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Bitter Taste Perception in Chickens

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Many behavioral studies and histological analyses of the sense of taste have been conducted in chickens, as it plays an important role in the ingestion of feed. In recent years, various taste receptors have been analyzed, and the functions of fatty acids, umami, and bitter taste receptors in chickens have become clear. In this review, the bitter taste sense in chickens, which is the taste quality by which animals reject poisons, is discussed among a variety of taste qualities. Chickens have taste buds in the palate, the base of the oral cavity, and the root of the tongue. Bitter taste receptors, taste receptor type 2 members 1, 2, and 7 (T2R1, T2R2, and T2R7) are expressed in these tissues. According to functional analyses of bitter taste receptors and behavioral studies, T2R1 and T2R7 are thought to be especially involved in the rejection of bitter compounds in chickens. Furthermore, the antagonists of these two functional bitter taste receptors were also identified, and it is expected that such antagonists will be useful in improving the taste quality of feed materials and poultry drugs that have a bitter taste. Bitter taste receptors are also expressed in extra-oral tissues, and it has been suggested that gastrointestinal bitter taste receptors may be involved in the secretion of gastrointestinal hormones and pathogen defense mechanisms. Thus, bitter taste receptors in chickens are suspected to play major roles in taste sensing and other physiological systems.

Key words: bitter taste receptor, bitterness, chicken, T2R

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Introduction

Animals' sense of taste plays a very important role in their choice of suitable feeds to ingest. The five basic tastes are known to comprise sweet, umami, bitter, sour, and salty taste qualities. Sweet and umami taste perception is involved in detecting energy sources such as carbohydrates and amino acids, respectively. Bitter and sour taste perception is involved in the detection and rejection of toxic and spoiled ingredients, respectively. Salty taste perception plays a role in maintaining mineral balance, and animals prefer suitable concentrations of sodium chloride (sodium taste) and avoid excess salt (high-salt taste). Sodium and high-salt tastes have different taste qualities (Oka *et al*., 2013). In addition, fatty acid and calcium tastes have been proposed as other taste qualities (Tordoff, 2001; Yasumatsu *et al*., 2019). Fatty acid taste perception is involved in the detection of fat and oil, which are energy sources. Calcium taste perception makes the ingestion of calcium more desirable. Each taste quality has a physiological purpose. In this review, the sense of taste in chickens, especially bitter taste perception, is discussed.

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Taste Sense in Chickens

On the chicken tongue, taste buds are present only at the root. Other taste buds in chickens are located mainly on the palate and on the base of the oral cavity (Kurosawa *et al*., 1983; Kudo *et al*., 2008). In chicks, there are about 300−400 taste buds on the palate and about 100−150 taste buds on the base of the oral cavity, as confirmed by scanning electron microscopy analysis (Rajapaksha *et al*., 2016). Taste buds gather in groups of 1−10 to form clusters, and these clusters are broadly distributed on the palate and the base of the oral cavity (Fig. 1a). There are two types of taste bud clusters. One type surrounds the salivary gland openings, and the other type does not. Moreover, in chickens, taste buds exist at high densities in oral locations where feed hits during swallowing (Berkhoudt, 1985). Because there are no taste buds at the tip or center of the tongue, it is presumed that the tongue plays a role in physically sending feed to the pharynx, specifically with regard to the partial digestion and swallowing of feed by means of salivary secretion from the

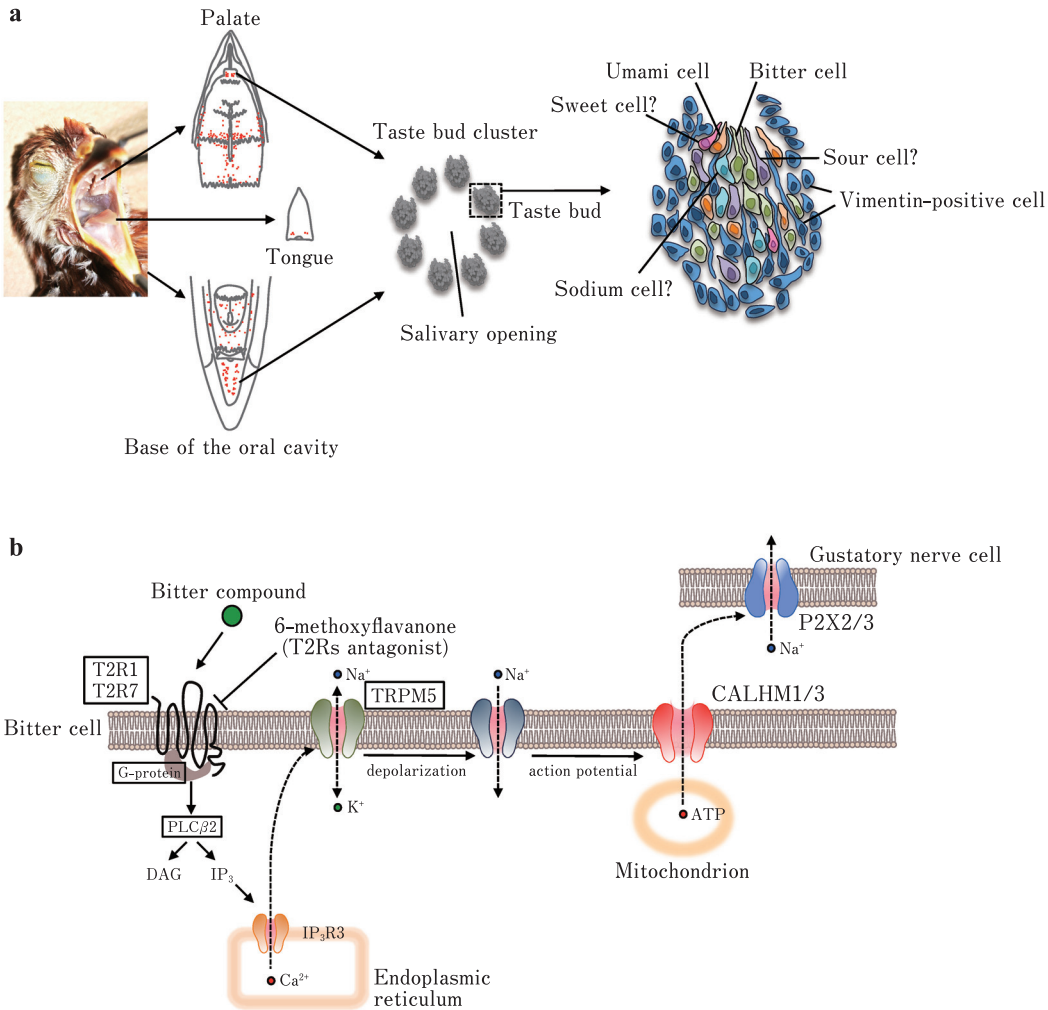

Fig. 1. **Taste bud distributions and signal transduction of bitter taste in chickens.** (**a**) Taste bud clusters are broadly distributed in the palate and the base of the oral cavity. However, in the tongue, a small number of taste buds are present at the root of the tongue only. The red dots show the taste pores. Many taste buds have a spindle shape, and all of the taste buds contain bitter taste cells. Bitter cells are mostly expressed in vimentin-negative cells. These figures are drawn with reference to previous studies (Kudo *et al*., 2008, Rajapaksha *et al*., 2016, Yoshida *et al*., 2019). (**b**) Signal transduction and neurotransmission of bitter taste cells in chickens. In mammals, bitter compounds activate T2Rs, and G-proteins coupled to T2Rs are dissociated. Subsequently, PLCβ2 is activated, IP_3 is produced, IP_3 activates IP_3R3, and then intracellular Ca^{2+} is increased. TRPM5 is activated by the Ca^{2+}, and depolarization *via* TRPM5 generates action potential through the activation of a voltage-gated Na^+ channel. After that, ATPs are released *via* the CALHM1/3 channel to P2X2/3 expressed in afferent taste nerves. This figure is drawn with reference to a previous study (Taruno *et al*., 2020). In the cascade, the molecules whose expression in the chicken taste organs have been confirmed are surrounded by lines. Two functional oral bitter taste receptors can be inhibited by 6-methoxyflavanone, which is the only known antagonist of chicken bitter taste receptors at present. T2R, taste receptor type 2 member; PLCβ2, phospholipase Cβ2; IP_3, inositol triphosphate; IP_3R3, inositol triphosphate receptor type 3; TRPM5, transient receptor potential melastatin 5; CALHM, calcium homeostasis modulator.

tongue gland.

Many reports have suggested that chickens do not prefer sweet substances (Ganchrow *et al*., 1990; Cheled-Shoval *et al*., 2017b). The major sweet taste receptor in mammals is the heterodimer of taste receptor type 1, members 2 and 3 (T1R2/T1R3). Because chickens have lost the *T1R2* gene (Shi and Zhang, 2006), they do not form T1R2/T1R3. Thus, chickens do not seem to prefer sweet compounds. However, because sweet taste sensors other than T1R2/T1R3 in taste cells, such as sodium-glucose cotransporter 1 (SGLT1) and

several glucose transporters (GLUT2, GLUT4, GLUT8, and GLUT9) have been identified in mice (Yee *et al*., 2011; Yasumatsu *et al*., 2020), it is possible that chickens can slightly perceive sweet taste with SGLT1 and GLUTs. On the other hand, many reports have found that chickens can taste bitter, sour, salty, and umami tastes (Ganchrow *et al*., 1990; Hirose *et al*., 2015; Yoshida *et al*., 2015; Dey *et al*., 2017; Yoshida *et al*., 2018a). Chicken taste receptor type 1 members 1 and 3 (T1R1/T1R3), which is an umami taste receptor candidate in chickens, is activated by L-alanine and L-serine in cell-based assays (Baldwin *et al*., 2014). Chicken otopetrin-1, which is a sour taste receptor candidate in chickens, is assumed to be activated by low pH, because otopetrin-1, its related genes, and its orthologs are activated by low pH in various species such as mice and *Drosophila* (Tu *et al*., 2018). In terms of fatty acid taste, it has been reported that chickens prefer lipids, and the functional fatty acid receptor, G-protein coupled receptor 120 (GPR120), and the fatty acid transporter, cluster of differentiation 36 (CD36), are expressed in the oral tissues of chickens (Sawamura *et al*., 2015; Kawabata *et al*., 2018). Furthermore, since lipase activity and the expression of several lipase genes have been confirmed in chicken oral tissues, it is assumed that lipids (triglycerides) are partly digested to fatty acids by oral lipases, and then fatty acids are detected by fatty acid taste receptors such as GPR120 and CD36 (Kawabata *et al*., 2018). In addition, oleic acid was aversive in the conditioned taste aversion test in chickens, suggesting that chickens can taste oleic acid in oral tissues (Kawabata *et al*., 2020b). Furthermore, when there is insufficient calcium intake from a chicken's main feed, the chicken can ingest sufficient calcium from other feed prepared separately from the main feed (Wilkinson *et al*., 2014). These results suggest that chickens can ingest calcium in correspondence with their physiological conditions. Thus, it is believed that chickens select feed on the basis of their taste sense, although there are some differences in the strength of their sensing ability for each taste quality. There are some excellent review articles of various studies on the sense of taste in chickens (Roura *et al*., 2013; Liu *et al*., 2018; Niknafs and Roura, 2018; Roura and Foster, 2018).

Functions of Bitter Taste Receptors in Chicken Oral Tissues

Red jungle fowl (*Gallus gallus*) have three intact bitter taste receptors (Go, 2006). These are taste receptor type 2 members 1, 2, and 7 (T2R1, T2R2, and T2R7). Hirose *et al*. (2015) cloned the chicken *T2R1* (c*T2R1*) gene from the Rhode Island Red strain and determined its nucleotide sequences (Hirose *et al*., 2015). The c*T2R1*-expressing vector was transiently transfected into HEK293T cells, and cT2R1-expressing cells were created. Calcium imaging confirmed that cT2R1 was activated by bitter compounds such as dextromethorphan and diphenidol. Furthermore, these cT2R1 activities were compatible with aversive behavior in response to these compounds in drinking tests. These results suggest that cT2R1 is one of the functional bitter taste receptors using which chickens reject bitter compounds (Fig. 1b) (Hirose *et al*., 2015).

Behrens *et al*. determined many bitter compounds that can activate cT2R1, cT2R2, and cT2R7 by calcium imaging (Behrens *et al*., 2014). Although there are 25 and 35 bitter receptors in humans and mice, respectively, there are three types of bitter receptors: broad, narrow, and intermediate (Meyerhof *et al*., 2010; Li and Zhang, 2014). Broad-type receptors can perceive many different bitter compounds (generalists), narrow-type receptors perceive specific bitter compounds (specialists), and the remainders are of the intermediate type. Behrens *et al*. (2011) showed that all chicken T2Rs are broad-type bitter receptors. Because the number of bitter taste receptors in chickens is much lower than that in humans and other animals such as mice, dogs, cows, opossums, and frogs (Go, 2006), it may be possible to reveal the effects of each bitter taste receptor on whole body function by making only three types of knockout animals, one without each of the three types of bitter receptors. Thus, the chicken is thought to be a useful model animal for studying the bitter taste perception of vertebrates (Cheled-Shoval *et al*., 2017a). The combination of *in silico* analysis and mutagenesis analysis of T2Rs showed that chicken T2Rs can receive approximately 50% of the bitter compounds that humans can receive, and some amino acid residues in chicken T2Rs that interact with bitter compounds have been determined (Di Pizio *et al*., 2017, 2018).

We performed a drinking test using bitter compounds from chicken T2R agonists as described by Behrens *et al*. (Behrens *et al*., 2014). We found that cT2R2 agonists were not disliked by chicks in the drinking test, although the concentrations of the test solution were sufficient to activate cT2R2. On the other hand, agonists of cT2R1 and cT2R7 at concentrations near those that activate cT2R1 and cT2R7 were disliked by chicks (Hirose *et al*., 2015; Dey *et al*., 2017). These results suggest that there are only two functional bitter taste receptors in chicken oral tissues that induce taste aversive behavior, cT2R1 and cT2R7 (Fig. 1b). Meanwhile, chickens rejected a solution with an extremely high concentration of caffeine, which is a cT2R2 agonist (Cheled-Shoval *et al*., 2017a). This result also suggests that cT2R2 is not substantially involved in bitter taste perception in chickens. Furthermore, although denatonium benzoate induces a strong bitter taste in humans, it does not activate cT2Rs (Meyerhof *et al*., 2010; Behrens *et al*., 2014). In a 5-min behavioral test, the intake of denatonium benzoate solution did not differ from the intake of water, which showed that chickens do not perceive the bitterness of denatonium benzoate (Yoshida *et al*., 2018b). These results also suggest that cT2R activity is linked with taste behaviors.

Interestingly, using a calcium imaging method, we found that 6-methoxyflavanone (6-meth) is an antagonist of the substantially bitter taste receptors, cT2R1 and cT2R7 (Figure 1b). Moreover, we also showed in a behavioral test that the intake of a bitter solution was increased by mixing the bitter solution with 6-meth (Dey *et al*., 2017). Six-meth is the only known antagonist that inhibits the bitter taste receptors in

chickens. To inhibit bitter taste, it is not necessary to use an antagonist of bitter taste receptors, because maskers that do not directly affect bitter taste receptors but modulate bitter taste perception indirectly can also be used (Jaggupilli *et al*., 2016). Furthermore, adjusting the taste of feed using preferable tastes such as sweet or umami may also be useful to change feed intake in poultry farming. For example, the mixture of saccharin, an artificial sweetener, with quinine (a bitter compound), increased the solution intake of quinine solution in chickens (Furuta *et al*., 2008, Yoshida *et al*., 2018a). It may be possible to develop new feedstuffs or poultry drugs by reducing the bitterness of materials that have good nutritional or pharmacological value but are not used because of their bitterness. Further studies are needed to identify new antagonists and maskers for bitter taste receptors and bitter perception in chickens.

Expressions of Bitter Taste Receptors and the Signal Transduction System for Bitterness in Chickens

In this section, histological analyses of chicken taste cells are discussed. α-gustducin is expressed in taste cells and is an essential G-protein subunit for transducing sweet, umami, and bitter tastes in mice (McLaughlin *et al*., 1992; Wong *et al*., 1996; Ruiz *et al*., 2003). Cells expressing α-gustducin are known to be type II taste cells (Boughter *et al*., 1997; Yang *et al*., 2000). Kudo *et al*. found that α-gustducin is also expressed in chicken taste buds (Kudo *et al*., 2010b, 2014). Furthermore, vimentin, an intermediate filament, is expressed in some chicken taste cells, and some vimentin-positive cells also express α-gustducin (Rajapaksha *et al*., 2016, Venkatesan *et al*., 2016). Because all taste buds have both vimentin- and α-gustducin-positive cells, these proteins have been used as markers for chicken taste buds. However, there are some taste cells that do not express both proteins. Yoshida *et al*. revealed that cT2R7 is frequently expressed in vimentin-negative cells in taste buds (Fig. 1a) (Yoshida *et al*., 2019). Since most taste buds in the palate and the base of the oral cavity have cT2R7-positive cells, and these cT2R7-positive cells are broadly distributed in oral tissues, it was supposed that chickens can detect bitter taste in a broad area of the oral tissues. Although the T1R1/T1R3 complex is the main umami receptor in mammals, T1R1 is also expressed in vimentin-negative taste cells in chickens (Yoshida *et al*., 2019). The differences between T2R7-positive and T1R1-positive cells have not been elucidated. In mammals, although it is known that umami taste cells differ from bitter taste cells, it is not known whether this is true in chickens. Further detailed studies are needed to analyze the expression patterns of the bitter taste receptor T2R7 and the umami taste receptor candidate molecules T1R1 and T1R3 in chickens. In mammals, taste cells are histologically and functionally divided into four types. Type I taste cells are glia-like cells (supporting type II and type III cells); type II cells are sweet, umami, and bitter taste cells; type III cells are sour taste cells; and type IV cells are immature cells (Kinnamon and Finger, 2019; Taruno *et al*., 2020). Revealing the types of taste cells present in chickens will help elucidate the chicken taste sense. In addition, no histological analysis of T2R1 and T2R2 in chicken taste cells has yet been reported; therefore, such analyses are needed. The expression of T2R2 in taste cells may be low because T2R2 agonists are not highly aversive in chickens, as mentioned above.

In mammals, the taste transduction systems of sweet, umami, and bitter tastes in taste cells are almost the same, but the taste receptors are different for each taste. Bitter compounds are perceived by T2Rs expressed in type II cells, and they proceed in the following cascade (Fig. 1b): the dissociation of G-protein coupled to T2Rs and subsequent activation of phospholipase Cβ2 (PLCβ2), the production of inositol triphosphate (IP_3), an increase in intracellular Ca^{2+} as a result of the release of Ca^{2+} from the intracellular Ca^{2+} store *via* inositol triphosphate receptor type 3 (IP_3R3), depolarization by the activation of transient receptor potential melastatin 5 (TRPM5) and TRPM4 by Ca^{2+}, and the generation of action potential by the activation of voltage-gated Na^+ channels (Taruno *et al*., 2020). After the action potential is generated, adenosine triphosphates (ATPs) are released *via* the calcium homeostasis modulator 1/3 (CALHM1/3) channel from bitter taste cells to afferent taste nerves (Taruno *et al*., 2013; Ma *et al*., 2018). The ATP receptors P2X2/3, expressed in afferent taste nerves, are then activated by ATP. Bitter taste information is transduced from taste cells to the brain *via* the transduction cascade. Among these transduction molecules, the expression of α-gustducin (a G-protein subunit), PLCβ2, and TRPM5 were confirmed in chicken oral tissues (Kudo *et al*., 2010b; Cheled-Shoval *et al*., 2014; Kudo *et al*., 2014; Cheled-Shoval *et al*., 2015). The mRNA expression of α-gustducin and TRPM5 in the palate, which contains many taste buds, was much higher than that in the tongue tip (Yoshida *et al*., 2018c). Furthermore, RNA-Seq analysis also showed that the expression of TRPM5 in the epithelium at the base of the oral cavity was significantly higher than that in the mesenchyme at the base of the oral cavity (Cui *et al*., 2017). This evidence suggests that these molecules are involved in bitter taste transduction in chickens.

Bitter Taste Receptor Activation by Canola Meal Extract and its Inhibition

This section provides an example of how physiological research on bitter taste has been applied to the problems of poultry farming. Although canola meal is a frequently used feed material, it is known to have slightly low palatability because it contains bitter compounds such as glucosinolate, sinapine, and tannin (Ismail *et al*., 1981; Khajali *et al*., 2011; Khajali and Slominski, 2012; Soares *et al*., 2013; Wieczorek *et al*., 2018). A recent report confirmed that canola meal extract activated both cT2R1 and cT2R7; thus, canola meal contains compounds that activate the chicken bitter taste receptors. The report also confirmed that chicks showed aversive behavior to canola meal extract solution in drinking tests. Furthermore, the study showed that 6-meth can inhibit the activity of canola meal extract on the bitter taste receptors

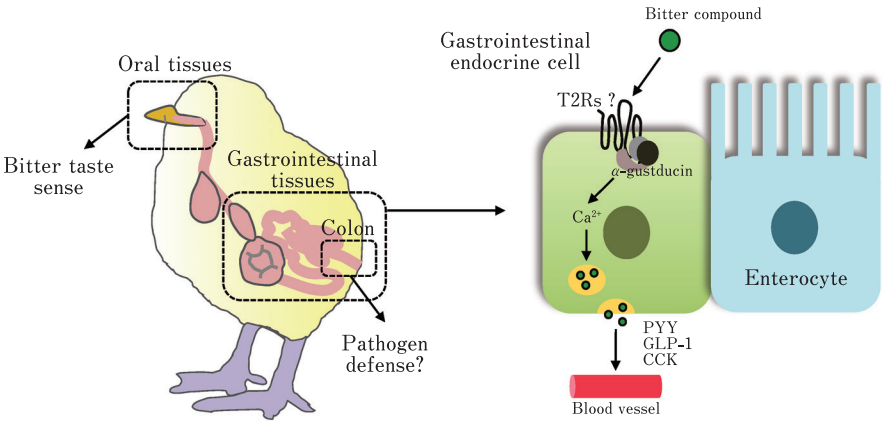

Fig. 2. **Expression patterns of T2Rs in chicken oral and gastrointestinal tissues.** T2Rs function as bitter taste receptors in oral tissues (palate, the base of the oral cavity, and the root of the tongue). mRNA expressions of T2Rs were also confirmed in the gizzard, duodenum, jejunum, ileum, cecum, and colon. Because α-gustducin is co-expressed with gastric hormones such as PYY, GLP-1, and CCK in gastrointestinal endocrine cells, gastrointestinal T2Rs may sense ingested feeds or toxins, digestion products of feeds, or metabolites of enterobacterium and regulate the secretions of these hormones. T2Rs, taste receptor type 2 members; PYY, peptide YY; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin.

and mitigate the reduction of the intake volume of canola meal extract in drinking tests. These results suggest that the inhibition of bitter taste receptors can improve the preference for canola meal in chickens (Kawabata *et al*., 2020a). In the future, the bitter taste characteristics of each feed material will probably be elucidated by examining how the currently used feed materials affect chicken bitter taste receptors.

Differences in Bitter Taste Sensitivity among Growth Stages, Chicken Strains, and Poultry Species

In a study of the differences in bitter taste sensitivity among growth stages using 0–1, 4–5, and 8–9 week-old chicks, behavioral tests showed that younger chicks are more sensitive to bitter compounds that activate cT2Rs (Dey *et al*., 2018). The study also suggested that higher expression of the mRNA of one of the bitter taste receptors, cT2R1, in younger chicks may be involved in their high sensitivity to bitterness. The finding that younger chicks have higher bitter taste sensitivity suggests that it may be important to care about bitter compounds in feed fed to younger chicks. Moreover, bitter taste sensitivity was also different among chicken strains. In 5-day-old chicks, sensitivity to quinine hydrochloride was highest in broiler chicks (Chunky), the second highest in the Rhode Island Red strain, and lowest in the White Leghorn strain among these three strains (Kudo *et al*., 2010a). In addition, the number of taste buds was highest in the broiler (Chunky), second highest in the Rhode Island Red, and lowest in the White Leghorn, suggesting that the number of taste buds is one of the factors that determine the bitter taste sensitivity in chickens. It has been reported that the taste bud number does not change from newborn chicks to adult chickens in the Rhode Island Red strain (Kudo *et al*., 2008). Furthermore, in broiler chicks, the number of taste buds hardly changes from hatching to 60 days of age (Ganchrow and Ganchrow, 1987). Thus, it is possible that the number of taste buds is related to the differences in bitter taste sensitivity among strains, while the differences in bitter taste sensitivity between growth stages in the same strain are related to the expression levels of the bitter taste receptors. In studies of other poultry species, Japanese quails showed little sensitivity to bitterness (Urata *et al*., 1992) and geese showed a bitter sensitivity (Takayama *et al*., 2018). By analyzing the bitter taste receptor functions in Japanese quails, the mechanisms underlying the differences in bitter taste sensitivity among poultry species could be elucidated.

Other Applications of Bitter Taste Research in Chickens

In layer chickens, feather pecking is known to be a problem. It was reported that applying a bitter compound, 4% quinine sulfate solution, to the wings reduced aggressive pecking. The chickens learned that feathers were not feed, and the effect of the bitter compound lasted for one to 2 weeks (Harlander-Matauschek *et al*., 2009). This study shows that it is possible to improve problematic chicken behaviors by utilizing the bitter taste sensation.

Bitter Taste Receptor Functions in Extra-oral Tissues in the Chicken

It has been reported that bitter taste receptors are also expressed in extra-oral tissues and are involved in the regulation of energy metabolism and defense against pathogens (Lu *et al*., 2017, Behrens and Meyerhof, 2019),

suggesting that cT2R2 may play a physiological role in extra-oral tissues (Fig. 2). Of course, it is possible that cT2R1 and cT2R7 also have other functions in extra-oral tissues. Cheled-Shoval *et al*. analyzed the mRNA expression levels of gastrointestinal T2Rs and found that the expression of T2Rs is rich in both oral tissues and the colon. Thus, it was suspected that T2Rs are involved in both taste sense and pathogen defense in chickens (Cheled-Shoval *et al*., 2015). In terms of their role in pathogen defense, it is supposed that gastrointestinal T2Rs detect some bacterial metabolites and toxins, and that this detection is involved in the flushing of harmful substances by enhancing fluid secretion, as in mammals. Furthermore, the administration of quinine to 3-day-old chicks increased the mRNA expression of T2R1 and T2R7 in the palate and all T2Rs (T2R1, T2R2, and T2R7) in the duodenum (Cheled-Shoval *et al*., 2014). In addition, the intake of saccharine for 21 days significantly increased the mRNA expression of T2R1, T2R2, and T2R7 in chick jejunum (Jiang *et al*., 2020). Although saccharine is an artificial sweetener, highly concentrated saccharine activates some human bitter taste receptors (Kuhn *et al*., 2004); it is supposed that saccharine directly activates cT2Rs and increases the expression of cT2Rs. Although cT2R2 may weakly function as a bitter taste sensor in oral tissues, it is assumed that cT2Rs are involved in the perception of bitter compounds in intestinal tissues. The intake of bitter compounds may modulate the oral and gastrointestinal expression of T2Rs in chickens, and it is possible that the modulation changes the bitter taste sensitivity and gastrointestinal functions physiologically. Further studies are needed to identify the role of each bitter taste receptor in gastrointestinal tissues. It has been reported that α-gustducin is co-expressed with gastrointestinal hormone granules such as peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK) in chicken gastrointestinal endocrine cells (Mazzoni *et al*., 2016, 2018). Thus, in gastrointestinal endocrine cells, after the perception of bitter compounds by T2Rs, α-gustducin and $\beta\gamma$-gustducin are dissociated, PYY, GLP-1, or CCK may be secreted from its downstream cascade (Fig. 2). It is known that GLP-1 is secreted by the activation of intestinal T2R in mice (Jeon *et al*., 2008), and a single nucleotide polymorphism of the bitter taste receptor TAS2R9 adversely affects glucose homeostasis in humans (Dotson *et al*., 2008).

Conclusions and Future Prospects

In this review, studies on the bitter taste sense in chickens are discussed. Recent studies have revealed that the chicken has two functional bitter taste receptors, T2R1 and T2R7, in its oral tissues; the bitter taste sensitivity is higher in younger chicks than in older chicks, and that the bitterness of canola meal, which is one of the representative feed materials, can be inhibited by the antagonist of the bitter taste receptors. These findings will contribute to the fundamental understanding of taste physiology in chickens and their application in poultry farming. However, much research is needed to fully elucidate the taste physiology of chickens and to identify its applications in the poultry industry.

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Conflicts of Interest

The author declares no conflict of interest.

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