

« Research Note »

## Fatty Acid Taste Receptor GPR120 Activation by Arachidonic Acid, Eicosapentaenoic Acid, and Docosahexaenoic Acid in Chickens

Fuminori Kawabata<sup>1</sup>, Yuta Yoshida<sup>2,3</sup>, Seiga Kuba<sup>3</sup>, Yuko Kawabata<sup>4</sup>, Shotaro Nishimura<sup>3</sup> and Shoji Tabata<sup>3</sup>

<sup>1</sup>Physiology of Domestic Animals, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan

<sup>2</sup>Department of Food and Life Sciences, Ibaraki University, Ami 300-0393, Japan

<sup>3</sup>Laboratory of Functional Anatomy, Faculty of Agriculture, Kyushu University, Fukuoka 819-0395, Japan

<sup>4</sup>Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

It has been reported that the supplementation of chicken diet with polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA) affects the qualities of eggs and meat. Previous studies have shown that a functional fatty acid taste receptor, G protein-coupled receptor 120 (GPR120), is broadly expressed in chicken oral and gastrointestinal tissues, and chickens have a gustatory perception of oleic acid, which is a chicken GPR120 agonist. The aim of this study was to elucidate the role of chicken GPR120 in response to PUFAs in chicken diets.  $Ca^{2+}$  imaging analyses revealed that chicken GPR120 was activated by AA, EPA, and DHA in a concentration-dependent manner. These results suggest that chickens can detect PUFAs *via* GPR120 in the oral and gastrointestinal tissues, implying that chickens have a gustatory perception of PUFAs.

**Key words:** arachidonic acid, chicken, docosahexaenoic acid, eicosapentaenoic acid, GPR120

*J. Poult. Sci.*, 59: 282–285, 2022

### Introduction

Lipids are one of the three major nutrients in feed; the other two are carbohydrates and proteins. Lipids enhance the palatability of feed in chickens. In fact, chickens prefer feed that contain long-chain triacylglycerol (LCT), and this preference is eliminated by oral anesthetization (Furuse *et al.*, 1996). Therefore, chickens can detect lipids *via* orosensory perception. Recently, studies on peripheral, cortical, and human perception levels have suggested that fatty acids from lipids elicit a unique taste (Running *et al.*, 2015; Yasumatsu *et al.*, 2019; Andersen *et al.*, 2020). A recent study revealed that a specific gustatory nerve fiber, the F-type fiber, responds best to fatty acids among the various tastants and transmits fatty acid taste quality information *via* G protein-coupled receptor 120 (GPR120) (Yasumatsu *et al.*, 2019).

In addition to the gene expression of several lipases that metabolize triacylglycerol to fatty acids, GPR120 is widely

expressed in chicken oral and gastrointestinal tissues (Kawabata *et al.*, 2018). We previously demonstrated that chicken GPR120 (cGPR120) is activated by oleic acid (OA) and linoleic acid (LA), and that chickens showed a preference in behavioral tests for feed that contain corn oil, which mainly contains OA and LA, over feed that contain mineral oil (Sawamura *et al.*, 2015). More recently, we found that chickens disliked the taste of OA when they were conditioned to feel bad at the ingestion of OA (Kawabata *et al.*, 2021). This suggests that chickens have a gustatory perception for OA, a GPR120 agonist.

Supplementation of chicken diets with polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) has been studied with the aim of enhancing egg and meat quality. Supplementation of the diet with AA improved the AA content and umami and kokumi taste intensity of thigh meat (Kiyohara *et al.*, 2011; Takahashi *et al.*, 2012). In addition, supplementation of chicken diets with fish oil, which contains EPA and DHA, enriches the EPA and DHA contents of the eggs and thigh meat (Huang *et al.*, 1990). However, it is unknown whether chickens have receptors for the detection of PUFAs as it has not been investigated. Elucidating the recognition mechanism of PUFAs in chickens is important not only for understanding the gustatory perception of the PUFA-enriched diet in chickens but also to clarify whether dietary supplementation with n-3 fatty acids such as EPA and

Received: September 10, 2021, Accepted: September 29, 2021

Released Online Advance Publication: November 25, 2021

Correspondence: Dr. Fuminori Kawabata, Physiology of Domestic Animals, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan. (E-mail: kawabata@hirosaki-u.ac.jp)

The Journal of Poultry Science is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. To view the details of this license, please visit (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

DHA triggers beneficial effects, such as the anti-inflammatory effects of the n-3 fatty acid receptor GPR120, which have been reported in rodents (Oh *et al.*, 2010). This study is aimed at investigating whether cGPR120 is activated by representative PUFAs such as AA, EPA, and DHA using  $\text{Ca}^{2+}$  imaging analyses.

## Materials and Methods

### Chemicals

LA, AA, EPA, DHA, and adenosine 5-triphosphate disodium salt hydrate (ATP) were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). LA and AA were dissolved in dimethyl sulfoxide (Nacalai Tesque, Kyoto, Japan), EPA and DHA were dissolved in ethanol (Nacalai Tesque), and ATP was dissolved in ultrapure water: these stock solutions were stored at  $-20^{\circ}\text{C}$ .

### Cell Culture

Human embryonic kidney (HEK)-derived 293T (HEK293T) cells were maintained in Dulbecco's modified Eagle's medium (DMEM high glucose, FUJIFILM Wako Pure Chemical Corporation) containing 10% fetal bovine serum (GE Healthcare, Buckinghamshire, UK) and penicillin-streptomycin solution ( $\times 100$ ) (FUJIFILM Wako Pure Chemical Corporation) at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$ .

### $\text{Ca}^{2+}$ Imaging

$\text{Ca}^{2+}$  imaging analyses were conducted as described in our previous report (Kawabata *et al.*, 2021). HEK293T cells were transfected with either empty vector pcDNA3.1(+) or cGPR120/pcDNA3.1(+) using ScreenFect A (FUJIFILM Wako Pure Chemical Corporation) on a 96-well clear-bottom black plate (Thermo Fisher Scientific, Waltham, MA, USA) coated with poly-D-lysine (0.1 mg/mL, FUJIFILM Wako Pure Chemical Corporation). After transfection, the cells were incubated for 48 h at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ . Fura 2-AM solution was then loaded into the wells according to the manufacturer's manual for the Calcium Kit II–Fura 2 (Dojindo Laboratories, Kumamoto, Japan). After incubation for 1 h in the dark at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ ,  $\text{Ca}^{2+}$  imaging was performed using a multi-mode microplate reader (FlexStation 3, Molecular Devices, San Jose, CA, USA). The assay was performed at approximately  $37^{\circ}\text{C}$ , and 5–50 mmol/L LA, 0.5–50 mmol/L AA, 0.5–35 mmol/L EPA, and 0.5–15 mmol/L DHA solutions diluted with standard bath solution (containing 140 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L  $\text{MgCl}_2$ , 2 mmol/L  $\text{CaCl}_2$ , 10 mmol/L HEPES, and 10 mmol/L glucose at pH 7.4, adjusted with NaOH just before each experiment) were applied. The final concentrations of fatty acid solutions in the well were 1–10 mmol/L LA, 0.1–10 mmol/L AA, 0.1–7 mmol/L EPA, and 0.1–3 mmol/L DHA after injections. Cell activity was analyzed based on the ratio between the fluorescence intensity excited at 340 nm and that excited at 380 nm before injection and the corresponding values after injection. Cell viability was confirmed using the response to 5  $\mu\text{mol/L}$  ATP.

### Statistical Analysis

The data are expressed as mean  $\pm$  SE. Statistical analyses were performed using two-way repeated analysis of variance and an unpaired *t*-test. The analyses, fitting with the Hill

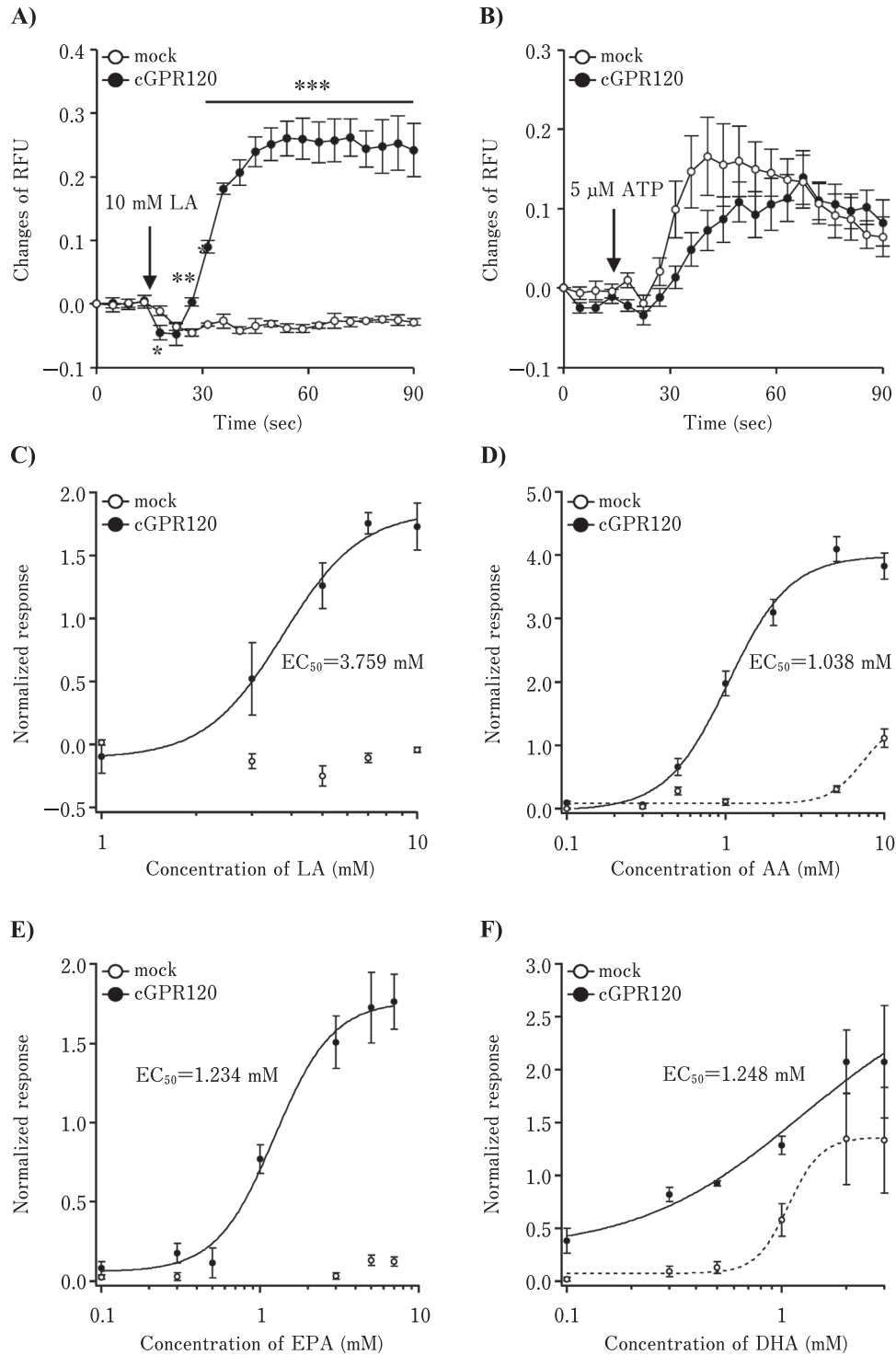
equation, and the calculation of the  $\text{EC}_{50}$  value were performed using the IGOR Pro software package (Version 6.34 J, WaveMetrics, Portland, OR, USA), and differences with *P*-values  $< 0.05$  were considered significant.

## Results and Discussion

First, we observed that the relative fluorescent units, which are an index of intracellular  $\text{Ca}^{2+}$ , increased owing to the injection of 10 mmol/L LA in HEK293T cells, transiently expressing cGPR120 (cGPR120 cells), but not in the HEK293T cells transfected with empty vector (mock cells) (Fig. 1A). The mock cells were activated by 5  $\mu\text{mol/L}$  ATP in the same way as the cGPR120 cells, suggesting that cell viabilities were not different between cGPR120 cells and mock cells (Fig. 1B). In addition, cGPR120 cells were activated in a dose-dependent manner by LA (Fig. 1C). These results corroborate our previous results (Sawamura *et al.*, 2015), suggesting that  $\text{Ca}^{2+}$  imaging analyses could successfully detect the activity of cGPR120 for fatty acids *in vitro*.

Therefore, we examined the activity of cGPR120 in PUFAs using  $\text{Ca}^{2+}$  imaging analyses. We observed that AA activated cGPR120 in a dose-dependent manner (Fig. 1D). Although we observed that mock cells were activated by a high concentration of AA (10 mmol/L), the  $\text{EC}_{50}$  of cGPR120 cells for AA was 1.038 mmol/L, suggesting that cGPR120 cells were activated by AA *via* cGPR120 (Fig. 1D). In addition, we observed that EPA and DHA activated cGPR120 in a dose-dependent manner (Fig. 1E, F). Similar to AA, a high concentration of DHA activated mock cells. Although cGPR120 cells were activated by a low concentration of DHA, mock cells were not (Fig. 1F). These results suggest that cGPR120 is activated by PUFAs such as AA, EPA, and DHA. The expression of *GPR120* has been observed in chicken oral tissues (Kawabata *et al.*, 2018), and chickens have gustatory perception of the GPR120 agonist, OA (Kawabata *et al.*, 2021). Furthermore, chickens prefer an LCT-containing diet (Furuse *et al.*, 1996) and corn-oil-rich diets, which contain OA and LA (Sawamura *et al.*, 2015). Therefore, it is possible that chickens have preference for a PUFA-rich diet and that the supplementation of the diet with PUFAs not only helps to improve the quality of the eggs and meat, but also help to improve the palatability of the diet to chickens.

The mRNA of *GPR120* is also expressed in the gastrointestinal tract tissues of chickens (Kawabata *et al.*, 2018). In mice, it has been reported that GPR120 is expressed in glucagon-like peptide-1 (GLP-1) secretion cells (L cells) and gastric inhibitory polypeptide (GIP) secretion cells (K cells) (Hara *et al.*, 2011). GLP-1 and GIP are mammalian incretin hormones, and chronic n-3 fatty acid intake elevates plasma GLP-1 levels and increases insulin response in obese mice (Pavlisova *et al.*, 2020). In chickens, proteins and amino acids such as lysine and methionine are triggers that induce GLP-1 secretion from ileal L cells (Hiramatsu, 2020); however, it has not been confirmed whether GPR120 is co-expressed with GLP-1 in chicken gastrointestinal tissues. Furthermore, it is not clear whether PUFA intake induces GLP-1 secretion from the gastrointestinal tissues of chickens.



**Fig. 1. Responses of HEK293T cells transfected with chicken GPR120 (cGPR120 cells) or an empty vector (mock cells) to linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexanoic acid (DHA) solutions.** (A) Ten mmol/L LA did not increase the relative fluorescent units (RFUs) of mock cells ( $n=4$  wells), whereas 10 mmol/L LA increased the RFUs of cGPR120 cells ( $n=4$  wells). (B) Five  $\mu$ mol/L ATP activated both cGPR120 and mock cells ( $n=4$  wells). (A, B) The arrows indicate the injection timing. Data were analyzed using two-way repeated analysis of variance, followed by an unpaired  $t$ -test. \*  $P<0.05$ ; \*\*  $P<0.01$ ; \*\*\*  $P<0.001$ . (C-F) The RFU changes in mock cells and cGPR120 cells for each concentration of LA (C), AA (D), EPA (E), and DHA (F) were normalized by each 5  $\mu$ mol/L ATP response on the same  $Ca^{2+}$  imaging plate. Normalized data were plotted and fitted with the Hill equation ( $n=4$  wells for each concentration). (A-F) Values are the means  $\pm$  SE.

Because cGPR120 is the receptor for fatty acids, it is possible that cGPR120 monitors the concentrations of fatty acids in gastrointestinal tissues and oral tissues. Further studies are needed to clarify the relationship between GPR120 and the secretion of GLP-1 and GIP, and the physiological role of GPR120 in chicken gastrointestinal tissues.

n-3 fatty acids induce potent anti-inflammatory effects *via* GPR120 in monocytes and macrophages (Oh *et al.*, 2010). The present study revealed that cGPR120 was activated by EPA and DHA, which are classified as n-3 fatty acids, hence, it is possible that dietary supplementation with n-3 fatty acids also induces anti-inflammatory effects *via* GPR120 in chickens. Although the addition of AA to chicken feed improves the taste and flavor of thigh meat (Takahashi *et al.*, 2012), the effects of adding AA to feed on the feed intake of chickens have not been elucidated. Further studies are needed to reveal whether AA addition to feeds increases palatability *via* cGPR120. It is also important to clarify whether AA addition to feeds brings about some physiological effects through cGPR120 other than taste in chickens. High AA content in chicken meat and eggs improves the flavor of meat and eggs (Takahashi, 2018). Furthermore, increasing the content of EPA and DHA in chicken meat and eggs also contributes to human health by making an important delivery route of n-3 fatty acids to humans (Konieczka *et al.*, 2017). Therefore, it is important that studies on the relationship between PUFA and GPR120 in chickens be deeply studied.

In conclusion, the present study demonstrated that representative PUFAs such as AA, EPA, and DHA, which have beneficial effects on the quality of chicken eggs and meat, activated chicken fatty acid taste receptor GPR120.

### Acknowledgements

This study was supported by grants to F. Kawabata from JSPS KAKENHI (#17K08047 and #19KT0005) and S. Tabata from JSPS KAKENHI (#18H02330).

### Conflict of Interest

The authors declare no conflict of interest.

### References

- Andersen CA, Nielsen L, Møller S and Kidmose P. Cortical response to fat taste. *Chemical Senses*, 45: 283–291. 2020.
- Furuse M, Mabayo RT and Okumura J. The role of gustation in oil preference in the chicken. *Japanese Poultry Science*, 33: 256–260. 1996.
- Hara T, Hirasawa A, Ichimura A, Kimura I and Tsujimoto G. Free fatty acid receptors FFAR1 and GPR120 as novel therapeutic targets for metabolic disorders. *Journal of Pharmaceutical Sciences*, 100: 3594–3601. 2011.
- Hiramatsu K. Chicken intestinal L cells and glucagon-like peptide-1 secretion. *Journal of Poultry Science*, 57: 1–6. 2020.
- Huang Z, Leibovitz H, Lee CM and Millar R. Effect of dietary fish oil on  $\omega$ -3 fatty acid levels in chicken eggs and thigh flesh. *Journal of Agricultural and Food Chemistry*, 38: 743–747. 1990.
- Kawabata Y, Kawabata F, Nishimura S and Tabata S. Oral lipase activities and fat-taste receptors for fat-taste sensing in chickens. *Biochemical and Biophysical Research Communications*, 495: 131–135. 2018.
- Kawabata F, Yoshida Y, Inoue Y, Kawabata Y, Nishimura S and Tabata S. Research Note: Behavioral preference and conditioned taste aversion to oleic acid solution in chickens. *Poultry Science*, 100: 372–376. 2021.
- Kiyohara R, Yamaguchi S, Rikimaru K and Takahashi H. Supplemental arachidonic acid-enriched oil improves the taste of thigh meat of Hinai-jidori chickens. *Poultry Science*, 90: 1817–1822. 2011.
- Konieczka P, Czauderna M and Smulikowska S. The enrichment of chicken meat with omega-3 fatty acids by dietary fish oil or its mixture with rapeseed or flaxseed—Effect of feeding duration: Dietary fish oil, flaxseed, and rapeseed and n-3 enriched broiler meat. *Animal Feed Science and Technology*, 223: 42–52. 2017.
- Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM and Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*, 142: 687–698. 2010.
- Pavlisova J, Horakova O, Kalendova V, Buresova J, Bardova K, Holendova B, Plecita-Hlavata L, Vackova S, Windrichova J, Topolcan O, Kopecky J and Rossmeisl M. Chronic n-3 fatty acid intake enhances insulin response to oral glucose and elevates GLP-1 in high-fat diet-fed obese mice. *Food & function*, 11: 9764–9775. 2020.
- Running CA, Craig BA and Mattes RD. Oleogustus: the unique taste of fat. *Chemical Senses*, 40: 507–516. 2015.
- Sawamura R, Kawabata Y, Kawabata F, Nishimura S and Tabata S. The role of G-protein-coupled receptor 120 in fatty acids sensing in chicken oral tissues. *Biochemical and Biophysical Research Communications*, 458: 387–391. 2015.
- Takahashi H, Rikimaru K, Kiyohara R and Yamaguchi S. Effect of arachidonic acid-enriched oil diet supplementation on the taste of broiler meat. *Asian-Australasian Journal of Animal Sciences*, 25: 845–851. 2012.
- Takahashi H. Association between arachidonic acid and chicken meat and egg flavor, and their genetic regulation. *Journal of Poultry Science*, 55: 163–171. 2018.
- Yasumatsu K, Iwata S, Inoue M and Ninomiya Y. Fatty acid taste quality information *via* GPR120 in the anterior tongue of mice. *Acta Physiologica*, 226: e13215. 2019.