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Is egg flavour changeable by chicken breeding? Association of chicken fatty acid desaturase 1 gene single-nucleotide polymorphisms with egg fatty acid profiles and flavour in a Japanese hybrid chicken

Shigeyuki Matsui¹ and Hideaki Takahashi^{2*}

Abstract: In Japan, hybrid crosses between Japanese native and exotic breeds, known as “Jidori” chickens, are sold at high prices to a limited market. Most Japanese recognize that eggs produced from Jidori chickens have a rich flavour, named *kokumi*, whereas the reason that Jidori eggs are *kokumi*-rich has not yet been elucidated. The main objective in this study was to elucidate why Jidori eggs have a rich flavour. We investigated whether polymorphisms in *fatty acid desaturase 1* and *2* (*FADS1* and *FADS2*) affected the fatty acid profiles of Suruga-shamo eggs, a chicken breed in Japan. A single-nucleotide polymorphism (SNP) in *FADS1* was significantly associated with arachidonic acid (ARA) content. The $n - 6/n - 3$ polyunsaturated fatty acid ratio of the yolk showed that the SNP allele that exhibited high ARA had a low $n - 6/n - 3$ ratio. We found that adding trace amounts of ARA, that correspond to the difference of the SNP genotypes, enhanced egg flavour intensity and continuity. We concluded that the SNP of *FADS1* could be used to develop strategies for improving egg flavour and decreasing the $n - 6/n - 3$ ratio in yolk.

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SM and HT designed research. SM collected the data. HT analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

PUBLIC INTEREST STATEMENT

In Japan, most chicken meat is produced from fast-growing broiler chickens. Since most Japanese chicken breeds have low meat yield and egg production, many of these breeds are in danger of disappearing. Recently, palatability of meat and eggs produced from native chickens has been reevaluated in the Japanese market. “Jidori”, that is translated as an indigenous chicken in a local, is far more delicious, firm in texture and expensive than the affordable broiler chicken. We would like to elucidate why Jidori meat and eggs have a rich flavour. Moreover, we would like to improve palatability of Jidori meat and eggs using molecular breeding and marker-assisted selection in the near future.

Subjects: Animal Physiology; Genetics; Sensory Science; Meat & Poultry; Physiology

Keywords: Suruga-shamo chicken; *fatty acid desaturase 1* gene; fatty acid profile; egg flavour; arachidonic acid

1. Introduction

In Japan, most chicken meat is produced from fast-growing broiler chickens. Furthermore, consumers in Japan recognize the taste of chicken native to Japan. To date, hybrid crosses between Japanese native and exotic breeds, known as “Jidori” chickens, are sold at high prices to a limited market. Suruga-shamo is a synthetic breed resulting from a cross between Japanese native (Shamo (Japanese Game), Nagoya, Hinai-dori, and Tosa-kukin) and exotic (Barred and White Plymouth Rock, and Rhode Island Red) breeds, which was developed by the Swine and Poultry Research Center, Shizuoka Prefectural Research Institute of Animal Industry (Kikugawa, Japan) in 1990. To date, the taste of both Suruga-shamo meat and eggs is recognized and gaining popularity in Japan.

Most Japanese recognize that eggs produced from Jidori chickens have a rich flavour, named *kokumi* (continuity, mouthfulness, and thickness; Yamamoto, Watanabe, Fujimoto, & Sako, 2009); however, the reason that Jidori eggs are *kokumi*-rich has not yet been elucidated. In a Jidori breed, Hinai-jidori chickens, we reported a significant difference in arachidonic acid (ARA, C20:4n – 6) content between Hinai-jidori and broiler meat, suggesting that the high ARA content is a characteristic feature of Hinai-jidori meat (Rikimaru & Takahashi, 2010). To elucidate the relationship between the ARA content and chicken taste, the effects of ARA-enriched oil supplements on the fatty acid content and sensory perceptions of meat were evaluated in Hinai-jidori (Kiyohara, Yamaguchi, Rikimaru, & Takahashi, 2011) and broiler (Takahashi, Rikimaru, Kiyohara, & Yamaguchi, 2012) chickens. Because of these experiments, direct evidence for significant positive associations among ARA and total taste intensity, umami (γ -glutamate taste), *kokumi*, and aftertaste from chicken meat were shown. To understand why Hinai-jidori meat has high ARA content, we further investigated genes encoding members of a metabolic pathway from linoleic acid (LA, C18:2n – 6) to ARA. Recently, we reported that single-nucleotide polymorphisms (SNP) of *fatty acid desaturase 1* and 2 genes (*FADS1* and *FADS2*), and *FADS1* and *FADS2* gene clusters affected ARA content in Hinai-jidori chickens. We speculated, therefore, that (1) ARA is a candidate substance related to the taste of Jidori eggs, and (2) *FADS1* and *FADS2* are the key genes that control ARA content of eggs.

Our main objective in the present study was to analyse polymorphisms of the *FADS1* and *FADS2* genes and test their association with the fatty acid profiles of Suruga-shamo eggs to elucidate why Jidori eggs have a rich flavour.

2. Materials and methods

2.1. Egg samples

All chickens received humane care as outlined in the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006). Suruga-shamo female chickens (154 individuals) were raised in the Swine and Poultry Research Center, Shizuoka Prefectural Research Institute of Animal Industry. The female chicks hatched on the same day (August 20, 2012) were housed in a start cage from 0 to 4 weeks of age; in a grow cage from 4 to 17 weeks of age; and in individual cages from 17 weeks of age in an open-sided house. Chicks were fed a starter diet (metabolizable energy (ME), 2,950 kcal/kg; crude protein (CP), 21% [w/w]) from 0 to 6 weeks; a starter/grower diet (ME, 2,850 kcal/kg; CP, 18%) from 6 to 10 weeks of age; a grower diet (ME, 2,880 kcal/kg; CP, 15%) from 10 to 17 weeks of age; and a layer diet (ME, 2,850 kcal/kg; CP, 17%) from 17 weeks of age onward. All diets were obtained from JA Higashinohon Kumiai Feed Co. (Ota, Japan). The company guaranteed that all diets were formulated to satisfy the nutrient requirements of the Japanese Feeding Standard for Poultry (National Agriculture & Food Research Organization, 2011). Chicks were fed *ad libitum* from 0 to 17 weeks of age, 90 g diet/day from 17 to weeks of age onward. Water was provided *ad libitum*.

One egg was randomly selected from each hen from 59 to 65 weeks of age. In total, 154 eggs were collected and their yolk was subjected to fatty acid analysis.

2.2. Preparation of chicken genomic DNA

Chicken blood samples were collected from the ulnar vein in the presence of heparin. The blood was spotted onto filter paper (FTA CloneSaver Card; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and air-dried. DNA was obtained from the FTA card according to Method No. 4, as described by Smith and Burgoyne (2004). Briefly, the spotted blood was cut out with the e-Core™ 1.2 mm electric punch kit (WB100052; GE Healthcare Bio-Sciences) and the disc was placed into a well of a 96-well polymerase chain reaction (PCR) plate (BRG-96S, Bio Regenerations Co., Yokohama, Japan). The disc was washed with 100 µL FTA purification reagent (WB120204; GE Healthcare Bio-Sciences) for 30 min, washed in 100 µL DNAzol BD (Molecular Research Center, Inc. Cincinnati, OH, USA), and then washed 3 times with 100 µL Milli Q water. Finally, 100 µL Milli Q water was added and heated to 90°C for 10 min using the thermal cycler (GeneAmp System 9700; Perkin-Elmer, Foster City, CA, USA) to release genomic DNA from the disc. The supernatant was transferred to the new 96-well PCR plate and subsequently used as a template DNA solution for PCR amplification.

2.3. SNP detection of fatty acid desaturase 1 and 2 genes

We tested rs73300323015 (A > G) of *FADS1* and LC060926 (g.25 A > G) of *FADS2*, that have been reported to be associated with the fatty acid profiles of chicken meat (Rikimaru, Egawa, Yamaguchi, & Takahashi, 2016). The SNPs were typed using a mismatch amplification mutation assay (MAMA, Cha, Zarbl, Keohavong, & Thilly, 1992). The PCR primers, reaction components, cycles, and genotyping procedures for the MAMA were described previously (Rikimaru et al., 2016).

2.4. Determination of fatty acid composition of the egg yolk

To determine fatty acid profiles, we extracted lipids from 2 mL of each yolk sample using 8 mL of chloroform:methanol (2:1, v/v) according to the method described by Folch, Lees, and Sloane-Stanley (1957). Following the addition of 2 mL of 0.5 N sodium methylate to methanol, the contents were heated at 60°C for 10 min. After cooling to room temperature, 4 mL of 2% acetic acid was added and mixed. Then, 5 mL hexane was added and mixed. After leaving the mixture for 1 h, the supernatant containing fatty acid methyl esters was recovered. The fatty acid methyl esters were separated using a GC2014 gas chromatograph (Shimadzu Co., Kyoto, Japan) and capillary column (FAMEWAX, RESTEC) (length = 30 m, i.d. = 0.25 mm, and film thickness = 0.25 µm). The column was set at an initial temperature of 130°C for 3 min, then increased from 130 to 250°C at 5°C/min, and maintained for 9 min. Other conditions included the following: injection port temperature, 240°C; flame ionization detector temperature, 230°C; high-purity nitrogen flow rate, 29.9 mL/min. The fatty acids were identified by comparison of retention times with FAME Mix Equity 1 (Sigma-Aldrich Co., St. Louis, MO, USA).

2.5. Statistical analysis

Allele frequencies were calculated by gene counting. Associations between SNPs and phenotypes were estimated using the Thesias program (Tregouet & Garelle, 2007) that is designed for testing the effects in unrelated subjects after adjusting for covariates and is based on the maximum likelihood model described by Tregouet et al. (2002). Differences between the SNPs were considered significant if $p < 0.05$.

3. Results

3.1. Association of SNPs with fatty acid composition of egg yolk

In the Suruga-shamo female population, rs73300323015 (A > G) of *FADS1* was polymorphic (G, 0.802; A, 0.198) (Table 1), whereas LC060926 (g.25 A > G) of *FADS2* was monomorphic (G, 1.0).

Estimates of haplotype effects of *FADS1* (rs73300323015) on fatty acid composition in yolk are shown in Table 1. The ARA composition was significantly higher in the G ($0.977 \pm 0.010\%$) than in the

Table 1. SNP effects of chicken fatty acid desaturase 1 (FADS1) on fatty acid profiles of yolk in Suruga-shamo chicken eggs

SNP		G	A
Frequency		0.802	0.198
Trait (%)	Abbreviation		
Myristic acid	C14:0	0.178 ± 0.002	0.167 ± 0.008
Myristoleic acid	C14:1	0.040 ± 0.001	0.040 ± 0.004
Pentadecanoic acid	C15:0	0.028 ± 0.001	0.026 ± 0.003
Palmitic acid	C16:0	12.440 ± 0.055	12.263 ± 0.178
Palmitoleic acid	C16:1	1.511 ± 0.032	1.353 ± 0.100
Heptadecanoic acid	C17:0	0.114 ± 0.003	0.131 ± 0.009
cis-10-Heptadecenoic acid	C17:1	0.136 ± 0.006	0.184 ± 0.022*
Stearic acid	C18:0	3.843 ± 0.041	3.885 ± 0.121
Oleic acid	C18:1	25.101 ± 0.114	25.138 ± 0.361
Linoleic acid	C18:2n – 6	4.536 ± 0.090	4.635 ± 0.253
α-Linolenic acid	C18:3n – 3	0.135 ± 0.004	0.131 ± 0.012
11-Eicosenoic acid	C20:1n – 9	0.132 ± 0.003	0.123 ± 0.013
Eicosadienoic acid	C20:2n – 6	0.039 ± 0.001	0.045 ± 0.005
cis-8,11,14-Eicosatrienoic acid	C20:3n – 6	0.080 ± 0.003	0.054 ± 0.028
Arachidonic acid	C20:4n – 6	0.977 ± 0.010	0.913 ± 0.031*
Docosadienoic acid	C22:2n – 6	0.183 ± 0.059	0.419 ± 0.122
Docosahexaenoic acid	C22:6n – 3	0.525 ± 0.007	0.495 ± 0.030
ΣSFA		16.604 ± 0.058	16.472 ± 0.191
ΣMUFA		26.920 ± 0.096	26.838 ± 0.302
Σn – 6 PUFA		4.838 ± 0.098	5.153 ± 0.265
Σn – 3 PUFA		0.661 ± 0.008	0.626 ± 0.265
n – 6/n – 3 PUFA		3.698 ± 0.069	4.093 ± 0.193*

Notes: ΣSFA, ΣMUFA, ΣPUFA = sum of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids.

*Statistically significant at $p = 0.05$ level.

A (0.913 ± 0.031%) allele. The cis-10-heptadecenoic acid (C17:1) composition was significantly lower in the G (0.136 ± 0.006%) than in the A allele (0.184 ± 0.022%). There were no significant differences between the A and G alleles in other fatty acid compositions in *FADS1*. The n – 6/n – 3 ratio was significantly lower in the G (3.698 ± 0.069) than in the A (4.093 ± 0.193) allele. Genotype difference between G/G and A/A in the n – 6/n – 3 ratio was estimated to be $0.790 = (4.093 - 3.698) \times 2$.

3.2. Effects of arachidonic acid on egg flavour

Because we previously found that chicken meat that contained higher levels of ARA tasted better than meat containing lower ARA content (Kiyohara et al., 2011; Takahashi et al., 2012), we assumed that the difference in ARA composition of the yolk affected the flavour. Japanese people feel strongly regarding the difference of egg *kokumi* when the yolk is not solidified. Of Japanese traditional foods, “Tamago Kake Gohan” is a daily food in Japan, and is composed of freshly cooked rice, raw eggs, and soy sauce. Thus, “yolk seasoned with soy sauce” was used as a model of Tamago Kake Gohan to assess the effect of ARA on egg flavour.

Eggs were obtained from a local market on the day of the experiment and stored at 4°C until sample preparation. After the eggs were broken, yolks were pooled in a beaker. The vitelline membrane was cut using ophthalmic scissors. The yolk was briefly mixed with a magnetic stirrer and then

Table 2. Sensory evaluation of yolk seasoned with soy sauce

Item	Score ¹
Egg odour	0.263
Egg flavour intensity	0.842**
Egg flavour continuity	0.579*

¹Average score of yolk with arachidonic acid-enriched oil (AAO) when yolk without AAO score is 0.

*Statistically significant at $p = 0.05$ level.

**Statistically significant at $p = 0.01$ level.

filtered with a tea strainer to remove vitelline membrane and chalaza. The yolk was mixed with a magnetic stirrer for 5 min then divided into two equal parts. Arachidonic acid-enriched oil (AAO, SUNTGA40S, Nippon Suisan Co., Tokyo, Japan) was added to one of them, according to the compositional difference detected from the genotypic differences in *FADS1* and *FADS2*. SUNTGA40S was extracted from a biomass of submerged fermented *Mortierella alpina* and it contained 40% ARA. We defined y as the volume (μL) of AAO added per 100 mL yolk.

$$y = (0.335 \times 0.96 \times x) \times \frac{1}{0.91} \times \frac{100}{40} \times 1000$$

where x is the percent difference detected in the genotype of *FADS1*. The value 0.335 is the proportion of lipid in yolk, 0.96 is the proportion of triglyceride and phospholipid in yolk lipids, 0.91 is the specific gravity of AAO, and 40 is the percentage of ARA in AAO.

Because the ARA difference between G/G and A/A genotypes of *FADS1* was estimated to be $0.128\% = (0.977 - 0.913) \times 2$, y was calculated to be 113 μL of AAO per 100 mL yolk. Then, 3.077 mL soy sauce (GTIN code 4964366440220, Teraoka yuki Jozo Co. Fukuyama, Japan) was added per 100 mL yolk. The final salt concentration of the yolk samples was estimated to be 1%. The yolk samples with and without AAO were stirred with a magnetic stirrer for 10 min. Afterward, 10 g of the yolk samples was divided and placed into disposable cups made of transparent plastic (110 mL capacity). The two samples were visually indistinguishable.

Sensory evaluation was performed by an analytical panel composed of 19 Japanese members at a third party institution (Daiwa Service Co., Sagami-hara, Japan). Immediately before serving, the reference and test samples were labelled with random 3-digit codes to avoid bias, and were served to the panelists according to the code. The yolk samples were served to the sensory panel within 1 h of the beginning of sample preparation. Water was provided for palate cleaning. The panelists evaluated three parameters, egg odour before tasting, egg flavour intensity (perceived intensity of mixed tastes with retronasal aroma), and egg flavour continuity. Panelists recorded whether the test sample was comparatively stronger (score = +2, +1) or weaker (score = -1, -2) than the reference sample (score = 0). Sensory analyses were performed in a room at 24°C with good lighting and ventilation. Sensory evaluation data were analysed by Wilcoxon's signed rank test, at a significance level of $p < 0.05$ or $p < 0.01$.

The parameter of egg flavour intensity of the yolk with AAO was scored significantly ($p < 0.01$) higher than that without AAO (Table 2). The parameter of egg flavour continuity of the yolk with AAO was scored significantly ($p < 0.05$) higher than that without AAO. There was no significant difference between the yolk with and without AAO in the parameter of egg odour.

4. Discussion

In chicken meat, Zhu et al. (2014) 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 resulting from the crossing of a Chinese indigenous breed with broiler chickens. Recently, we reported that polymorphisms in the *FADS1* and *FADS2* genes, and *FADS1* and *FADS2* gene clusters affected the

fatty acid profile, especially ARA and docosahexaenoic acid (DHA, C22:6n – 3), in the thigh meat in a Japanese Jidori breed, Hinai-jidori (Takahashi et al., 2016). Furthermore, in poultry eggs, Khang et al. (2007) 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 in Japanese quail; however, there have been no studies on the associations between the genetic variants of *FADS1* and fatty acid profiles of chicken eggs. In the present study, we found that an SNP of *FADS1* affected the fatty acid profile, i.e. ARA, in the yolk.

Because health consciousness is rising worldwide, reduction of the n – 6/n – 3 ratio is a special concern of the poultry egg industry. Therefore, feed additives, e.g. flaxseed and fish oil, are used for this purpose (Fraeye et al., 2012). However, the increase in amounts of n – 3 polyunsaturated fatty acids (PUFA) in the egg yolk has been paralleled by a decrease in n – 6 PUFA, especially ARA (Bean & Leeson, 2003; Hayat, Cherian, Pasha, Khattak, & Jabbar, 2009). The organoleptic quality of the n – 3 PUFA enriched eggs tends to be similar to regular eggs, although in some cases panelists are able to detect off-flavours (Caston, Squires, & Leeson, 1994; Elswyk, Sams, & Hargis, 1992). In contrast, we found that the allele exhibiting high ARA, also has a low n – 6/n – 3 ratio in *FADS1*. A similar effect on *FADS2* for the n – 6/n – 3 ratio was reported by Khang et al. (2007) in Japanese quail. These data suggest that the n – 6/n – 3 ratio of eggs is genetically changeable by utilizing gene polymorphisms of enzymes in the pathway from LA to ARA, without using feed additives.

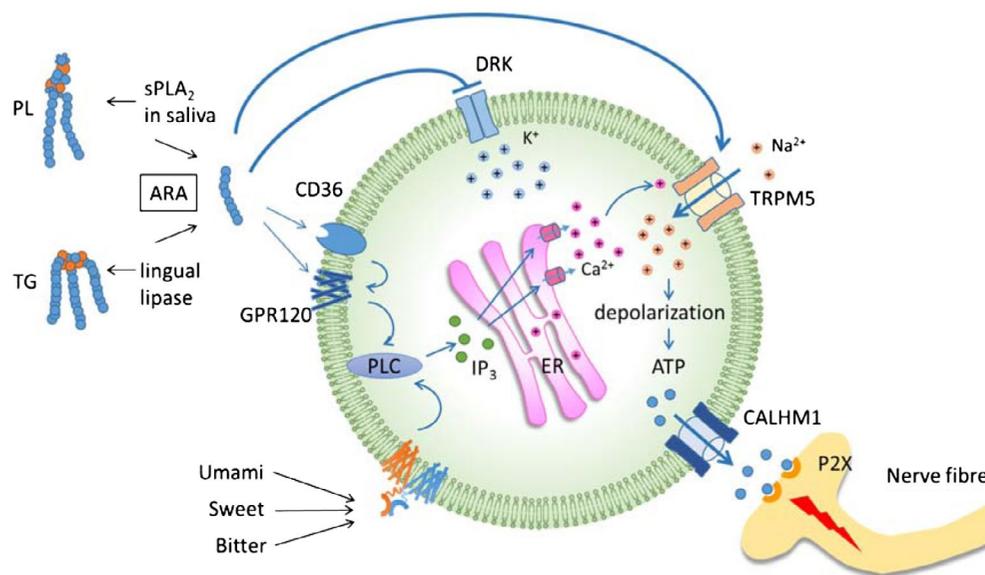
The fact that the addition of AAO to cooked foods improves the taste is widely recognized in Japan. For example, when foods, such as vegetable soup, croquettes, and fried rice, are cooked in vegetable oil containing AAO, the palatability index of the foods increased (Kiyohara, Yamaguchi, Ushio, Shimomura, & Ichikawa, 2009; Yamaguchi, Tajima, & Matsuzaki, 2005). AAO-supplemented cooking and frying oils are commercially available from J-OIL MILLS, Inc. (Yokohama, Japan) in the Japanese market. However, a mechanism to explain the effect of ARA regarding the enhancement of food taste has not been yet satisfactorily elucidated.

Dietary fat including AAO is predominantly in the form of triglycerides, which are not effective taste stimuli. Kawai and Fushiki (2003) proposed that lingual lipase yields free fatty acids (FFA) from triglycerides rapidly enough to enable detection by a fat sensor on the tongue surface. Recently, the CD36 and the G-protein coupled receptors (i.e. GPR120 and GPR40) have been identified as putative FFA taste receptors (Cartoni et al., 2010; Laugerette et al., 2005). Because CD36 is expressed in some type II (sweet, bitter, and umami) receptor cells in mouse taste buds (Laugerette et al., 2005), and GPR120 and GPR40 are mainly expressed in type II and type I (salty) receptor cells (Cartoni et al., 2010), FFA may affect taste perception of sweet, bitter, umami, and salty based on taste receptor distribution. However, the presence of GPR40 has not been confirmed in the gustatory papillae of humans (Galindo et al., 2012). Gilbertson, Fontenot, Liu, Zhang, and Monroe (1997) reported that PUFAs, especially LA, ARA, DHA, and eicosapentaenoic acid (EPA), were able to inhibit the delayed rectifying K⁺ (DRK) channels. The inhibition of DRK channels may elicit a fast cell depolarization due to transient accumulation of positive charges in taste bud cells, since K⁺ is the major intracellular monovalent cation. Oike et al. (2006) reported that ARA activates the TRPM5 cation channel, which is a component of the sweet, bitter, and umami taste pathways of type II receptor cells. In addition, TRPM5-null mice showed no licking response to a sweet tastant, a diminished preference ratio for sweet and umami tastants, and a reduced response to bitter taste (Damak et al., 2006). Together, these data suggest that ARA may serve as a flavour enhancer for type II receptor cells by modulating the TRPM5 channel. We proposed the following model of fatty acid signal transduction in type II receptor cells (Figure 1). In the present study, AAO added to the yolk seasoned with soy sauce may be digested by lingual lipase rapidly and the resultant free ARA may enhance egg flavour intensity and continuity.

Of yolk lipids, except cholesterol, triglycerides and phospholipids constitute approximately two-thirds and one-thirds, respectively (Awad, Bennink, & Smith, 1997). In yolk, ARA presents almost entirely in the second carbon group of glycerol of phospholipids (Gładkowski et al., 2011). Therefore,

Figure 1. Proposed signalling pathway in type II receptor cells.

Notes: ARA inhibits DRK channel and activates TRPM5 channel. Intracellular K^+ ions and influx of Na^+ ions through TRPM5 cause depolarization of the cells and firing of action potentials. ARA: arachidonic acid; CALHM1; calcium homeostasis modulator 1; DRK: delayed rectifying K^+ channels; ER: endoplasmic reticulum; IP_3 : type 3 isoform of the inositol 1,4,5-trisphosphate; P2X: purinergic receptors P2X; PL: phospholipids; PLC: phospholipase C; $sPLA_2$: secreted phospholipase A_2 and TG: triglycerides. Source: We propose a model referring to Bensard, Passilly-Degrace, and Khan (2016).



to release ARA from the second carbon group of glycerol of phospholipids, secreted phospholipase A_2 ($sPLA_2$) is required in saliva. Indeed, $sPLA_2$ activity has been reported in acinar cells and the apical plasma membrane fraction from salivary glands in the rat (Mizuno-Kamiya, Inokuchi, Kameyama, Yashiro, & Fujita, 2001; Takuma & Ichida, 1997). Komiyama et al. (2009) reported that type V $sPLA_2$ ($sPLA_2$ -V) was expressed in the human parotid and submandibular glands under disease-free conditions. The salivary $sPLA_2$ -V is suggested to be responsible for innate immunity together with other saliva-derived antimicrobial agents, e.g. lysozyme and bactericidal permeability-increasing proteins, because $sPLA_2$ -V exerts bactericidal activity toward Gram-positive bacteria (Koduri et al., 2002). We hypothesize that yolk phospholipids are digested by the salivary $sPLA_2$ -V, free ARA is released, and the resultant ARA affects the taste perception of the yolk. We conclude that free ARA generated in the oral cavity is a key substance that enhances egg flavour, even if experimental demonstration of the effects of ARA on egg flavour was performed using the triglyceride form of ARA in this study.

5. Conclusion

In conclusion, this is the first report to demonstrate the possibility of using a SNP of the *FADS1* as a selection marker for Suruga-shamo chickens to improve fatty acid profiles, especially ARA composition and the $n - 6/n - 3$ ratio. The data suggested that a breeding strategy for improving the flavour and $n - 6/n - 3$ ratio of eggs could be developed using a *FADS1* SNP as a selection marker in Suruga-shamo chickens. However, further studies are needed to determine whether the SNP effects are applicable to the other chicken strains. Moreover, this report provides evidence that ARA content in yolk is positively associated with egg flavour.

Abbreviations

AAO	arachidonic acid-enriched oil
ARA	arachidonic acid
CALHM1	calcium homeostasis modulator 1
CP	crude protein
DHA	docosahexaenoic acid
DRK	delayed rectifying K^+ channels
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum

FADS1	fatty acid desaturase 1 gene
FADS2	fatty acid desaturase 2 gene
FFA	free fatty acids
LA	linoleic acid
IP3	type 3 isoform of the inositol 1,4,5-trisphosphate
MAMA	mismatch amplification mutation assay
ME	metabolizable energy
MUFA	monounsaturated fatty acids
P2X	purinergic receptors P2X
PL	phospholipids
PLC	phospholipase C
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SNP	single-nucleotide polymorphism
sPLA ₂	secreted phospholipase A2
TG	triglycerides

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Competing Interests

The authors declare no competing interest.

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee of Shizuoka Prefectural Research Institute of Animal Industry. All animals received humane care as outlined in the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006). Sensory evaluation in this study was performed at a third party institution (Daiwa Service Co., Sagami-hara, Japan). The company approved the sensory evaluation. All sensory panelists understood details of the research and consented to participation.

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Cover image

Source: Author.

References

- Awad, A. C., Bennink, M. R., & Smith, D. M. (1997). Composition and functional properties of cholesterol reduced egg yolk. *Poultry Science*, 76, 649–653. <http://dx.doi.org/10.1093/ps/76.4.649>
- Bean, L. D., & Leeson, S. (2003). Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens. *Poultry Science*, 82, 388–394. <http://dx.doi.org/10.1093/ps/82.3.388>
- Bensard, P., Passilly-Degrace, P., & Khan, N. A. (2016). Taste of fat: a sixth taste modality? *Physiological Reviews*, 96, 151–176.
- Cartoni, C., Yasumatsu, K., Ohkuri, T., Shigemura, N., Yoshida, R., Godinot, N., ... Damak, S. (2010). Taste preference for fatty acids is mediated by GPR40 and GPR120. *Journal of Neuroscience*, 30, 8376–8382. <http://dx.doi.org/10.1523/JNEUROSCI.0496-10.2010>
- Caston, L. J., Squires, E. J., & Leeson, S. (1994). Hen performance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax. *Canadian Journal of Animal Science*, 74, 347–353. <http://dx.doi.org/10.4141/cjas94-047>
- Cha, R. S., Zarbl, H., Keohavong, P., & Thilly, W. G. (1992). Mismatch amplification mutation assay (MAMA): Application to the c-H-ras gene. *Genome Research*, 2, 14–20. <http://dx.doi.org/10.1101/gr.2.1.14>
- Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Perez, C. A., Shigemura, N., ... Margolskee, R. F. (2006). Trpm5 null mice respond to bitter, sweet, and umami compounds. *Chemical Senses*, 31, 253–264.

- Elswyk, M. E., Sams, A. R., & Hargis, P. S. (1992). Composition, functionality, and sensory evaluation of eggs from hens fed dietary menhaden oil. *Journal of Food Science*, 57, 342–344.
<http://dx.doi.org/10.1111/jfds.1992.57.issue-2>
- Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K., & Foubert, I. (2012). Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Research International*, 48, 961–969.
<http://dx.doi.org/10.1016/j.foodres.2012.03.014>
- Galindo, M. M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J. D., Hofmann, T., ... Behrens, M. (2012). G protein-coupled receptors in human fat taste perception. *Chemical Senses*, 37, 123–139.
<http://dx.doi.org/10.1093/chemse/bjr069>
- Gilbertson, T. A., Fontenot, D. T., Liu, L., Zhang, H., & Monroe, W. T. (1997). Fatty acid modulation of K⁺ channels in taste receptor cells: gustatory cues for dietary fat. *American Journal of Physiology - Cell Physiology*, 272, C1203–C1210.
- Gladkowski, W., Kielbowicz, G., Chojnacka, A., Gil, M., Trziszka, T., Dobrzański, Z., & Wawrzęńczyk, C. (2011). Fatty acid composition of egg yolk phospholipid fractions following feed supplementation of Lohmann Brown hens with humic-fat preparations. *Food Chemistry*, 126, 1013–1018.
<http://dx.doi.org/10.1016/j.foodchem.2010.11.112>
- Hayat, Z., Cherian, G., Pasha, T. N., Khattak, F. M., & Jabbar, M. A. (2009). Effect of feeding flax and two types of antioxidants on egg production, egg quality, and lipid composition of eggs. *The Journal of Applied Poultry Research*, 18, 541–551.
<http://dx.doi.org/10.3382/japr.2009-00008>
- Kawai, T., & Fushiki, T. (2003). Importance of lipolysis in oral cavity for orosensory detection of fat. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 285, R447–R454.
<http://dx.doi.org/10.1152/ajpregu.00729.2002>
- Khang, N. T., Jennen, D. G. J., Tholen, E., Tesfaye, D., Mennicken, L., Hoelker, M., ... Wimmers, K. (2007). Association of the FADS2 Gene with ω -6 and ω -3 PUFA Concentration in the egg yolk of Japanese Quail. *Animal Biotechnology*, 18, 189–201.
<http://dx.doi.org/10.1080/10495390701201390>
- Kiyohara, R., Yamaguchi, S., Rikimaru, K., & Takahashi, H. (2011). Supplemental arachidonic acid-enriched oil improves the taste of thigh meat of Hinai-jidori chickens. *Poultry Science*, 90, 1817–1822.
<http://dx.doi.org/10.3382/ps.2010-01323>
- Kiyohara, R., Yamaguchi, S., Ushio, H., Shimomura, M., & Ichikawa, T. (2009). Effect of adding arachidonic acid to cooked foods. *Journal of Cookery Science of Japan*, 42, 294–299. (in Japanese).
- Koduri, R. S., Gronroos, J. O., Laine, V. J., Le Calvez, C., Lambeau, G., Nevalainen, T. J., & Gelb, M. H. (2002). Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A2. *Journal of Biological Chemistry*, 277, 5849–5857.
<http://dx.doi.org/10.1074/jbc.M109699200>
- Komiyama, K., Tsuruta, T., Mukae, S., Amano, Y., Okazaki, Y., Matsumoto, M., ... Ishii, T. (2009). In vivo localization of secretory type V phospholipase A2 (sPLA2-V) in human salivary glands under normal and pathological conditions. *Oral Medicine & Pathology*, 13, 99–104.
- Laugerette, F., Passilly-Degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J. P., & Besnard, P. (2005). CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *Journal of Clinical Investigation*, 115, 3177–3184.
<http://dx.doi.org/10.1172/JCI25299>
- Mizuno-Kamiya, M., Inokuchi, H., Kameyama, Y., Yashiro, K., & Fujita, A. (2001). Ca²⁺-independent phospholipase A₂ activity in apical plasma membranes from the rat parotid gland. *Archives of Oral Biology*, 46, 789–799.
[http://dx.doi.org/10.1016/S0003-9969\(01\)00050-4](http://dx.doi.org/10.1016/S0003-9969(01)00050-4)
- National Agriculture and Food Research Organization (2011). *Japanese Feeding Standard for Poultry*. Tokyo: Central Association of Livestock Industry. (in Japanese).
- Oike, H., Wakamori, M., Mori, Y., Nakanishi, H., Taguchi, R., Misaka, T., ... Abe, K. (2006). Arachidonic acid can function as a signaling modulator by activating the TRPM5 cation channel in taste receptor cells. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1761, 1078–1084.
<http://dx.doi.org/10.1016/j.bbali.2006.07.005>
- Rikimaru, K., Egawa, Y., Yamaguchi, S., & Takahashi, H. (2016). 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. *Journal of Fisheries & Livestock Production*, 4, 202. doi:10.4172/2332-2608.1000202
- Rikimaru, K., & Takahashi, H. (2010). Evaluation of the meat from Hinai-jidori chickens and broilers: Analysis of general biochemical components, free amino acids, inosine 5'-monophosphate, and fatty acids. *The Journal of Applied Poultry Research*, 19, 327–333.
<http://dx.doi.org/10.3382/japr.2010-00157>
- Science Council of Japan. (2006). *Guidelines for proper conduct of animal experiments*. Retrieved from <http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf>
- Smith, L. M., & Burgoyne, L. A. (2004). Collecting, archiving and processing DNA from wildlife samples using FTA[®] databasing paper. *BMC Ecology*, 4, 4.
<http://dx.doi.org/10.1186/1472-6785-4-4>
- Takahashi, H., Rikimaru, K., Kiyohara, R., & Yamaguchi, S. (2012). Effect of arachidonic acid-enriched oil diet supplementation on the taste of broiler meat. *Asian Australasian Journal of Animal Sciences*, 25, 845–851.
<http://dx.doi.org/10.5713/ajas.2011.11517>
- Takuma, T., & Ichida, T. (1997). Role of Ca²⁺-independent phospholipase A2 in exocytosis of amylase from parotid acinar cells. *Journal of Biochemistry*, 121, 1018–1024.
<http://dx.doi.org/10.1093/oxfordjournals.jbchem.a021688>
- Tregouet, D. A., Barbaux, S., Escolano, S., Tahri, N., Golmard, J. L., Tiret, L., & Cambienet, F. (2002). Specific haplotypes of the P-selectin gene are associated with myocardial infarction. *Human Molecular Genetics*, 11, 2015–2023.
<http://dx.doi.org/10.1093/hmg/11.17.2015>
- Tregouet, D. A., & Garelle, V. (2007). A new JAVA interface implementation of THEIAS: Testing haplotype effects in association studies. *Bioinformatics*, 23, 1038–1039.
<http://dx.doi.org/10.1093/bioinformatics/btm058>
- Yamaguchi, S., Tajima, Y., & Matsuzaki, N. (2005). *Body taste improver comprising long-chain highly unsaturated fatty acid and/or ester thereof and vegetable fat composition containing the same*. Japanese Patent, No. 3729272. Tokyo: Japan Patent Office.
- Yamamoto, T., Watanabe, U., Fujimoto, M., & Sako, N. (2009). Taste preference and nerve response to 5'-inosine monophosphate are enhanced by glutathione in mice. *Chemical Senses*, 34, 809–818.
<http://dx.doi.org/10.1093/chemse/bjp070>
- Zhu, S. K., Tian, Y. D., Zhang, S., Chen, Q. X., Wang, Q. Y., Han, R. L., & Kang, X. T. (2014). Adjacent SNPs in the transcriptional regulatory region of the FADS2 gene associated with fatty acid and growth traits in chickens. *Genetics and Molecular Research*, 13, 3329–3336.
<http://dx.doi.org/10.4238/2014.April.29.11>



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