim. Vet. Adv. 11 578
[14] Basumatary K, Das B, Borah P, Barkalita L, Bharali K and Tamuly S 2019 J. Entomol. Zool. Stud. 7 922
[15] Zhang L, Zhu Q, Liu D, Yin H, Wang Y, Yang Z, Wang Z, Yuan Y and Zhao X 2015 Asian Australas. J. Anim. Sci. 28 763
[16] Sun K, Yang W, Huang Y, Wang Y, Xiang L and Qi J 2013 PLoS One 8 e75462.
[17] Ding Y, Xu Y, Lin Y, Yue Y, Jin S, Li Y and Zheng Y 2012 J. Appl. Anim. Res. 40 311
[18] Kou J, Wang W X, Liu H H, Pan Z X, He T, Hu J W, Li L and Wang J W 2012 Poult. Sci 91 2588
[19] Eichie FO 2018 Int. J. Biomed. Adv. Res. 9 96
[20] Deng M, Zhu F, Yang Y, Yang F, Hao J, Chen S and Hou Z 2019 BMC Genomics 20 1
[21] Zhang H, Shen L, Xu Z, Kramer L M, Yu J, Zhang X, Na W, Yang L, Cao Z, Luan P, Reecey J M and Li H 2020 Poult. Sci 99 2349
[22] Susanti R and Yuniastuti A 2020 J. Phys.: Conf. Ser. 1567 032042
[23] Susanti R and Yuniastuti A 2020 Biodiversitas 21 605
[24] Fan W, Liu W, Liu H, Meng Q, Xu Y, Guo Y, Wang B, Zhou Z and Hou S 2020 BMC Genomics 21 58
[25] Zhang Y, Li W and Wang D 2016 Pakistan J. Zool. 48 1459