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E-mail: editorijps@gmail.com

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Research Article
Potential Functional Variants for Fatness, Carcass and Meat Quality Traits in Exon 3 of Fat Mass and Obesity-Associated Gene in Indonesian Ducks

[1]Asep Gunawan, [2]Anggraeni, [1]Kasita Listyarini and [1]Cece Sumantri

[1]Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, 16680 Bogor, Indonesia
[2]Department of Animal Husbandry, Faculty of Agriculture, University of Djuanda, 16720 Bogor, Indonesia

Abstract

Background and Objective: The fat mass and obesity-associated gene (FTO) regulates glucose metabolism, body weight and fat content, it is also involved in DNA repair, fatty acid (FA) metabolism and post raslational modification. Due to these functions, FTO may affect FAs, carcass and meat quality in ducks. The objective of this study was to identify functional variants of FTO associated with fatness, carcass and meat quality and to investigate the tissue expression profile of the FTO gene in ducks. **Methodology:** Fifty-seven Indonesian Cihateup ducks were used in this study. Tissues from breast muscle and liver were used to evaluate genomic DNA and mRNA expression. Fatness traits, which include FA composition, carcass and meat quality, were evaluated at 12 weeks. **Results:** A SNP in exon 3 of the SCD gene was significantly associated with breast muscle for carcass traits and lauric acid (C12:0) for FA composition, however, there was no significant association with meat quality traits. To measure the mRNA expression of FTO, ducks were divide into three genotypes (AA, AG and GG). Compared to the AG and GG genotype, the AA genotype ducks had greater breast muscle weight and higher lauric acid levels (C12:0) for carcass and FA traits, respectively. FTO mRNA expression was significantly higher in genotype AA and GG ducks. **Conclusion:** The SNP of FTO in exon 3 is a functional SNP that regulates carcass and FAs specific for breast muscle weight and lauric acid levels in ducks.

Key words: Fat mass and obesity-associated gene, mRNA expression, fatty acids, carcass, ducks

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Corresponding Author: Asep Gunawan, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, 16680 Bogor, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The fat mass and obesity-associated (FTO) gene is an alpha-ketoglutarate-dependent dioxygenase and plays a role in glucose metabolism, body weight and fat content. FTO genes are also involved in DNA repair, fatty acid (FA) metabolism and posttranslational modifications[1]. A genome-wide association and chromosomal position indicated that the FTO genes is located in a QTL region for obesity[2,3] and plays an important role in growth and FA synthesis[4]. The FTO gene is highly expressed in the brain, especially in the hypothalamus[5]. In humans, several single nucleotide polymorphisms (SNPs) of the FTO gene are associated with body mass index that contribute to obesity and related diseases[6,7].

FTO plays a role in programmed cell death, limb development, craniofacial development and control of left-right asymmetry[8]. Simopoulos[2] reported that FTO variants are associated with a cellular phenotype consistent with obesity in primary human adipocytes, including decreased mitochondrial energy and increased triglyceride accumulation as well as activation of FA synthesis[2,9,10]. FTO over-expression enhanced lipogenesis in primary cultured human myotubes by increasing the expression of FAS and ACC, genes that play important roles in FA metabolism and reduce mitochondrial oxidative function[11] mRNA expression of FTO is closely connected to lipid metabolism[12,13]. FTO mRNA and protein levels are higher in the liver of rats with non-alcoholic fatty liver disease and FTO causes lipid overexpression in human hepatic cells. Furthermore, FTO may serve as a molecular link between inflammation, growth and lipid metabolism in the liver.

Several variant SNPs of the FTO gene are associated with growth and meat quality in farm animals and aquaculture species. Polymorphisms of the FTO gene are associated with backfat thickness and muscle area in indigenous Chinese cattle breeds[14]. An FTO SNP is significantly associated with backfat thickness, intramuscular fat, lean cuts and abdominal fat weight in pigs[15,16]. In the Asian seabass, FTO SNPs play an important role in growth and FA synthesis[4]. Functional and positional studies have suggested that FTO could be an important candidate gene for regulating fatness traits, such as FAS and growth traits like carcass and meat quality.

Carcass, meat quality and FA composition are economially important phenotypes in ducks. The ability to genetically select for animals with higher meat quality as well as those that contain high levels or unsaturated FAs and low saturated FAs to benefit human health, to meet customer needs would benefit the industry. FAs, which includes polyunsaturated fatty acids (PUFAs) and monounsaturated FAs (MUFAs), play an important role in meat quality, especially in nutrition and flavor[17,18]. High proportions of PUFAs and MUFAs can increase levels of hepatic low-density lipoprotein (LDL), which reduce cholesterol levels[19]. In contrast, the composition and total amount of saturated FAs (SFAs) have been associated with coronary heart disease[20].

A candidate gene approach can accelerate genetic improvement of economically important traits through DNA markers[21,22]. SNP detection within genes affecting carcass, meat quality and FA composition and their gene expression is a widely used tool to characterize candidate genes. Due to its role in glucose metabolism, body weight and fat content, FTO is a candidate gene for carcass, meat quality and FA traits in ducks.

Indonesian local ducks are of the laying type and are similar to the Indian runner, which live mostly in Sumatra, Java, Bali, West Nusa Tenggara and Kalimantan[23]. The Cihateup duck is a local Indonesian duck, which originated from West Java and is raised for layer or meat. Muzani *et al.*[24] reported that Cihateup male ducks at 8 weeks of age can produce a cut carcass weight of 1323.87 g with 812.13 g (61.36%)[24]. The fleshy parts of Cihateup ducks (thigh and chest) can reach 27.14 and 24.97% of body weight, thigh weight of Cihateup ducks is greater than that of Alabio ducks (25.22%)[25].

To our knowledge, variant analysis and functional study through mRNA expression of the FTO gene has not been evaluated to determine associations with carcass, meat quality and FA traits. The aim of this study was to identify variants of FTO associated with carcass, meat quality and FA traits in Indonesian Cihateup ducks. Furthermore, differential mRNA expression in the FTO gene was measured in the liver across genotypes.

MATERIALS AND METHODS

Animals and phenotypes: This study was conducted using 57 Indonesian Cihateup ducks. The ducks were reared under the same environment conditions in the Field Laboratory of Poultry, Department Animal Production and Technology, at Bogor Agricultural University and were slaughtered at 12 weeks. Carcass and meat quality data were collected according to the guidelines of the Indonesian performance test with the number 13-2016 IPB[26]. Tissues from breast muscles were used for genomic DNA isolation, carcass, meat quality and FA analysis.

Carcass and meat quality analysis: Carcass weight (CW), head weight (HW), wing weight (WW), neck weight (NW), breast muscle weight (BMW) and 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). A meat cut ratio of five parts (wings, back, neck, breast and legs), was calculated by dividing with live weight. The breasts were placed in plastic bags and refrigerated (4-6°C). At the laboratory, samples were frozen at -18°C until analysis.

Meat quality traits included drip loss, cooking loss and pH were measured. Conductivity and pH-values were analysed using Star-series equipment (Rudolf Matthaeus Company, Germany) in breast meats. Drip loss was scored according to a bag-method with a size-standardized sample from breast meats collected at 24 h that was weighed, suspended in a plastic bag, held at 4°C for 48 h and then re-weighed[27]. To measure cooking loss, breast meat cube was taken from the muscle, weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 min. The pH values of breast meat were analyzed 6 h post slaughter using a pH meter (Model 340, Mettler-Toledo, Greifensee, Switzerland). Meat color parameters (L*, lightness, a*, redness, b*, yellowness) were measured using a photoelectric spectrocolorimeter (CR-300, Minolta Camera Co.) (Table 1).

Fatty acids analysis: Fourty-two Indonesian Cihateup ducks were used for FA analysis. Muscle samples of approximately 100 g were collected and content was measured for each sample using the extraction method as previously described by Folch *et al.*[28]. FA composition was quantified using gas chromatography (GC-2010 GC-2010 Plus-Shimadzu AOC 201 autoinjector). The FAs were expressed as a percentage of the total FAs, including fat content, SFAs, MUFAs and PUFAs as described by Anggraeni *et al.*[26] (Table 2).

Table 1: Descriptive statistics of carcass characteristics

Traits	N	Mean	SE	Minimum	Maximum
Body weight (g)	57	1474.200	15.70	1224.00	1775.00
Head (g)	57	85.370	1.05	70.00	102.00
Neck (g)	57	82.770	1.31	62.00	100.00
Shank (g)	57	49.000	1.12	36.00	93.00
Carcass traits					
Carcass weight (g)	57	928.200	15.70	709.00	1270.00
Wing (g)	57	132.300	1.74	110.00	178.00
Breast (g)	57	239.840	6.91	130.00	390.00
Foot (g)	57	230.300	3.66	179.00	296.00
Carcass (%)	57	25.060	0.46	20.08	36.57
L color	57	39.124	0.18	35.28	40.86
A color	57	18.815	0.18	16.24	21.94
Meat quality					
B color	57	3.191	0.07	2.15	4.60
pH	57	5.496	0.02	5.00	5.80
SM	57	48.046	0.58	35.00	56.94
H_2O (%)	57	27.336	0.33	21.35	33.43

Table 2: Descriptive statistics of fatty acid composition

Traits	N	Mean	SE	Minimum	Maximum
Lauric acid (C12:0)	42	0.076	0.006	0.039	0.264
Myristic acid (C14:0)	42	0.520	0.007	0.415	0.618
Pentadecanoic acid (C15:0)	42	13.390	2.190	0.040	30.740
Palmitic acid (C16:0)	42	12.050	1.820	0.040	28.530
Palmitoleic acid (C16:1)	42	0.133	0.015	0.007	0.620
Heptadecanoic acid (C17:0)	42	2.097	0.331	0.077	5.040
Stearic acid (C18:0)	42	2.472	0.359	0.080	6.615
Elaidic acid (C18:1n9t)	42	0.118	0.005	0.064	0.221
Oleic acid (C18:1n9c)	42	43.873	0.371	38.083	48.994
Linoleic acid (C18:2n6c)	42	18.377	0.204	16.083	21.992
Paullinic acid (C20:1)	42	0.735	0.020	0.443	1.005
Eicosadienoic acid (C20:2)	42	0.175	0.009	0.098	0.295
Arachidonic acid (C20:4n6)	42	0.226	0.009	0.067	0.361
SFA	42	30.742	0.582	22.569	36.629
MUFA	42	46.837	0.403	41.112	53.168
PUFA	42	18.490	0.209	16.423	22.359

Table 3: GenBank accession numbers and primer sequences

Gene names	Accession number	Primer sequence	Application	Size (bp)	Ta (°C)	Enzyme
FTO	NW_004676795.1	F: 5'-TTT ACC CCA GTG TCT CGT AT-3' R: 5'-GAC TCA AGC AAA TTT TGT CC -3'	Genotyping	215	60	NlaIII
FTO	XM_021270536.1	F: 5'-GCC TAA GAA CAT GAA TCT GC-3' R: 5'-ATG GCT ACA TGT TGG AAA TC-3'	qRT-PCR	164	60	
GAPDH	XM_005016745.3	F:5' TCC TCA TCT GCA TCT CTT TT 3' R: 5' CAT TCC CGT TAA TCA CAA GT3'	qRT-PCR	217	60	

Polymorphism study: As a SNP, the arginine (A) to guanine (G) trasversion of FTO at g. 387 G>A in exon 3 reported by Gan *et al.*[29] was further investigated in this study. For PCR amplification, the forward and reverse primer pairs were designed covering exon 3 of ducks FTO genomic sequence using the primer 3 tool (Table 3)[30]. PCR was performed in a 20 µL volume containing 2 µL of genomic DNA, 1×PCR buffer (with 1.5 mM $MgCl_2$), 0.25 mM of dNTP, 5 pM of each primer and 0.1 U of Taq DNA polymerase (GeneCraft). Genotyping of the Indonesian Cihateup duck population was performed using the PCR-RFLP method. PCR product was analyzed using 1.5% agarose gel (Fischer Scientific Ltd) and digested by using the restriction enzymes of NlaIII for FTO (New England Biolabs). Digested PCR-RFLP products were resolved in 2% agarose gels. The details of PCR-RFLP pattern, GenBank accession numbers and primer sequences used in this study are listed in Table 3.

mRNA expression of the FTO gene: Sample tissues from nine duck livers were isolated for mRNA analysis based on genotype results. The nine ducks were divided into three groups (AA, AG and GG genotypes) and differential gene expression was performed using PROC GLM test with SAS. For the qRT-PCR analysis, cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen). Gene-specific primers for FTO for the qRT-PCR were designed using Primer3 software (Table 3) [30]. For each run, the 96-well microtiter plate contained each cDNA sample and no-template control. The qRT-PCR was performed according to Gunawan *et al.*[31,32]. The housekeeping gene GAPDH was used as reference for the target genes (Table 3).

Statistical analysis: Association analyses of the FTO gene were performed using the General Linear Model (GLM) using SAS. 9.2 software. The model of GLM was as follows:

$$Yijkl = \mu+genotypei+sexj+eijk$$

where, Yijkl is the carcass, meat quality and FA traits, µ is overall mean, genotype i is the fixed effect of i-th genotype (i = 1, 2 and 3), sex j is the fixed effect of j-th sex (j = male/female), which is the combination of location and penning (group, individual), eijk is the residual error.

Least square mean values for the loci genotypes were compared by t-test and $p<0.05$ values were adjusted using the Tukey-Kramer correction[33,34].

Gene expression study of FTO by qRT-PCR: The delta Ct (Ct) method was used to calculate the difference between target genes and reference genes ($Ct = Ct_{target}-Ct_{housekeeping\ genes}$) as described previously[35]. The results were quantified as fold change calculated from delta Ct-values. The differences between phenotypes in high and low carcass, meat quality and FA traits of FTO gene expressions were compared using t-tests. The $p<0.05$ was considered statistically significant.

RESULTS

SNP identification and genotyping: A synonymous FTO SNP at g. 387 G>A was confirmed in exon 3 in Indonesian Cihateup duck population (Fig. 1). The DNA restriction fragments obtained for the FTO-NlaIII polymorphism was 215 bp for the AA genotype, 215, 130 and 85 bp for the AG genotype, 130 bp and 85 bp for the GG genotype (Fig. 2, Table 4). The homozygote GG and heterozygote AG genotypes were more frequent, the AA genotype was rare in this population. The genotype frequency of polymorphism in FTO SNP at g. 387 G>A deviated from the Hardy Weinberg Equilibrium ($p>0.05$) (Table 4).

Associations between SNP in exon 3 of FTO with carcass and meat quality traits: Analysis of FTO g. 387 G>A with carcass and meat quality traits revealed significant associations with breast muscle weight ($p<0.05$). Genotype AA had significantly higher breast muscle weight than genptypes AG and GG (Table 5). The FTO gene was not significantly associated with all meat quality traits [pH, cooking loss, drip loss, lightness (L*), redness (a*) and yellowness (b*)].

Associations between SNP in exon 3 of FTO with fatness traits: Analysis of FTO g. 387 G>A with FA composition showed significant associations with lauric acid levels (C12:0)

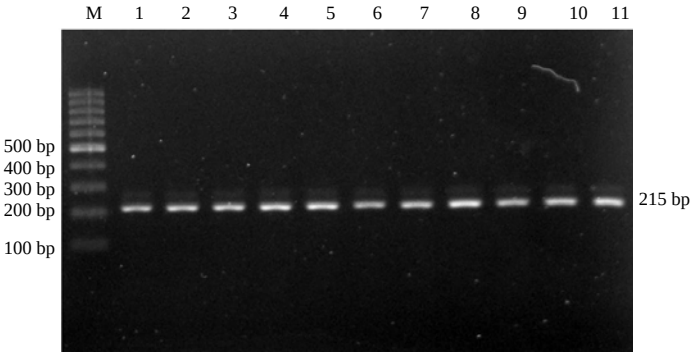

Fig. 1: PCR product for the FTO gene

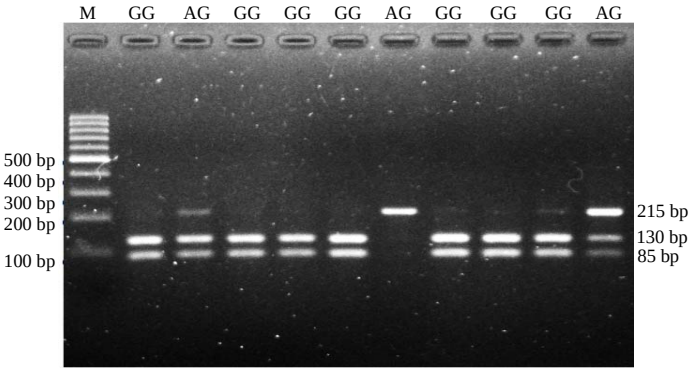

Fig. 2: PCR-RFLP genotyping results for the FTO gene

Table 4: Number of animals per genotype and allele frequency

		Genotype frequency			Allele frequency		
Polymorphism	N	AA	AG	GG	A	G	χ^2
FTO	57	0.05 (3)	0.42 (24)	0.53 (30)	0.26	0.74	0.418*

*Significantly different (χ^2 0.05 = 3.841), χ^2 = Chi-square

Table 5: Genotype and association analysis of the FTO gene with carcass characteristics

	Genotype (μ±SE)		
Traits	AA (n = 3)	AG (n = 24)	GG (n = 30)
Carcass traits			
Body weight (g)	1478.00±49.30	1478.40±21.2	1470.40±24.40
Head (g)	77.67±3.84	86.58±1.47	85.17±1.54
Neck (g)	76.00±5.51	83.50±2.16	82.87±1.73
Shank (g)	43.33±3.84	48.67±1.27	49.83±1.82
Carcass weight (g)	940.00±40.00	935.90±24.40	920.90±22.50
Wing (g)	129.00±8.62	132.58±2.31	132.40±2.68
Breast (g)	312.30±20.80*	238.60±10.70*	233.60±8.97*
Foot (g)	243.30±13.00	227.96±5.79	230.87±5.09
Carcass (%)	25.96±1.55	24.54±0.64	25.38±0.72
Meat quality traits			
L color	39.94±0.27	39.15±0.27	39.02±0.26
A color	19.29±0.61	18.48±0.28	19.01±0.25
B color	2.62±0.25	3.28±0.11	3.17±0.11
pH	5.59±0.09	5.51±0.02	5.47±0.03
Cooking loss	48.66±3.33	48.07±1.07	47.96±0.68
Drip loss	28.78±1.05	27.26±0.45	27.24±0.51

*p<0.05

Table 6: Genotype and association analysis of the FTO gene with fatty acid composition

Traits	Genotypes		
	AA (n = 3)	AG (n = 17)	GG (n = 22)
Lauric acid (C12:0)	0.124±0.070*	0.072±0.007*	0.073±0.007*
Myristic acid (C14:0)	0.547±0.029	0.511±0.011	0.523±0.009
Pentadecanoic acid (C15:0)	9.670±9.630	15.010±3.540	12.650±3.020
Palmitic acid (C16:0)	15.010±7.560	10.940±2.940	12.510±2.540
Palmitoleic acid (C16:1)	2.409±0.342	1.911±0.131	2.224±0.082
Heptadecanoic acid (C17:0)	1.520±1.410	2.347±0.532	1.981±0.460
Stearic acid (C18:0)	3.290±1.540	2.210±0.564	2.562±0.506
Elaidic acid (C18:1n9t)	0.115±0.009	0.118±0.009	0.119±0.007
Oleic acid (C18:1n9c)	45.010±1.420	44.481±0.571	43.247±0.506
Linoleic acid (C18:2n6c)	18.856±0.588	18.389±0.317	18.302±0.300
Paullinic acid (C20:1)	0.632±0.099	0.723±0.026	0.759±0.029
Eicosadienoic acid (C20:2)	0.212±0.044	0.155±0.010	0.185±0.010
Arachidonic acid (C20:4n6)	10.330±2.840	12.850±2.450	12.850±2.450
SFA	30.320±2.340	31.227±0.943	30.424±0.809
MUFA	48.170±1.830	47.233±0.658	46.350±0.526
PUFA	19.364±0.594	18.692±0.347	18.215±0.277

*p<0.05

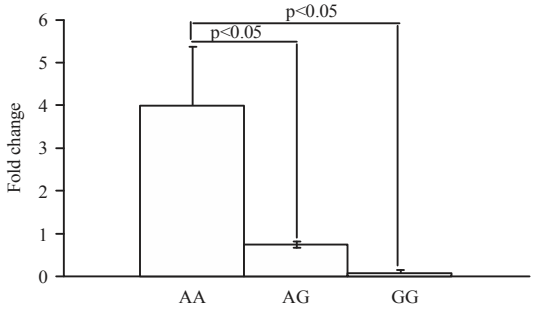

Fig. 3: mRNA expression of the FTO gene

(p<0.05) (Table 6). Genotype AA had higher lauric acid (C12:0) levels than genotypes AG and G, this association should be confirmed and validated in a larger population.

mRNA expression of FTO in different carcass and fatness traits: Since the association study revealed that the AA genotype had relatively greater breast muscle and lauric acid levels (C12:0) compared to AG and GG genotype, we measured mRNA expression using qRT-PCR and found that the FTO mRNA was differentially expressed in genotype AA (high breast muscle weight and high lauric acid) and genotypes AG and GG genotype (low breast muscle weight and low lauric acid) in the muscle (p<0.05) (Fig. 3). Higher mRNA expression was detected in the muscles of genotype AA (p<0.05).

DISCUSSION

This study revealed an association between FTO and carcass traits (breast muscle weight) and fatness traits (lauric acid (C12:0)) in Indonesian Cihateup ducks. These results indicate that FTO variants can be used as a candidate gene. Several FTO variants are significantly associated with growth and meat quality in livestock. In cattle, FTO variants are associated with body weight, average daily gain (ADG) and hot carcass weight in crossbreed beef cattle[36]. Additionally, FTO gene expression is associated with backfat thickness and muscle area in Chinese indigenous cattle breeds[14]. Fontanesi *et al.*[37] showed that the FTO SNP in intron 4 is associated with intramuscular fat deposition in Italian Duroc pigs. In the Cherry valley duck×Liancheung white duck mix, genotype AA is associated with greater leg muscle weight compared to genotypes GG and AG[29]. In addition, SNPs of FTOs are also associated with growth, oil content, omega-3 content and omega-3/6 ratio in the Asian Seabass[4].

Present study showed that FTO variants in exon 3 had a significant effect on carcass traits and FA composition. To our best knowledge, this is the first study that has demonstrated an association between FTO in exon regions with carcass and FA traits in ducks. To further analyze the association between the FTO gene with carcass and fatness traits, mRNA expression levels were measured in liver tissues collected from AA, AG and GG genotypes. Higher FTO mRNA expression was detected in ducks with greater breast muscle and higher lauric acid levels (C12:0). In addition, there was significant up-regulation of FTO in the AA genotype compared to AG and GG genotype groups. Several studies have reported that FTO SNPs are associated with fatness traits in pig populations[38-40]. Furthermore, Xia *et al.*[41] found 53 suggestive QTLs for FA traits close to the FTO gene position. These findings are in agreement with our SNP and suggest that lauric acid could be used as a marker to accelerate genetic improvement of these traits.

The differences between breast muscle weight suggested that muscle fiber characteristics are an important component

of duck meat quality. Estimation of aerobic capacity data has suggested that breast and leg muscle are mainly composed of fast and slow muscle fibers, respectively, in poultry[42]. The quality of fresh meat can be determined by the modification of muscle fiber characteristics and the IMF content is positively correlated with the amount of red muscle fiber but is negatively correlated with the amount of white muscle fiber[43]. Li *et al*.[44] reported that the development of the breast muscle always lags behind that of the leg muscle in embryonic stages of the Peking duck. Therefore, the different effects of FTO polymorphisms on carcass traits between the breast muscle and the leg muscle could be correlated with their different physiological characteristic and anatomical positions.

Consumption of meat with SFAs like lauric acid (C12: 0) can also result in insulin resistance, increased cholesterol production or hyperinsulinemia in the human liver[45].The HDL cholesterol increasing effect of lauric and myristic acids strongly influences the LDL/HDL cholesterol ratio, elevated plasma cholesterol contributes to cardiovascular disease[46,47]. MUFAs are more effective at decrease blood cholesterol levels than PUFAs[45], thus, consumption of SFAs should be limited between 0-10%, with 16% MUFAs and 7% PUFAs, cholesterol intake should not be more 300 mg per day[48]. Furthermore, consumption of SFAs, trans MUFAs and cholesterol exceeding normal requirements can increase body weight, contributing to cardiovascular disease, atherosclerosis and other conditions[48].

Greater breast muscle meat in Cihateup ducks is beneficial for human health because it contains lower saturated FA levels. Therefore, the FTO gene could be used for rapid selection in breeding to improve meat quality and produce greater breast muscles of ducks in farm and industry. However, this study must be validated in other animal populations to evaluate its potential in selective breeding.

CONCLUSION

FTO might be an important candidate gene to select for carcass and FA composition, especially for breast muscle and lauric acid (C12:0). However, this study must be validated in other animal populations to evaluate its potential for selective breeding.

SIGNIFICANCE STATEMENT

The present study describes, for the first time, the expression of FTO gene polymorphisms and their association with fatness, carcass and meat quality traits in Cihateup ducks, implicating FTO as an important candidate gene for selection of ducks with greater breast muscle and low saturated fatty acids.

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