

Identification of Polymorphism and Association Analyses of FMO3 Gene Related With Carcass and Meat Quality in Cihateup Duck

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Abstract. The aim of this study was to identify the association polymorphisms of the FMO3 gene related to carcass and meat quality in Indonesian Cihateup ducks. A total of 76 Indonesian Cihateup ducks were used in this study. Tissues from breast muscle were used for genomic DNA. Association analysis showed that the SNP g.849A>G was highly significantly associated ($P < 0.01$) with live weight (LW), carcass weight (CW), breast muscle weight (BMW), pH, cooking loss, drip loss (DL), lightness (L^*) and redness (a^*), TBARS and TMA. Compared to the GG genotype, the AG genotype exhibited greater levels ($P < 0.05$) LW, CW, pH, a^* , TBARS and TMA but not DL and L^* . These results will improve the understanding of the functions of the FMO3 gene in carcass and meat quality within the liver and will shed light on FMO3 as a candidate gene in the selection of ducks with good carcass and meat quality traits

Keywords: FMO3 gene, PCR-RFLP, carcass and meat quality, Indonesian duck

Abstrak. Tujuan dari penelitian ini untuk mengidentifikasi asosiasi dan ekspresi dari gen FMO3 terhadap sifat karkas dan kualitas daging pada Itik Cihateup. Total itik yang digunakan pada penelitian ini berjumlah 76 ekor. Jaringan otot dada dan hati digunakan masing-masing untuk analisis genomik DNA. Hasil asosiasi menunjukkan gen FMO3 pada titik mutasi SNP g.849A>G menunjukkan secara signifikan ($P < 0.01$) berhubungan dengan bobot hidup (LW), berat karkas (CW), berat otot dada (BMW), pH, susut masak, drip loss, kecerahan daging (L^*), tingkat warna merah (a^*), TBARS dan TMA. Apabila dibandingkan dengan genotip GG, genotip AG memiliki bobot komponen karkas dan kualitas daging lebih besar dalam hal LW, CW, pH, a^* , TBARS dan TMA namun lebih kecil untuk nilai drip loss dan L^* . Hasil studi ini memperjelas tentang mekanisme dan fungsi dan peran gen FMO3 terhadap sifat karkas dan kualitas daging itik serta peranannya FMO3 untuk dapat digunakan sebagai kandidat gen dalam memilih sifat karkas dan kualitas daging itik yang baik.

Kata kunci: Gen FMO3, PCR RFLP, sifat karkas, kualitas daging, itik cihateup

Introduction

Carcass and meat quality traits are economically important traits in duck. The ability to genetically select for animals that better suit a consumer's needs in terms of the amount of meat quality that can be predicted would benefit the farmers. Carcass and meat quality traits are important in animal breeding programs, and include live weight (LW), carcass weight (CW), pH, meat color (MC), drip loss (DL), and other traits. However, these traits are difficult to improve using conventional selection (Boukha et al. 2011). Moreover, their measurement is both expensive and difficult, and can only be conducted after slaughtered. Selection on the basis molecular genetic such as variant analysis single nucleotide polymorphism

(SNPs) of candidate gene approach has been already successfully applied to investigate several DNA markers (van der Steen et al. 2005). Identification of SNPs within genes influencing meat quality traits and their association and gene expression is the important and generally applied tools to characterize candidate genes. Therefore, it is very necessary to study the related gene mechanism regulating in carcass and meat quality in meat ducks.

One of the candidate genes that propose an important role in regulating in carcass and meat quality is flavin monooxygenase family member 3 (FMO3). The FMO family of enzymes converts lipophilic compounds into more polar metabolites and decreases the activity of these

compounds (Cashman, 2005). The FMO3 enzyme is the predominant enzyme in the adult human liver involved in the degradation of trimethylamine (TMA). FMO3 was reported to be downregulated in tissues with higher fat deposition and it could be speculated that the higher fat deposition may be caused by diminished FMO3 and related with fatty acid oxidation or increased adipogenesis of adipocytes in muscle (Wang et al. 2013).

Numerous studies have shown that FMO3 and other family of FMO gene plays an important role in regulating fishy off-flavor and taint pigs, cows, and chickens (Gunawan et al. 2013; Gleen et al., 2007; Mo et al. 2013; Honkatukia et al., 2005;), which are important references in the study of duck meat quality metabolism. Glenn et al. (2007) reported that FMO3 gene was located in a QTL region which associated with off-flavor in pigs . A nonsense mutation (R238X) of FMO3 gene was associated to underlie the fishy off-flavor in cow milk (Honkatukia et al. 2005). In chickens, a nonsynonymous mutation in the chicken FMO3 gene (T329S) has been investigated to be associated with elevated levels of TMA and fishy taint in the chicken egg yolk. However, no study has investigated the association study of these gene with regard to carcass and meat quality in duck. Notably , functional and positional studies have suggested that these genes could be important candidate genes for carcass and meat quality. Therefore, the aim of this research was to study FMO3 gene through association analysis to prove its candidacy for carcass and meat quality traits in ducks.

Materials and Methods

Animal and Phenotypes

Seventy Six Indonesian Cihateup ducks were used in this study. The ducks were reared under the same feeding conditions until they were 12 weeks old and had approximately 1.6 kg of slaughter weight per duck. The carcass and

meat quality data were collected according to the guidelines of the Indonesian performance test with the number 13 – 2016 IPB.

Carcass and Meat Quality Analysis

The carcass traits included carcass weight (CW), head weight (HW), wing weight (WW), neck weight (NW), breast muscle weight (BMW), leg muscle weight (LMW) were measured after slaughter. Carcass yield was calculated by dividing carcass weight (flesh plus bones) by live weight (by removing head, viscera and foot weights). Meat cut ratio of 5 five parts viz. wings, back, neck, breast and legs, was calculated by dividing it with live weight, and then ratio of each part was calculated. The breasts were placed in plastic bags and refrigerated (4°C to 6°C). At the laboratory, the samples were frozen at –18°C until they were analyzed.

Meat quality traits analyzed in this study covered drip loss, cooking loss, pH. Conductivity and pH-values were measured using Star-series equipment (Rudolf Matthaeus Company, Germany) in breast meats. Drip loss was scored based on a bag-method with a size-standardized sample from breast meats collected at 24 h p.m. that was weighed, suspended in a plastic bag, held at 4°C for 48 h, and thereafter re-weighed (Kayen et al. 2013). To determine cooking loss, a breast meats cube was taken from the muscle, weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 min. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion. The pH values of breast meats were measured 6 h post slaughter using a pH meter (Model 340, Mettler-Toledo, Greifensee, Switzerland). Briefly, 1 g of breast meat was cut into small pieces and homogenized with 9 mL of distilled water for 1 min in an Ultra-Turrax (Model No. T25, Janke and Kunkel, Staufen, Germany). Each sample was measured in triplicate and the mean values were used. Meat colour parameters (L*,

lightness; a^* , redness; and b^* , yellowness) were measured using a photoelectric spectrophotometer (CR-300, Minolta Camera Co.)

The meat samples were determined for level of pH using method of Van Lack et al.(2000). Cooking loss was measured referring to Bouton et al. (1971) method. Determination of drip loss was conducted by using Hamm (1972) method. While TMA analysis were according to AOAC (2005). Measurements of TBARS were done using procedure of Sorensen and Jogersen (1996).

DNA extraction , PCR reaction analysis of SNP

Single nucleotide polymorphism described in Wang et al.(2013) (SNP) in the duck FMO3 gene was genotyped in the Cihateup population. For PCR amplification, the primer was designed from the duck FMO3 genomic sequence using the Primer3 tool (Rozen and Skaletsky, 2000) (Table 2). For genotyping, genomic DNA was isolated from the breast muscle (BM) tissue of the Indonesian Cihateup ducks according to the standard phenol-chloroform method (Gunawan et al. 2011). *In silico* analysis of the genomic sequence, performed by comparing several sequences from a publicly available database (NCBI), revealed the possible targets for PCR amplification. A working solution with a final concentration of 50 ng/ μ l was prepared and stored at 4 °C for further analysis. Polymerase chain reactions (PCRs) were performed in a 20 μ l volume containing 2 μ l of genomic DNA, 1 \times PCR buffer (with 1.5 mM MgCl₂), 0.25 mM of dNTPs, 5 pM of each primer and 0.1 U of Taq DNA polymerase (GeneCraft). The genotyping of the Indonesian Cihateup duck population was performed by the PCR-RFLP method. The PCR product was analyzed using 1.5% agarose gel (Fischer Scientific Ltd.) and digested by using the restriction enzyme *A/wNI* for FMO3 (New England Biolabs). After digestion, PCR-RFLP products were resolved on a 3% agarose gel. The fragments were visualized under ultraviolet

light, and the sizes and number of the fragments were analyzed using molecular analyst software (Bio-Rad). PCR-RFLP patterns for FMO3 alleles were as follows: genotype AA resulted in two fragments (144 and 96 bp) while genotype GG resulted in one fragment (240 bp). The details of the PCR RFLP pattern, Genbank, accession numbers and primer sequences used in this study was described according to Anggraeni et al. (2017).

Statistical analysis

Genotype and allele were and the Hardy weinberg equilibrium of the SNP was performed by the Chi square test using SAS. The association analysis between genotype of FMO3 with carcass and meat quality were performed using the generalized linear model (PROC GLM) of SAS (version 9.2 SAS Inst Inc., Cary, USA) was used. The model was as follows:

$$Y_{ikl} = \mu + \text{genotype}_i + e_{ik}$$

Y_{ikl} is the carcass and meat quality; μ is the overall mean; genotype_i is the fixed effect of the i -th genotype ($i=1$ and 2); which is the combination of location and penning (group, individual); and e_{ik} is the residual error. Least-squares mean values for the loci genotypes were compared by t-tests, and p-values were adjusted by the Tukey-Kramer correction (Cinaret al. 2012 ;Kayan et al. 2011).

Results and Discussions

Phenotype of carcass and meat quality

Descriptive parameters for carcass and meat quality traits data are given in Table 1. For the studied population, the mean age was 12 weeks. Mean live weight (LW) of the ducks was 1472.73 g. The average Carcass weight (CW), breast weight (BW), wing weight (WW) and leg weight (LMW) were 938.28 ± 116.07 , 238.72 ± 51.51 , 132.42 ± 12.97 and 216.37 ± 28.29 , respectively (Table 1). This result is lower than most previous duck carcass measurement in the literature. Gan et al. (2015) obtained CW and LW in Cherry Valley duck and Liancheng white

were 1775.39 g and 1620.90 g. Zhang et al. (2013) reported CW at 8 weeks in Peking duck was 2737 g. Differences found among result are probably due to breed differences, selection pressure within population, sample size and environmental effect (Abdullah & Olutogun, 2006).

The drip loss, cooking loss, pH, redness and lightness and color of breast meat from Cihateup ducks are presented in Table 2. The average drip loss (DL), cooking loss (CL), pH, lightness (L*), redness (a*) and yellowness (b*) were 29.64 ± 1.83 %, 43.43 ± 2.95 %, 5.47 ± 0.14 , 39.03 ± 1.46 , 18.92 ± 1.44 and 3.19 ± 0.58 respectively (Table 2). We found that neither slaughter age nor slaughter weight affected the carcass and meat quality traits in the studied population. This result was in agreement which

reported by Gan et al. (2015) that have measured meat quality in chinese duck crossbreed. Gan et al. (2015) revealed pH, L*, a* and b* were 5.4; 35.29; 18.11 and 4.23 respectively.

It is necessary to characterize more precise carcass and meat quality standards for native breeds, including Indonesian Cihateup duck breed, to ensure their expanding consumption. The cooking loss of breast meat in this study was 43.43%, which showed higher compare to commercial meat-type ducks, Cherry Valley. Qiao et al. (2017) reported cooking loss in Cherry Valley was range 34.5% to 35.6%. Higher cooking loss in duck meat despite a higher proportion of oxidative fiber may be related to water holding capacity of duck (Ali et al. 2007).

Table 1. The average Carcass weight, breast, wing and leg weight

Component of carcass	Means \pm STD	Min	Max
Live weight (g)	1472.73 ± 120.17	1224	1775
Head weight (g)	84.22 ± 8.18	70	102
Neck weight (g)	81.83 ± 10.26	60	100
Shank weight (g)	48.08 ± 8.10	36	93
Carcass weight (g)	938.28 ± 116.07	709	1270
Carcass percentage (%)	63.64 ± 4.90	54.16	74.94
Breast weight (g)	238.72 ± 51.51	160	390
Breast percentage (%)	16.17 ± 2.98	9.26	24.90
Wing weight (g)	132.42 ± 12.97	110	178
Breast muscle (g)	197.33 ± 48.25	125	347
Leg weight (g)	216.37 ± 28.29	169	272
Leg muscle (g)	179.55 ± 25.74	128	233

Table 2. Traits and Quality Meat Duck

Traits	Means \pm STD	Min	Max
L*	39.03 ± 1.46	35.23	40.86
a*	18.92 ± 1.44	16.24	21.94
b*	3.19 ± 0.58	2.15	4.6
pH	5.47 ± 0.14	5.21	5.87
Cooking Loss	43.43 ± 2.95	38.12	50.27
Drip Loss	29.64 ± 1.83	26.21	33.43

L*= Lightness; a*=Redness; b*= Yellowness

Polymorphism Study of FMO3 gene

The SNP of FMO3 at g.849A>G was confirmed by PCR-RFLP. Figure 1. depicts results of FMO3 gene amplification of PCR method. Animals of this population were genotyped at g.849A>G in exon 1, which was the SNP segregating within Cihateup populations. In this study, two genotypes AG and GG were found with SNPs at g.849A>G in Cihateup population. The calculated genotype and allele frequencies of the FMO3 gene in the duck population are shown in Table 3. The homozygote GG was more frequent, and the heterozygote AG was

rare in our population. The chi-square test showed that the locus of FMO3 was in Hardy-Weinberg equilibrium in this Indonesian Cihateup duck population (Table 3). It is important to note that there is low allele frequency (0.06%) for A on FMO3 (Table 3).

The genotype segregated into 2 genotypes, we convinced that these associations are reliable. This SNP characterized by PCR-RFLP method were in agreement with previous reports (Kramer et al. 2003; Malek and Lamont 2003; Tohidi et al. 2012). Results of amplification of FMO3 gene using PCR-RFLP method is presented in Figure 2.

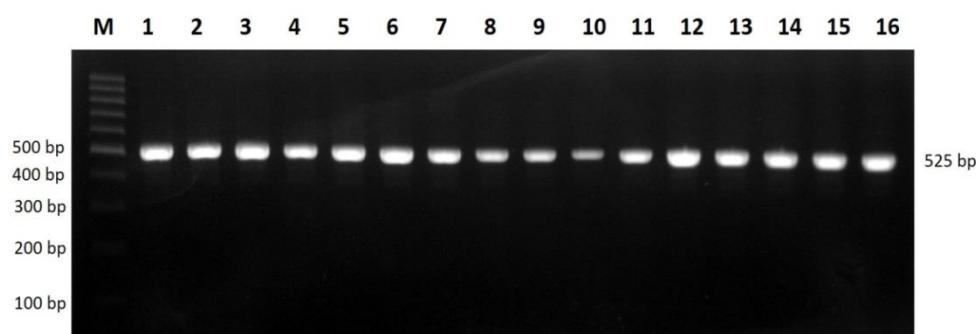


Figure 1. Amplification of FMO3 gene with the PCR method.

Table 3. Genotype, allele frequencies, and the chi-square test of FMO3 using RFLP

Sample	N	Genotype frequency			Allele frequency				χ^2
		AA	AG	GG	A	G	He	Ho	
Cihateup	60	0.000	0.12 (7)	0.88(51)	0.06	0.94	0.10	0.15	0.22

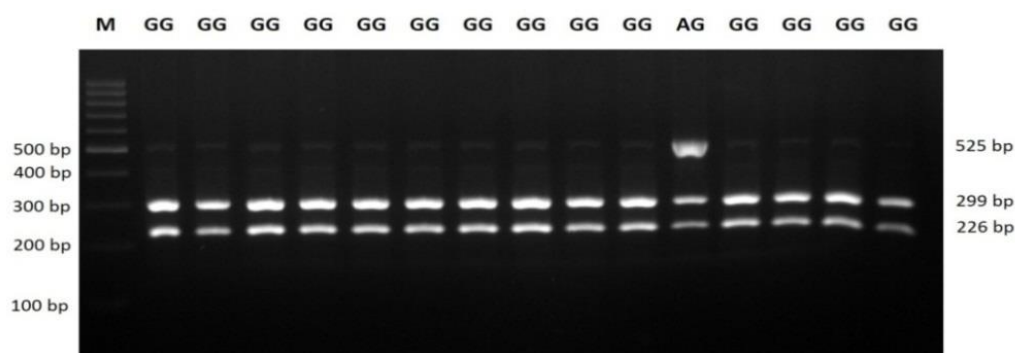


Figure 2. Results of FMO3 gene amplification using PCR-RFLP method.

Cihateup ducks populations were polymorphic with two alleles found (A and G). Nei and Kumar (2000) and Allendroft et al. (2013) mentioned that populations may be classified as polymorphic if there are more than one allele at the locus and if the most common allele frequencies are less than 0.99. The χ^2 analysis showed that Cihateup duck populations were deviated/in from Hardy-Weinberg equilibrium. Allendroft et al. (2013) explained that χ^2 is in Hardy-Weinberg equilibrium if the genetic variation, allele and genotype frequencies in a population remain constant from one generation to the next in the absence of disturbing factors

Association of FMO3 polymorphisms with carcass quality

In the present study, FMOg.849A SNP was significantly associated with carcass traits including live weight (LW), carcass weight (CW), carcass percentage (%), breast weight (BW),breast percentage (%) and breast muscle (g) (Table 4). The AG genotype exhibited the highest values for the carcass traits, which were significantly higher than the GG genotype for LW, CW, and were significantly higher than the GG and GA genotypes for BW (P < 0.05).

Considering the effect of the FMO3 gene, we were chosen to perform an association study with meat and carcass quality traits according to its physiological roles and chromosomal position that linked to several QTL for meat quality traits in in duck. The FMO family of enzymes converts lipophilic compounds into more polar metabolites and decreases the activity of these compounds (Cashman, 2005).The SNP of FMO3 was reported previously in pig, birds and mammals, but association study were performed with fishy and boar taint (Gunawan et al. 2013; Glenn et al. 2007; Honkatukia et al. 2005; Lunden et al. 2002). In pigs, the FMO3 gene was located in a QTL region correlated with off-flavor, which identified of an association between the FMO3 gene and off-flavor in pigs. Gunawan et al. (2013) reported association other FMOs family of FMO5 with androstenone levels (boar taint). A nonsynonymous mutation in the chicken FMO3 gene (T329S) was reported to be associated with elevated levels of TMA and fishy taint in the chicken egg yolk. In cattle, a nonsense mutation (R238X) in the cattle FMO3 gene was revealed to underlie fishy off-flavor in cow milk.

Table 4. Association of FMO3 polymorphisms with carcass traits

Component of carcass	Genotype	
	AG (7)	AA (69)
Live weight (g)	1575 ± 59.65a	1459.23 ± 119.92b
Head weight (g)	84.14 ± 7.08	84.23 ± 8.38
Neck weight (g)	86.57 ± 8.87	81.21 ± 10.34
Shank weight (g)	48.14 ± 6.20	48.08 ± 8.37
Carcass weight (g)	1076.71 ± 102.71a	920.00 ± 105.59b
Carcass percentage (%)	68.26 ± 4.32a	63.03 ± 4.67b
Breast weight(g)	309.86 ± 55.34a	229.32 ± 43.42b
Breast percentage (%)	20.06± 3.36a	15.95 ± 2.42b
Wing weight (g)	140.71±8.62	131.32±13.11
Breast muscle (g)	253.86±34.40a	189.87±44.92b
Leg weight(g)	229.14±33.67	214.68±27.43
Leg muscle (g)	182.43±28.75	179.17±25.59

Carcass and meat quality could be affected by LW (Galián et al. 2009; Choi et al. 2013), which is what we found in SNP g.849A>G. The quality of fresh meat can be controlled by the modification of muscle fiber characteristics, and that the IMF content is positively correlated with the amount of red muscle fiber but is negatively correlated with the amount of white muscle fiber (Joo et al. 2013). In this study, The GG genotype was associated with increased BM than AG genotype. It could be mentioned that there was differences between the BM, which may indicate that muscle fiber characteristics is also an important aspect of duck meat quality. Estimation of aerobic capacity data have recommended that the BM and LM are mainly composed of fast and slow muscle fibers, respectively, in poultry (Turner and Butler, 1988).

Association of FMO3 polymorphisms with meat quality

Association analysis of the g.849A>G SNP with meat quality revealed significant ($P < 0.01$) associations with pH, drip loss, lightness (L^*) redness (a^*), TBARS and TMA. Compared to the GG phenotype ($P < 0.05$), the genotype AG exhibited greater compositions of pH, redness (a^*), TBARS and TMA but not drip loss and lightness (L^*) (Table 5).

FMO3 is major member of the FMO family and is primarily present in the adult human liver, where it plays an important role in the metabolism of xenobiotic-containing TMA (Wang et al. 2013). TMA is the main compound

causing the human metabolic disorder TMAU, the fishy off-flavor in dairy milk and the fishy taint in chicken eggs. The genotype AG exhibited greater compositions of pH, redness (a^*), TBARS and TMA but not drip loss and lightness (L^*). The colour redness (a^*) differences found in this study might be explained by differences in Fe or haematin content. Furthermore, protein matrix microstructure, the chemical states of myoglobin, or oxygen diffusion might be other influencing factor that responsible for controlling the colour in meat quality (Sañudo et al. 1997; Swatland, 2012), The lightness (L^*) of meat quality was different between AG and GG genotype (Table 5). The discrepancy between this genotype, could be explained by differences in duck muscle composition or structure that affect light scattering, oxygen diffusion and/or myoglobin changes (Mateo et al. 2017). The TBARS of meat quality was different between AG and GG genotype (Table 5). These differences could be propertized to the concentration of muscle compounds that affect lipid oxidation, that is, released Fe from myoglobin, PUFA or tocopherols (Min and Ahn, 2005). TBARS is also considered as the acceptable oxidation level of raw meat quality before cooking (Campo et al. 2006). The higher expression of FMO3 in ducks with high carcass and meat quality (LW, CW, pH, redness (a^*), TBARS and TMA) suggested that FMO3 might be involved in regulating in carcass and meat quality traits.

Table5. Association of FMO3 polymorphisms with meat quality

Traits	AG (7)	AA (69)
L^*	38.20 ± 1.75	39.14 ± 1.41
a^*	20.90 ± 0.95a	18.66 ± 1.29b
b^*	3.31 ± 0.55	3.18 ± 0.59
pH	5.83 ± 0.10a	5.30 ± 0.23b
Cooking Loss	46.05 ± 3.51a	43.08 ± 2.72b
Drip Loss	28.60 ± 1.54	29.78 ± 1.84
TBARS	1.36 ± 0.24a	1.09 ± 0.22b
TMA	8.53 ± 0.55a	7.36 ± 1.09b

L^* = Lightness; a^* =Redness; b= Yellowness

Conclusions

This study demonstrated that polymorphisms in FMO3 at g.849A>G might contribute to the selection of carcass and meat quality traits in Indonesian Cihateup ducks. In general, the genotype AG was higher in carcass and meat quality with the characteristic (high LW, CW, pH, redness (a*), TBARS and TMA) in ducks. These results have implications for genomic selection because the FMO3 gene is associated with carcass and meat quality traits in ducks. However, the sample size used to determine the association was small, so validation in a larger sample size is necessary

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