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Association of Chicken Fatty Acid Desaturase 1 and 2 Gene Single-Nucleotide Polymorphisms with the Fatty Acid Composition of Thigh Meat in Japanese Hinai-dori Crossbred Chickens

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Abstract

Hinai-jidori chicken, a cross between Hinai-dori (a breed native to Akita Prefecture, Japan) sires and Rhode Island Red dams, is a popular chicken brand in Japan. We previously reported that a high arachidonic acid (ARA) content is a characteristic feature of Hinai-jidori chicken and demonstrated that chicken meat with higher ARA contents had a much better taste perception than that with low ARA contents using Hinai-jidori and broiler chickens. To investigate the genes affecting fatty acid profiles, including ARA, in Hinai-jidori chicken, we genotyped polymorphisms of the *fatty acid desaturase 1 and 2* (*FADS1* and *FADS2*) genes and investigated their association with the fatty acid profile in Hinai-jidori meat. 5′-flanking regions, all the exons, and 3′-untranslated regions of the *FADS1* and *FADS2* genes in three chicken breeds, i.e., Hinai-dori, Rhode Island Red, and White Plymouth Rock, were amplified via PCR, after which their nucleotides were sequenced and SNPs were identified. Of the 71 and 46 SNPs found in the *FADS1* and *FADS2* genes, respectively, two SNPs were chosen from each gene, and their associations with fatty acid profiles of Hinai-jidori meat were analyzed. Hinai-jidori female chickens hatched on the same day and reared under identical environmental conditions for the same duration were used in this study. In each SNP of *FADS1* and *FADS2*, the ARA and docosahexaenoic acid (DHA) compositions were significantly higher in the G than in the A allele, respectively. Moreover, an association of *FADS1* and *FADS2* haplotypes with the fatty acid composition was observed. For example, the ARA and DHA composition of the G-G-haplotype were significantly higher than those of the A-A-haplotype. Thus, we concluded that SNPs in the *FADS1* and *FADS2* gene cluster are useful to increase ARA and DHA, and can be used to develop strategies for improving the taste of Hinai-jidori chicken.

Keywords: Hinai-jidori chicken; Marker gene; *Fatty acid desaturase 1*; *Fatty acid desaturase 2*; Fatty acid profile

Introduction

Globally, most chicken meat is obtained from limited fast-growing broiler strains provided by commercial breeding companies that use intensive fatting systems to ensure high meat yields. Meanwhile, some consumers are willing to pay a high selling price for better quality chicken meat, known as "Jidori" chicken in Japan. Most Jidori chickens were initially bred by crossing native Japanese breeds with highly selected lines with rapid growth. Since Jidori chickens require a relatively long growing time at a considerably high production cost, their selling price can be 2-5 times more than that of broilers. The Hinai-jidori chicken, a cross between Hinai-dori (a chicken breed native to the Akita Prefecture of Japan) sires and Rhode Island Red dams, is a popular brand of Jidori chicken in Japan [1].

A sensory evaluation report showed increased palatability of the Hinai-jidori chicken over broiler chickens [2]. Past studies showed that free amino acid (FAA) contents, including glutamic acid (Glu) [3] and inosine 5′-monophosphate (IMP) [4] could be correlated with chicken meat palatability. For example, Matsuishi et al. [3] reported that chicken soup made from broiler chicken is more palatable than that from a Jidori chicken brand (Nagoya Cochin), suggesting that it reflects the high FAA content of broiler meat; however, most Japanese consumers recognize that Jidori meat is more palatable than broiler meat. These authors removed the fat from the chicken soup and then subjected the soup to a sensory evaluation; thus, we speculated that fat contains key substances. To define candidate substances related to chicken meat palatability, we reared Hinai-jidori and broiler chickens under identical environmental and time conditions, and then compared the meat quality traits, e.g., FAA and IMP content and fatty acid composition, of their thigh meat. We concluded that high arachidonic acid (ARA, C20:4n-6) and docosahexaenoic acid (DHA, C22:6n-3) content is characteristic of Hinai-jidori chicken meat [2]. Then, we demonstrated that ARA content in chicken meat could be manipulated by an ARA diet supplement in Hinai-jidori [5] and broilers [6], and chicken meat containing higher levels of ARA tasted much better than that containing low ARA contents. Koriyama et al. [7] reported that DHA suppressed sourness and bitterness, but increased sweetness and umami tastes. These data suggest that ARA and DHA are fundamental for the taste perception of chicken meat.

ARA originates from both dietary sources and the elongation-desaturation process of its precursor, linoleic acid (LA, C18:2n-6). The δ-5 (D5D) and δ-6 (D6D) desaturases are key enzymes involved in this pathway (Figure 1) [8]. D6D catalyzes the conversion of LA to γ-linolenic acid (GLA, C18:3n-6), which is then elongated to dihomo-

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γ-linolenic acid (DGLA, C20:3n-6) by elongases (Figure 1). In turn, C18:3n-6 is desaturated to ARA by D5D. D6D, D5D, and elongases are also involved in the n-3 fatty acid pathway (Figure 1), which favors the conversion of α-linolenic acid (ALA, C18:3n-3) into DHA. D5D and D6D are encoded by fatty acid desaturase 1 and 2 genes (*FADS1* and *FADS2*), respectively. The *FADS1* and *FADS2* genes are clustered in a back-to-back direction on chicken chromosome 5 [9,10]. Therefore, we speculated that *FADS1* and *FADS2* are the key genes that control both ARA and DHA in chicken meat.

Our main objective in this study was to analyze the polymorphism of the *FADS1* and *FADS2* genes and test its association with the fatty acid profiles of Hinai-jidori chickens to effectively understand why Hinai-jidori meat has high ARA and DHA contents.

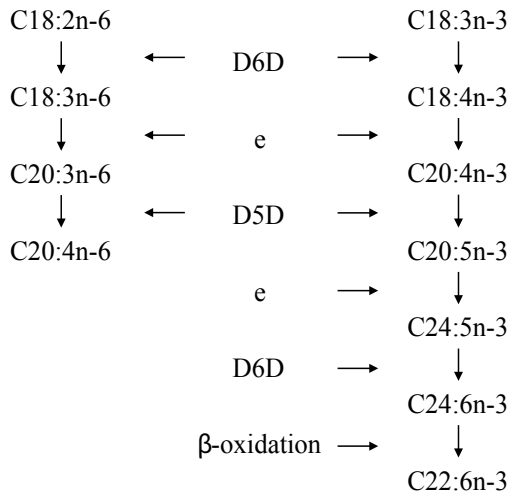

Figure 1. Synthetic pathway of long chain unsaturated fatty acids. D6D: δ-6 desaturase; D5D: δ6 desaturase; e: elongases

Materials and Methods

Identification of DNA polymorphisms of *FADS1* and *FADS2*

A draft sequence of the chicken genome, available in established databases [9,10], was used in the present study. To detect DNA polymorphisms of *FADS1* and *FADS2*, unrelated chickens belonging three breeds, i.e. Hinai-dori (3 individuals), Rhode Island Red (3), and White Plymouth Rock (3), were used. Hinai-dori and Rhode Island Red breeds were used, since they are the parents of Hinai-jidori chickens. White Plymouth Rock, which is a founder of broiler chickens, was used as an outlier breed.

The blood samples were collected from the ulnar vein. Genomic DNA was purified from blood using the SepaGene kit (EIDIA, Tokyo, Japan). The nucleotide sequences of the regulatory regions (promoters and 5′ and 3′ UTRs) and twelve exons each of *FADS1* and *FADS2* in the nine individuals were determined by polymerase chain reaction (PCR) amplification followed by direct sequencing using the same procedure as described previously [11]. Primers for the PCR and direct sequencing were listed in Table 1. The DNA sequences of the *FADS1* and *FADS2* genes were analyzed using the GENETYX program (Software Development Co., Tokyo, Japan) and DNA polymorphisms were identified. Linkage disequilibrium (LD) block analysis and haplotype estimation were performed using Haploview software [12].

Hinai-jidori chicken samples

Unrelated female Hinai-jidori chickens (32 individuals) were raised in the Akita Prefectural Livestock Experiment Station. The chicks hatched on the same day were housed in an open-sided poultry shed and given access to a grass paddock until 22 wk of age. Chicks were fed a starter diet (ME, 3,000 kcal/kg; CP, 24% [wt/wt]) from 0 to 4 wk, grower diet (ME, 2,850 kcal/kg; CP, 18%) from 5 to 10 wk,

Set	Locus	Forward (5′→3′)	Reverse (5′→3′)	Product (bp)	Annealing temperature (°C)
FADS1 gene					
1	5′ UTR and Exon 1	GCGGGCCAATGGGCGTGGAG	TTCCTTACGGAGCGCGCAGCTGA	458	68
2	Exon 2	GCAGCAATATCAGATCCTGCCAA	TTGGGTTTGAGAAGCCCCATCT	651	60
3	Exon 3	AGTCAGCTAAGAAAGTATCCCGGAA	AGCATGAAGCCTGCTCTACCAA	587	61
4	Exon 4	AAGCAAAGGCTCCTAGCTCTTCT	GAGGCAGAAATGAGAATACAGTGCC	332	61
5	Exon 5	CTGTTCTCCTGGGTAACTGTG	CCAAACCAACTGGTCTCTTGT	501	57
6	Exons 6 and 7	CACATCCAAGGCAGGGAGAA	CCACCAAACATTCTCTCCCTGA	634	59
7	Exon 8	GGTGTGATGTGGTTGTCCAG	CAGACGGAAAAGATAACCAGGAG	647	58
8	Exon 9	ACAAGTGCTTTGTACTGACTCGTT	GCTGCTGTGATCAGCTCTCTTG	251	60
9	Exon 10	GTTGTGTCTGACTCGTGTAAGAGAA	GTACCTAATCTCAGGAGGCACATAG	503	60
10	Exon 11	GTAGGGGAACTCTGCAAGGCAA	CTCTACGTCCCTTGCTTGTTCACTC	257	62
11	Exon 12 and 3′ UTR	CTCTCTTCTACCACGCTTGCTC	TTCATCACTGGAATTAAGCTGTGTC	447	59
FADS2 gene					
12	5′ UTR and Exon 1	CGTGCCGTCGGGGCGAGGGT	GCGTGCTCCCCGGCATGCCCTAA	930	71
13	Exon 2	AATTGGAAGGGGCTCTTAAAGGCCA	GGATCCCTATTGCTCCTACCGCTT	594	64
14	Exon 3	TGGTGTAGCCAAACAAAGCAAGA	GAAGGAAAGGCACGGGAGATAAG	516	60
15	Exons 4 and 5	TGTCTATTTTCTTTCATGCTCAACT	TCTTAGCACTCTTGTAAGCGG	642	56
16	Exon 6	AATACAAAGAAGCTGTCAGCATCA	CCAGAGGTTACTTCCCAGTCTC	554	58
17	Exons 7 and 8	AGCACATCACTTCTTACACCA	ATAAAACAACACAGTGTGGCAAA	621	56
18	Exon 9	GGGATAATTGCATTAGTCCAG	GTCTTATCCAACCTTAACGATT	530	54
19	Exon 10	TAAAGCTTCCCATGCTGCAGT	GAGAAGGTGTTAGGCAATCTCGT	475	60
20	Exon 11	CAGCAGGAGAATCGACGTATTC	GTAGTGACACCAGATTACAAAACAC	468	58
21	Exon 12 and 3′ UTR	CTCAGACTGAGTAACAGAGTTCTCC	CATTTGCGGTTACACGCGATT	666	59

Table 1: Primers for chicken fatty acid desaturase 1 (*FADS1*) and fatty acid desaturase 2 (*FADS2*) sequencing.

and finisher diet (ME, 2,900 kcal/kg; CP, 16%) from 11 to 22 wk; the diets were specially prepared for Hinai-jidori chickens (Kitanihon Kumiai Feed Co., Sendai, Japan). Water and feed was provided and libitum for the duration of the experiment. All animals received human care as outlined in the Guidelines for Proper Conduct of Animal Experiments [13].

At 22 wk of age, the chickens were fasted for 18 h, and then slaughtered. The chickens were bled and plucked; their carcasses were manually eviscerated and washed, followed by immediate cooling in ice-cold water until a temperature of 8°C was reached. They were then removed from the water and drained for 10 min. Carcasses were dissected and the thigh meat was deboned after skin removal; the thigh meat from one leg was minced using a domestic meat chopper (No.5-A, Veritas, Tokyo, Japan). Meat samples were stored at -30°C until further analysis.

Determination of fatty acid composition of the thigh meat

To determine fatty acid profiles, we extracted lipids from 0.1 g of each minced meat sample using 3 mL chloroform:methanol (2:1, v/v) according to the method described by Iverson et al. [14]. The extract was thoroughly mixed with 1.5 mL hexane. Following the addition of 200 µL 2 M potassium hydroxide in methanol, the contents were vortexed for 30 s. Next, 2 ml saturated sodium chloride solution was added and mixed thoroughly. The sample was then centrifuged at 1,000×g for 5 min, and the supernatant containing fatty acid methyl esters was recovered. The fatty acid methyl esters were separated using a GC2010 Gas Chromatograph (Shimadzu Co., Kyoto, Japan) and capillary column (DB-23, Shimadzu) (length=30 m, i.d.=0.25 mm, and film thickness=0.25 µm). The column was set at an initial temperature of 80°C for 2 min, then increased from 80 to 160°C at 35°C/min, 160 to 185°C at 2°C/min, followed by 10°C/min to a maximum temperature of 230°C, which was maintained for 9 min. Other conditions included the following: injection port temperature, 250°C; flame ionization detector temperature, 250°C; helium flow rate, 1.49 ml/min. The fatty acids were identified by comparison of retention times with FAME Mix Equity1 (Sigma-Aldrich Co., St. Louis, MO, USA).

Statistical analysis

Comparisons between two groups were performed using a Student's *t*-test. Comparisons among groups were performed using Tukey's multiple-comparison test. Haplotypes were inferred using the Thesias program [15] that is designed for testing haplotype effects in unrelated subjects when adjusting for covariates. This computer program is based on the maximum likelihood model described by Tregouet et al. [16]. Differences between the groups were considered significant when $P < 0.05$.

Results

Seventy-one and forty-six SNPs were found in the *FADS1* and *FADS2* genes, respectively (Tables 2 and 3). Of the SNPs, seven have not been previously identified. The nucleotide sequences containing the new SNPs were registered in the DNA data bank of Japan (DDBJ) and the accession numbers of the sequences containing the new SNPs are shown in Tables 2 and 3. All SNPs found in the coding regions of the *FADS1* and *FADS2* genes were synonymous substitutions without changing amino acids. No LD blocks were detected in *FADS1* (Figure 2), whereas an LD block was detected between the 5′-upstream region and intron between exons 1 and 2 in *FADS2* (Figure 3).

Of the SNPs in the *FADS1* gene, we selected rs733003230 (A > G) as a candidate SNP for testing associations between its type and fatty acid profile of Hinai-jidori chicken meat, since it is located at exon 1 and the distribution of alleles at the SNP sites is possibly uneven between Hinai-jidori founder (Hinai-dori and Rhode Island Red) and White Plymouth Rock breeds (Table 4). Meanwhile, of the SNPs in the *FADS2* gene, we selected LC060926 (g.25 A > G) as a candidate SNP, since it was found in the 5′-upstream regulatory region within one large LD block and its SNP distribution indicates possible breed differentiation (Table 4). Therefore, a mismatch amplification mutation assay (MAMA) PCR protocol was developed that detects the rs73300323015 and LC060926 SNPs described by Cha et al. [17]. We designed PCR primers to distinguish the SNPs of *FADS1* and *FADS2*, and PCR and genotyping were performed as described in Table 5.

As shown in the Table 5, Bases shown in lower case with a capital represent induced mismatches. Bases shown in lower case at the 3′-end represent target single nucleotide polymorphisms (SNPs). The SNP that can or cannot be amplified by PCR for each primer set are shown as '+' or '–', respectively. For the PCRs of FADS1-A and FADS2-G, we used 10-µL reaction volumes containing the following: 2 pmol of each primer for each marker, 200 µM of each dNTP, 0.5 units of Paq5000DNA Polymerase (Agilent Technologies, La Jolla, CA, USA), 1× reaction buffer (containing 2 mM $MgCl_2$) provided by the manufacturer, and 10 ng genomic DNA. Reactions were performed in a 96-well plate in a thermal cycler (GeneAmp System 9700; Perkin-Elmer, Foster City, CA, USA) using the following conditions: initial denaturation at 95°C for 2 min; and 35 cycles at 95°C for 20 s, at 67°C for 30 s, at 72°C for 30 s (FADS1-A), or 35 cycles at 95°C for 20 s, at 57.5°C for 30 s, at 72°C for 30 s (FADS2-G). For the PCRs of FADS2-A, we used 10-µL reaction volumes containing the following: 2 pmol of each primer, 200 µM of each dNTP, 0.5 units of KOD plus polymerase (TOYOBO, Tokyo, Japan), 1 × reaction buffer provided by the manufacturer, 1 mM $MgSO_4$, and 10 ng genomic DNA. Reactions were performed in a 96-well plate in the thermal cycler using the following conditions: initial denaturation at 94°C for 2 min; and 35 cycles at 94°C for 20 s, at 60°C for 30 s, at 72°C for 30 s. For the PCRs of FADS1-G, PCR amplification was performed in an 8-µL reaction volumes containing the following 2 pmol of each primer, and 4 µL of 2 × PCR mix (EmeraldAmp; Takara, Otsu, Japan), and 10 ng genomic DNA. Reactions were performed in the thermal cycler (GeneAmp System 9700; Perkin-Elmer, Foster City, CA, USA) using the following conditions: 30 cycles of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s. The PCR products were electrophoresed on a 2.0% agarose gel with 1 × Tris-acetate EDTA (TAE) buffer and stained with ethidium bromide. The combination of these results enabled us to identify the genotype of each individual.

Estimates of association for SNPs in *FADS1* (rs73300323015) and *FADS2* (LC060926) with fatty acid composition in the thigh meat are shown in Table 6. In both *FADS1* and *FADS2*, the ARA and DHA compositions were significantly higher in the G than in the A allele. In *FADS1*, stearic acid (SA, C18:0) and LA compositions were significantly higher in the G than in the A allele. Meanwhile, myristic (MA, C14:0), palmitic (PA, C16:0), and palmitoleic (POA, C16:1) acid compositions were significantly lower in the G than in the A allele. There were no significant differences between the A and G alleles in the other fatty acid compositions in either *FADS1* or *FADS2 TAB 5*.

The association of *FADS1* and *FADS2* haplotypes with fatty acid compositions is shown in Table 7. The ARA and DHA compositions of the G-G-haplotype were significantly higher than those of the A-A-haplotype. The LA composition of the A-A-haplotype was lower than that of G-A haplotype. The POA composition of the A-A-haplotype was higher than that of the G-A haplotype. The MA and PA compositions

No.	Location and characteristics	SNP_ID (accession number in DDBJ)	Base position in chicken chromosome 5
1	5′ upstream	rs737673984	16770615
2	5′ UTR	rs316315792	16770519
3	Exon1	rs733003230	16770503
4	Exon1	NR[1] (LC061130, g.233 C > T)	16770386
5	Intron between exons 1 and 2	rs735910165	16770292
6	Intron between exons 1 and 2	NR (LC061130, g.327 C > T)	16770238
7	Intron between exons 1 and 2	rs316695951	16768904
8	Intron between exons 1 and 2	rs315355848	16768883
9	Intron between exons 1 and 2	rs739885036	16768857
10	Intron between exons 1 and 2	rs316813933	16768840
11	Intron between exons 2 and 3	rs317184242	16768644
12	Intron between exons 2 and 3	rs312607775	16768620
13	Intron between exons 2 and 3	rs316637108	16768590
14	Intron between exons 2 and 3	rs314280740	16768519
15	Intron between exons 2 and 3	rs313466490	16768487
16	Intron between exons 2 and 3	rs16472277	16768480
17	Intron between exons 2 and 3	rs16472276	16768459
18	Intron between exons 2 and 3	rs16472275	16768449
19	Exon3, synonymous substitution	rs16472273	16768243
20	Intron between exons 3 and 4	rs740812406	16767689
21	Intron between exons 3 and 4	rs318222659	16767644
22	Intron between exons 3 and 4	NR (LC061135, g.169 C > T)	16767626
23	Intron between exons 3 and 4	rs736122139	16767615
24	Intron between exons 3 and 4	rs734502299	16767573
25	Intron between exons 3 and 4	rs16472268	16767528
26	Intron between exons 3 and 4	rs312267702	16767513
27	Intron between exons 3 and 4	rs16472267	16767500
28	Intron between exons 4 and 5	rs314115979	16767293
29	Intron between exons 4 and 5	rs734153233	16767188
30	Intron between exons 4 and 5	rs316098909	16766910
31	Intron between exons 5 and 6	rs16472263	16766660
32	Intron between exons 5 and 6	rs16472262	16766645
33	Intron between exons 5 and 6	rs16472241	16766105
34	Intron between exons 5 and 6	rs315789178	16766076
35	Intron between exons 5 and 6	rs312905121	16766070
36	Intron between exons 5 and 6	NR (LC061137, g.79 A > T)	16766033
37	Intron between exons 5 and 6	rs314740868	16765937
38	Intron between exons 5 and 6	NR (LC061137, g.459 C > T)	16765653
39	Intron between exons 7 and 8	rs316317531	16765623
40	Intron between exons 7 and 8	rs314512343	16765596
41	Intron between exons 7 and 8	rs315716526	16765575
42	Intron between exons 7 and 8	rs314580393	16765553
43	Intron between exons 7 and 8	rs316698751	16765379
44	Intron between exons 7 and 8	rs313988812	16765084
45	Intron between exons 7 and 8	rs315678178	16765004
46	Intron between exons 7 and 8	rs313458459	16764983
47	Intron between exons 7 and 8	rs741298367	16764943
48	Exon8, synonymous substitution	rs736455876	16764941
49	Exon8, synonymous substitution	rs734538614	16764932
50	Exon8, synonymous substitution	rs740633346	16764847
51	Intron between exons 8 and 9	rs313383381	16764846
52	Intron between exons 8 and 9	rs734385913	16764836
53	Intron between exons 8 and 9	NR (LC061138, g.563 A > C)	16764605
54	Intron between exons 8 and 9	rs314576839	16764468
55	Exon9, synonymous substitution	rs318122562	16764416
56	Intron between exons 9 and 10	rs313188516	16764399
57	Intron between exons 9 and 10	rs313138210	16764386
58	Intron between exons 9 and 10	rs317616317	16763711
59	Intron between exons 10 and 11	rs730997463	16763703
60	Intron between exons 10 and 11	rs734399599	16763695

61	Intron between exons 10 and 11	rs313846569	16763655
62	Intron between exons 10 and 11	rs741458532	16763653
63	Intron between exons 10 and 11	rs736622224	16763636
64	Intron between exons 10 and 11	NR (LC061141, g.283 G > T)	16763589
65	Intron between exons 10 and 11	rs316671622	16763509
66	Intron between exons 10 and 11	rs312786975	16763416
67	Intron between exons 10 and 11	rs315852578	16763401
68	Intron between exons 11 and 12	rs314477937	16763001
69	Intron between exons 11 and 12	rs314676526	16762972
70	3′ UTR	rs735594367	16762693
71	3′ UTR	rs316625828	16762520

[1]NR: Not Reported.

Table 2: SNPs in chicken fatty acid desaturase 1 (*FADS1*).

No.	location and characteristics	SNP_ID (accession numbers in DDBJ)	Base position in chicken chromosome 5
1	5′ upstream	NR[1] (LC060926, g.25 A > G)	16777160
2	Exon 1, synonymous substitution	rs10722582	16777539
3	Intron between exons 1 and 2	NR (LC060926, g.521 C > G)	16777656
4	Intron between exons 1 and 2	rs315346254	16780804
5	Intron between exons 1 and 2	rs733308658	16780921
6	Intron between exons 1 and 2	rs736789598	16780923
7	Intron between exons 1 and 2	rs740152791	16780924
8	Intron between exons 2 and 3	rs314358722	16781127
9	Intron between exons 2 and 3	rs15673187	16781129
10	Intron between exons 2 and 3	rs312643892	16781260
11	Intron between exons 2 and 3	rs735043547	16781832
12	Intron between exons 2 and 3	rs312319790	16781849
13	Exon 3, synonymous substitution	rs732319615	16782027
14	Intron between exons 3 and 4	rs16472308	16782103
15	Intron between exons 3 and 4	rs317747268	16782118
16	Intron between exons 3 and 4	rs733216524	16782166
17	Intron between exons 3 and 4	rs313324908	16782214
18	Intron between exons 3 and 4	rs316303425	16782233
19	Intron between exons 3 and 4	rs317328157	16782238
20	Intron between exons 3 and 4	rs738216895	16782243
21	Intron between exons 4 and 5	rs736383930	16782968
22	Intron between exons 4 and 5	rs16472310	16783011
23	Intron between exons 5 and 6	rs312510513	16785052
24	Intron between exons 5 and 6	rs312795090	16785069
25	Intron between exons 5 and 6	rs314644465	16785075
26	Intron between exons 5 and 6	rs317565335	16785130
27	Exon 7, synonymous substitution	rs317214584	16785758
28	Intron between exons 8 and 9	rs315961674	16786173
29	Intron between exons 8 and 9	rs313217440	16788575
30	Intron between exons 8 and 9	rs315529969	16788585
31	Intron between exons 9 and 10	rs317944267	16788628
32	Intron between exons 9 and 10	rs741640292	16788894
33	Intron between exons 9 and 10	rs734093311	16788947
34	Intron between exons 9 and 10	rs738755803	16789009
35	Intron between exons 9 and 10	rs15673219	16789863
36	Intron between exons 10 and 11	rs15673221	16789989
37	Intron between exons 10 and 11	rs314090105	16791017
38	Intron between exons 10 and 11	rs316938331	16791028
39	Intron between exons 10 and 11	rs312449387	16791061
40	Intron between exons 10 and 11	rs737848558	16791075
41	Intron between exons 10 and 11	rs315447773	16791162
42	Exon 11, synonymous substitution	rs317337151	16791219
43	Exon 11, synonymous substitution	rs10727332	16791234
44	Intron between exons 11 and 12	rs312748222	16792072
45	3′ downstream	rs15673236	16792140
46	3′ downstream	rs314733839	16792222

[1]NR: Not Reported.

Table 3: SNPs in chicken fatty acid desaturase 2 (*FADS2*).

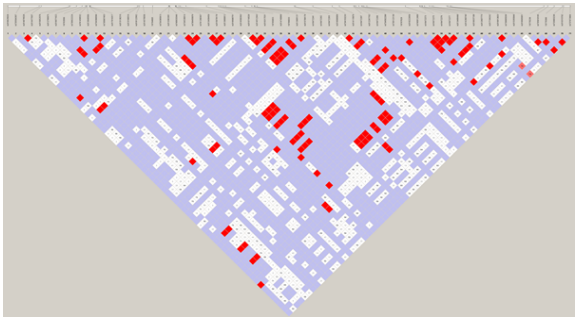

Figure 2: Linkage disequilibrium (LD) plot of fatty acid desaturase 1 (*FADS1*) SNPs. Red squares show the high correlation coefficients (R^2) between two SNPs.

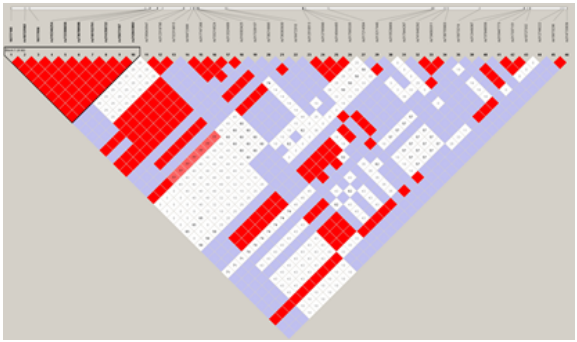

Figure 3: Linkage disequilibrium (LD) plot of fatty acid desaturase 1 (*FADS1*) SNPs. Red squares show the high correlation coefficients (R2) between two SNPs. Red squares show the high correlation coefficients (R^2) between 2 SNPs. One big LD block was identified from the 5′-upstream regulatory region to intron between exon 2 and 3 shown on the left side.

Gene	Fatty acid desaturase 1 (*FADS1*)	Fatty acid desaturase 2 (*FADS2*)
SNP	rs733003230 (A/G)	LC060926 (g.25 A > G)
Locus	Exon 1, synonymous	5′-upstream regulatory region
Sample		
Hinai-dori breed 1	A/A	A/A
Hinai-dori breed 2	A/G	A/A
Hinai-dori breed 3	A/A	A/A
Rhode Island Red breed 1	G/G	A/A
Rhode Island Red breed 2	A/G	A/A
Rhode Island Red breed 3	G/G	A/G
White Plymouth Rock breed 1	A/A	G/G
White Plymouth Rock breed 2	A/A	A/G
White Plymouth Rock breed 3	A/A	G/G

Table 4: Genotypes of selected SNPs in the sequenced individuals.

of the A-A-haplotype were higher than those of the G-A- and G-G-haplotypes, respectively. There were no significant differences among the haplotypes with respect to other fatty acid compositions.

Discussion

To date, most research concerning *FADS1* and *FADS2* has focused on humans. For example, Tanaka et al. [18] reported that an SNP near *FADS1* was significantly associated with the plasma concentrations of ARA, eicosapentaenoic acid (EPA, C20:5n-3), and eicosadienoic acid (EDA, C20:2n-6) in a human population (InCHIANTI) living in the Chianti region of Tuscany, Italy. Schaeffer et al. [19] reported

	Primers (5′ → 3′)	Product (bp)	SNP	
			A	G
FADS1-A	ccggcgtagtggctgatgac	195	+	–
	ggCggggagagccatgCaA			
FADS1-G	ccggcgtagtggctgatgac	195	–	+
	ggAggggagagccatgTaG			
FADS2-A	tcgcacatagctccgtGtT	274	+	–
	aaatcctgccgcagagaag			
FADS2-G	aaccttccgctctatcacca	397	–	+
	tgggccgagcttgccGcG			

Table 5: The primers and target position in chicken fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) genes for the mismatch amplification mutation assay.

that polymorphisms of the *FADS1* and *FADS2* gene cluster showed significant associations with the level of the n-6 fatty acids, LA, GLA, EDA, DGLA, ARA, dodecylthioacetic acid (DTA, C22:4 n-6), and n-3 fatty acids, ALA, EPA, and docosapentaenoic acid (DPA, C22:5n-3) in serum phospholipids. Moltó-Puigmartí et al. [20] reported that polymorphisms of the *FADS1* and *FADS2* gene cluster showed significant associations with the level of the n-6 fatty acids, LA, GLA, ARA, DGLA, DTA, and n-3 fatty acid, DHA, in serum phospholipids, whereas the gene cluster showed significant associations with the level of the n-6 fatty acids, DGLA, ARA, DTA, and n-3 fatty acids, EPA, DPA and DHA, in human milk. Together, these data suggest that the *FADS1* and *FADS2* gene cluster affect not only n-6 but also n-3 fatty acids, especially LA, ARA, and DHA in humans.

In poultry, associations between genetic variants of the *FADS2* gene and fatty acid profile in Japanese quail eggs and chicken meat have been reported; however, no studies on the associations between the genetic variants of the *FADS1* and *FADS2* gene clusters and fatty acid profiles in meat have been reported. Khang et al. [21] reported that an SNP of *FADS2* showed significant associations with the level of the n-6 fatty acids LA and ARA and the n-3 fatty acid DHA in egg yolk using Japanese quail lines selected for high and low n-6/n-3 polyunsaturated fatty acid (PUFA) ratios. Zhu et al. [22] reported that two SNPs of *FADS2* showed significant associations with the level of the n-6 fatty acids, LA and ARA, in the muscle of an F_2 resource population crossing a Chinese indigenous breed and broiler chickens, although the meat portion sampled was not documented. In the present study, we found that polymorphisms of the *FADS1* and *FASD2* genes, and *FADS1* and *FADS2* gene clusters affected the fatty acid profile, i.e., ARA and DHA in the thigh meat in Hinai-jidori chickens. Since chickens with higher ARA contents are tastier than those with lower ARA contents [6], the data in the present study suggested that a breeding strategy for improving the taste of Hinai-jidori meat could be developed using SNPs of *FADS1* and *FASD2* as selection markers. However, further studies are needed to determine whether the SNP effect is applicable to the other chicken strains and if similar effects are observed in chicken eggs.

Rikimaru and Takahashi [2] reported that the ARA and DHA compositions of Hinai-jidori chickens at the age of 22 wk were significantly higher than those of broiler chickens at the age of 8 and 22 wk. Sirri et al. [23] compared fatty acid profiles of breast and thigh meat among fast- (Cobb 700), medium- (Naked neck Kabir), and slow- (Brown Classic Lohman) growing strain chickens slaughtered at the age of 81 d. The SA, ARA and DHA compositions of the slow-growing strain were significantly higher than those of the fast- and medium-growing strains, whereas the MA, POA, and oleic acid (OA, C18:1) compositions of the slow-growing line were significantly lower than those of the fast- and medium-growing lines both in breast and thigh

Gene	**Fatty acid desaturase 1 (*FADS1*)**		**Fatty acid desaturase 2 (*FADS2*)**	
Locus	rs733003230 (A > G)		LC060926（g.25 A > G）	
SNP type	A	G	A	G
SNP Frequency	0.453	0.547	0.813	0.188
Fatty acid % of total analyzed fatty acid				
Myristic acid (C14:0)	0.35 ± 0.01	0.32 ± 0.01**	0.34 ± 0.00	0.31 ± 0.01
Palmitic acid (C16:0)	12.02 ± 0.27	11.02 ± 0.16**	11.64 ± 0.11	10.78 ± 0.46
Palmitoleic acid (C16:1)	2.36 ± 0.28	1.71 ± 0.13*	2.11 ± 0.10	1.56 ± 0.30
Heptadecanoic acid (C17:0)	0.07 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
Stearic acid (C18:0)	3.65 ± 0.22	4.09 ± 0.09*	3.80 ± 0.12	4.29 ± 0.30
Oleic acid (C18:1)	19.58 ± 0.58	18.71 ± 0.35	19.24 ± 0.26	18.52 ± 0.75
Linoleic acid (C18:2n-6)	9.33 ± 0.68	11.07 ± 0.36*	10.07 ± 0.30	11.21 ± 0.98
γ-Linolenic acid (C18:3n-6)	0.07 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.03
α-Linolenic acid (C18:3n-3)	0.32 ± 0.02	0.34 ± 0.01	0.33 ± 0.01	0.35 ± 0.03
Eicosenoic acid (C20:1)	0.14 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02
Eicosadienoic acid (C20:2)	0.09 ± 0.03	0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.03
Eicosatrienoic acid (C20:3(n-3+n-6))	0.10 ± 0.03	0.06 ± 0.01	0.08 ± 0.01	0.03 ± 0.03
Arachidonic acid (C20:4n-6)	1.01 ± 0.15	1.33 ± 0.07*	1.10 ± 0.07	1.55 ± 0.19*
Lignoceric acid (C24:0)	0.09 ± 0.03	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.03
Docosahexaenoic acid (C22:6n-3)	0.25 ± 0.04	0.35 ± 0.02*	0.28 ± 0.02	0.40 ± 0.06*
Unidentified FA	0.56 ± 0.05	0.57 ± 0.03	0.56 ± 0.02	0.60 ± 0.07

*statistically significant at P=0.05; **statistically significant at P=0.01.

Table 6: SNP effects of chicken fatty acid desaturase 1 and 2 *(FADS1* and *FADS2*) on fatty acid profiles of Hinai-jidori thigh meat.

Combined haplotypes of *FADS1* and *FADS2*	A-A	G-A	G-G
Frequencies of plausible haplotypes under linkage equilibrium	0.453	0.359	0.188
Fatty acid % of total analyzed fatty acid			
Myristic acid (C14:0)	0.35 ± 0.01^{a}	0.33 ± 0.01^{b}	0.31 ± 0.01^{b}
Palmitic acid (C16:0)	12.04 ± 0.15^{a}	11.14 ± 0.28^{b}	10.75 ± 0.35^{b}
Palmitoleic acid (C16:1)	2.38 ± 0.22^{a}	1.78 ± 0.30^{b}	1.54 ± 0.46^{ab}
Heptadecanoic acid (C17:0)	0.07 ± 0.01	0.09 ± 0.02	0.10 ± 0.03
Stearic acid (C18:0)	3.63 ± 0.17	4.00 ± 0.22	4.30 ± 0.32
Oleic acid (C18:1)	19.60 ± 0.36	18.80 ± 0.58	18.49 ± 0.86
Linoleic acid (C18:2)	9.31 ± 0.49^{b}	10.99 ± 0.69^{a}	11.26 ± 1.05^{ab}
γ-Linolenic acid (C18:3n-6)	0.08 ± 0.01	0.05 ± 0.02	0.03 ± 0.03
α-Linolenic acid (C18:3n-3)	0.32 ± 0.02	0.34 ± 0.03	0.35 ± 0.04
Eicosenoic acid (C20:1)	0.14 ± 0.01	0.13 ± 0.02	0.14 ± 0.02
Eicosadienoic acid (C20:2)	0.09 ± 0.02	0.06 ± 0.03	0.05 ± 0.03
Eicosatrienoic acid (C20:3(n-3+n-6))	0.10 ± 0.02	0.07 ± 0.03	0.03 ± 0.03
Arachidonic acid (C20:4n-6)	0.99 ± 0.12^{b}	1.24 ± 0.15^{ab}	1.56 ± 0.24^{a}
Lignoceric acid (C24:0)	0.09 ± 0.02	0.09 ± 0.03	0.09 ± 0.04
Docosahexaenoic acid (C22:6n-3)	0.25 ± 0.04^{b}	0.32 ± 0.04^{ab}	0.40 ± 0.07^{a}
Unidentified FA	0.56 ± 0.03	0.56 ± 0.06	0.60 ± 0.07

^{a,b}Means within a row with different superscript letters are significantly different at P=0.05.

Table 7: Haplotype effects of chicken fatty acid desaturase 1 and 2 *(FADS1* and *FADS2*) on fatty acid profiles of Hinai-jidori thigh meat.

meat. Jayasena et al. [24] reported that Korean native chickens at the age of 100 d showed significantly higher compositions of LA, ARA, and DHA than broilers at the age of 32 d. Boschetti et al. [25] reported that medium-growing (Kabir Red) and particularly slow-growing (Hy-line W36) lines showed a greater expression of the *FADS1* and *FADS2* genes in hepatic tissue than a fast-growing line (Cobb 500) at 81 d of age, although they did not speculate associations between *FADS1* and *FADS2* gene polymorphisms and fatty acid profiles in the meat. These reports may suggest that there is a significant strain difference in the fatty acid profile of meat; however, we would like to refer to sex differences of samples in these reports. Rikimaru and Takahashi [2] used Hinai-jidori females because almost 100% of the Hinai-jidori chickens sold commercially are females, whereas Sirri et al. [23], Jayasena et al. [24], and Boschetti et al. [25] used males. In fact, Sirri et al. [26] reported that the ARA, DPA, and DHA composition of breast and thigh meat of cocks were significantly higher than those of capons at the age of 180 d. To explain the difference between cocks and capons, the authors supposed that D6D activity is affected by testosterone, since Clejan et al. [27] found a decrease of ARA and DHA in castrated rats owing to the lack of testosterone and showed that the administration of testosterone to castrated rats could bring the ARA content to normal values. It is known that testosterone exists in plasma before the onset of puberty in cockerels [28-30]. Together, the difference of fatty acid profiles detected among Cobb 700, Naked neck Kabir, and Brown Classic Lohman at 81 d of age [23], and between 100-d-old Korean native chickens and 32-d-old broilers [24] may simply reflect the plasma testosterone concentration of each strain at slaughter age, although this information is unknown. Meanwhile, this study has some advantages over previous studies in assessing the effect of the *FADS1* and *FADS2* genes on the fatty acid profiles of the meat. In particular, the effects of testosterone on fatty acid profiles and environmental factors are negligible, since we used female chickens that hatched on the same

day and were reared under identical environmental conditions for the same duration.

Conclusion

In conclusion, this is the first report to show the possibility of using polymorphisms of the *FADS1* and *FASD2* gene, and *FADS1* and *FADS2* gene clusters as selection markers for Hinai-Jidori chickens to improve fatty acid profiles, especially ARA and DHA. Moreover, this report provides an additional line of evidence that *FADS1* and *FADS2* polymorphisms affect fatty acid profiles in vertebrates.

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