Behavioral Responses of Chicks to a Saccharin-Quinine Mixture

Tatsuyuki Yoshida, Yuichi Ito and Hiroki Furuta

Department of Animal Science, Nippon Veterinary and Life Science University
Musashino-shi Tokyo 180-8602, Japan

Running title: Behavioral Responses of Chicks to Saccharin

Correspondence: Hiroki Furuta, Department of Animal Science, Nippon Veterinary and Life Science University, Kyounan-cho 1-7-1 Musashino-shi Tokyo 180-8602, Japan.
(Email: fhiroki@nvlu.ac.jp)
To date, few reports have been published on the sensitivity of birds to sweet tastes. Therefore, in this study, the behavioral responses of White Leghorn chicks to the sweet taste of saccharin and the bitter taste of quinine were assessed. Three chicks were provided with a solution of 3.0 mM quinine and a mixture of 3.0 mM quinine mixed with 0.1, 0.5, 1.0, or 10.0 mM saccharin in a two-bottle choice test for 48 h. It was found that the chicks consumed more of the 0.5 mM saccharin/3.0 mM quinine mixture but significantly less of the 10.0 mM saccharin/3.0 mM quinine mixtures than the quinine solution alone (P < 0.05). The aversive behavior of 3.0 mM quinine solution was eased when mixed with 0.5 mM saccharin, indicating that chicks are detecting the sweetness associated with the 0.5 mM saccharin. The aversion to the 1.0 and 10.0 mM saccharin solutions might be stronger than to the 3.0 mM quinine solution alone. These findings suggest that chicks are able to detect this artificial sweetener.

**Keywords:** chick, leghorn, quinine, saccharin, taste
**Introduction**

Taste is an important sense for recognizing nutritional value and discriminating between safe and dangerous foods. Taste receptors are able to detect five different tastes: salty, acidic, sweet, umami, and bitter. Among these, a bitter taste is related to harmful and toxic substances (Go et al., 2005). The sensitivity of mice to sweet and umami tastes has previously been investigated using mixtures of sweet or umami substances and quinine hydrochloride (QHCl), which is an aversive, bitter-tasting substance, and have demonstrated that sweet or umami flavors can mask the bitter taste (Hsiao and Fan, 1993; Contreras et al., 1995; Murata et al., 2003). However, large differences in sensitivity to sweet and umami substances have been observed among mouse strains. It has been reported that G protein-coupled receptors in the taste receptor type 1 (T1R) family are related to sweet and umami tastes. Located on the 4th chromosome in mice and on the 1st chromosome in humans, T1R3 is a SAC receptor gene that confers a sensitivity to saccharine (Hoon et al., 1999; Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz, et al., 2001). However, it has been proposed that, though the T1R2/T1R3 heterodimer detects sweetness, the T1R1/T1R3 heterodimer responds to umami taste in humans, suggesting that T1Rs are critical for both sweet and umami tastes (Max et al., 2001; Nelson et al., 2001).

In chicks, the gustatory system appears to function before hatching, and newly-hatched chicks are able to sense taste. Chickens have three bitter taste receptors that can be activated in a cell-based assay: T2R1, T2R2, and T2R7 (Behrens et al., 2014; Hirose et al., 2015). It has previously been shown that chicks consume lower amounts of a quinine solution than they do water, indicating that they show an aversion to bitter-tasting substances (Gentle, 1975). Although it has been considered that birds are also able to taste sweetness, there have been few reports of chicks sensing the taste of artificial sweeteners. Therefore, the present study examined the behavioral responses of White Leghorn chicks to an artificial
sweetener by offering them a choice between a solution of quinine mixed with saccharine and quinine alone in a two-bottle choice test.

**Materials and Methods**

*Behavioral responses of chicks to saccharin*

Three male White Leghorn chicks (Japan Layer, Gifu, Japan) aged 2 - 20 d were placed in a cage (20 cm × 18 cm × 16 cm) in a windowless room under 24-h lighting and a temperature of 30 °C. The food was free intake. From days 2 – 4, the chicks were provided water from a bottle (CLEA Japan, Tokyo, Japan) with a nipple drinker for chicks used in the poultry industry. From days 5 – 20, chicks were provided with solutions of 3.0 mM quinine (Quinine Hydrochloride Dihydrate: Tokyo Chemical Industry, Tokyo, Japan) and a solution of 3.0 mM quinine mixed with either 0.1, 0.5, 1.0, or 10.0 mM saccharin (Saccharin Sodium Salt Hydrate: Sigma-Aldrich, Tokyo, Japan) for 48 h in bottles with nipple drinkers. The behavioral responses of the chicks to the drinking solutions were then compared using a two-bottle choice test by recording the amount of solution that was consumed by each chick. To prevent their position from affecting choice, the bottles were switched after 24 h. The examination was repeated five times, and the amount of intake solution was measured every 24 h.

*Statistical analysis*

The data were analyzed using Student’s t-test to determine whether there was a significant difference between treatments. Results are presented as means ± SE.
Results and Discussion

The amounts of the saccharin/quinine mixtures and quinine solution alone that were consumed in each choice test were: 48.2 ± 7.5 g vs. 50.9 ± 9.4 g, respectively, for the 0.1 mM saccharin mixture; 73.0 ± 7.9 g vs. 48.3 ± 10.6 g, respectively, for the 0.5 mM saccharin mixture; 35.5 ± 9.8 g vs. 60.15 ± 9.4 g, respectively, for the 1.0 mM saccharin mixture, and 34.0 ± 6.8 g vs. 64.5 ± 10.3 g, respectively, for the 10.0 mM saccharin mixture (Fig. 1). The chicks consumed significantly less of the 1.0 and 10.0 mM saccharin mixtures than the 3.0 mM quinine solution (P < 0.05). This result indicates that taste may affect food intake, which would subsequently affect the health of the chicks. Compared to the 3.0 mM quinine, the chicks consumed twice as much of the 0.5 mM saccharin mixture (P < 0.05) but half as much as the 10.0 mM saccharin mixture (P < 0.05) (Fig. 2).

It has been previously shown that mice exhibit a weak aversion to the bitter taste of quinine, which is alleviated by the addition of sweeteners (Bachmanov et al., 2001). Mixtures of sweet and bitter compounds have also been used in various other behavioral experiments, demonstrating that beagles are able to taste saccharin when it is mixed with quinine (Furuta et al., 2010). Although it has been found that chickens avoid bitter tastes (Gentle, 1975; Furuta et al., 2008), there have been few reports on their ability to detect sweetness when artificial sweeteners are mixed with quinine, as observed in mice.

In this experiment, no significant changes were observed amount of the mix solution and the QHCl to compare with the 1st day and the 2nd day. Bottle positioning had no influence on the results of this experiment. It was clear that 0.1 mM saccharin was not sufficient for chicks to detect a difference from quinine alone. By contrast, there was a tendency for chicks to drink more of the 0.5 mM saccharin mixture than quinine alone (P > 0.05), indicating that they could detect the sweetness of the saccharin, which weakened their aversion to the quinine. However, the chicks consumed significantly less of the 1.0 and 10.0 mM saccharin mixtures (P
< 0.05), possibly due to the combination of sweet and bitter tastes also being aversive to them. Similarly, it has been reported that beagles are more averse to the taste of a mixture of saccharin and quinine than to the bitterness of quinine alone (Furuta et al., 2010).

These findings indicate that White Leghorn chicks are able to detect the artificial sweetener saccharin. Since different strains of mice have different sensitivities to sweetness, it is conceivable that different breeds of chickens would also exhibit such differences. This experimental method should be used to investigate the sweetness sensitivities of chicken breeds, as such information would be useful in improving chicken feed by catering to the preferences of the different breeds.
References

Bachmanov AA, Tordoff MG and Beauchamp GK. Sweetener preference of C57BL/6ByJ and 129P3/J mice. Chemical senses. 26: 905-913. 2001.

Behrens M, Korsching SI and Meyerhof W. Tuning properties of avian and frog bitter taste receptors dynamically fit gene repertoire sizes. Molecular biology and evolution. 31: 3216-3227. 2014.

Contreras RJ, Carson CA and Pierce CE. A novel psychophysical procedure for bitter taste assessment in rats. Chemical senses. 20: 305-312. 1995.

Furuta H, Izumi T, Dodo K, Yahata K, Nishimoto S and Bungo T. Response to sweetener-quinine mixtures in chicks: short-term fluid intake test. Journal of applied animal research, 33: 133-136. 2008.

Furuta H, Nakase A, Yuzuhara A, Saeki K, Oda H, Mizukoshi M, Azakami D, Ishioka K, Yoshida T and Sako T. Behavioral responses of beagles to saccharin mixed quinine. J. Journal of Pet Animal Nutrition, 13: 7-11. 2010.

Gentle MJ. Gustatory hyposensitivity to quinine hydrochloride following diencephalic lesions in Gallus domesticus. Physiology and behavior, 14: 265-270. 1975.

Go Y, Satta Y, Takenaka O and Takahata N. Lineage-specific loss of function of bitter receptor genes in humans and nonhuman primates. Genetics, 170: 313-326. 2005.

Hirose N, Kawabata Y, Kawabata F, Nishimura S and Tabata S. Bitter taste receptor T2R1 activities were compatible with behavioral sensitivity to bitterness in chickens. Biochemical and biophysical research communications, 460: 464-468. 2015.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba, NJ and Zuker CS. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell, 96: 541-551. 1999.
Hsiao S and Fan RJ. Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats. Behavioral neuroscience. 107: 317-326. 1993.

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y and Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochemical and biophysical research communications. 283:236-242. 2001.

Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H, Damak S and Margolskee RF. Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. Nature genetics, 28: 58-63. 2001.

Montmayeur JP, Liberles SD, Matsunami H and Buck LB. A candidate taste receptor gene near a sweet taste locus. Nature genetics, 4: 492-498. 2001.

Murata Y, Nakayama K, Yamada A, Shigemura N, Sasamoto K and Ninimiya Y. Gurmarin suppression of liking responses to sweetner-quinine mixtures in C57BL mice. Chemical senses. 28: 237-243, 2003.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ and Zuker CS. Mammalian sweet taste receptors. Cell, 106: 381-390. 2001.

Sainz E, Korley JN, Battey JF and Sullivan SL. Identification of a novel member of the T1R family of putative taste receptors. Journal of neurochemistry. 77: 896-903. 2001.
Explanation of figures

Fig. 1. Mean fluid intakes (g) of a 0.1 mM saccharin/3.0 mM quinine mixture and a 3.0 mM quinine solution by chicks in a two-bottle choice test. Data were obtained from three chicks and are expressed as means ± SE. Means with different letters signify significant differences (P < 0.05).

A: 0.1 mM saccharin/3.0 mM quinine. B: 0.5 mM saccharin/3.0 mM quinine. C: 1.0 mM saccharin/3.0 mM quinine. D: 10.0 mM saccharin/3.0 mM quinine.

Fig. 2. The relative change of fluid intakes of a saccharin/quinine mixture and a 3.0 mM quinine solution that designates the quinine as standard.

Means with different letters signify significant differences (P < 0.05) by comparison with quinine.
Fig. 1
Fig. 2