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Influence of Oral Administration of a High-Calcium Solution into a Marine Teleost (Nibbler Fish) and a Freshwater Teleost (Goldfish) on Their Plasma Calcium Levels

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Abstract: In this study, the influence of the oral administration of a high-calcium solution on plasma calcium levels in a marine teleost has been investigated and compared with those in a freshwater teleost. The calcium consomme solution (calcium concentration was 0.6 M) was administered into each fish at a rate of 10 μl/g body weight. Blood samples were collected from the caudal vessels of each individual with heparinized syringes at 3, 9, and 24 h after injection. Thereafter, plasma calcium concentrations were measured. In nibbler fish (marine teleost), the plasma calcium levels tended to increase at 3 h after the injection of a high-calcium consomme solution, although there was no significant difference between experimental and control groups. At 9 and 24 h after injection, plasma calcium levels in nibbler fish did not change as compared with those in control fish. In goldfish (freshwater teleost), plasma calcium concentrations increased significantly at 3 and 9 h after the administration of a high-calcium consomme solution as compared with those of control fish. At 24 h after injection, plasma calcium concentrations in goldfish decreased to the control levels. To confirm the above results in nibbler fish, solutions of different calcium concentrations (0.1 and 1 M) were injected orally into nibbler fish. As a result, the ingested calcium (0.1 and 1 M) consomme solutions did not influence plasma calcium levels in nibbler fish as compared with those in control fish at 3 h after injection. It has been known that seawater-adapted teleosts produce insoluble calcium precipitates to excrete *via* the intestine extra-calcium ingested by drinking seawater. The calcium precipitates were formed by a chemical reaction of Mg^{2+} and Ca^{2+} derived from ingested seawater with bicarbonate (HCO_3^-). In nibbler fish, Ca^{2+} ingested orally may bind to HCO_3^- and then form calcium precipitates. Therefore, we strongly suggest that the regulation of orally administered calcium is different between seawater- and freshwater-adapted teleosts.

Keywords: High-calcium solution, Plasma calcium, Calcium precipitates, Oral administration, Nibbler fish, Goldfish, Seawater adaptation

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Introduction

It is well-known that calcium is an essential mineral for maintaining cell viability and plays vital role in animals' life (Srivastav *et al.*, 2000). In all vertebrates, blood calcium levels are strictly kept at a constant concentration (around 2.5 mM) in spite of changing the internal milieu or external environment (Dacke, 1979; Bentley, 1998). The diet is a physiologically important source of calcium, although teleosts have the ability to absorb a large part of the necessary calcium from the environmental water (Dacke, 1979; Bentley, 1998). Therefore, we previously examined the effects of the oral administration of a high-calcium solution (consomme soup containing $CaCl_2$) into goldfish (*Carassius auratus*) (Sasayama *et al.*, 1996) and eels (*Anguilla japonica*) (Suzuki *et al.*, 1999) on their plasma calcium levels. As a result, plasma calcium concentrations increased significantly in both goldfish (Sasayama *et al.*, 1996) and eels (Suzuki *et al.*, 1999). In freshwater teleosts such as goldfish and eels, thus, calcium contained in the diet is an important source in a low calcium environment, such as fresh water.

However, the influence of the oral administration of a calcium solution into marine teleosts on plasma calcium levels has not yet been fully elucidated because marine teleosts inhabit seawater that contains around 3–5 times the calcium in their blood (Björnsson and Nilsson, 1985; Suzuki *et al.*, 1999; Sato *et al.*, 2017; Zanaty *et al.*, 2020). In the present study, we examined the effects of the oral administration of a high-calcium consomme solution on the plasma calcium levels of marine teleost (nibbler fish) and compared them with those of freshwater teleost (goldfish).

Materials and Methods

Animals

Nibbler fish (*Girella punctata*) (both sexes, n = 16, 263 ± 22.2 g) (Fig. 1A) were captured by fishing in Tsukumo Bay of the Noto Peninsula (Ishikawa Prefecture). After acclimation for 2 weeks, these fish were used in the present experiments. Freshwater teleost, goldfish (*Carassius auratus*) (both sexes, n = 16, 21.0 ± 1.44 g) (Fig. 1B) were purchased from a commercial source (Higashikawa Fish Farm, Yamatokoriyama, Japan) and used in the present study.

All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Kanazawa University.

Experiment I: Comparison of the oral administration of a high-calcium solution on the plasma calcium levels of marine and freshwater teleosts:

Nibbler fish (marine teleost) were divided into two groups. One group was anesthetized with 0.03% tricaine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and administered a high-calcium solution directly into the digestive tract through the oral cavity by an oral sonde with a syringe (Fig. 1C). The calcium solution consisted of a consomme cube and $CaCl_2$, and the final calcium concentration was 2.4 g Ca/100 ml (0.6 M). The high-calcium consomme solution was given to each fish at a rate of 10 μl/g body weight. The other group (control) was given only the consomme solution in the same way. In the case of freshwater teleost (goldfish), fish were divided into two groups and administered a high-calcium consomme solution (experimental) or a consomme solution (control) into the digestive tract through the oral cavity by an oral sonde with a syringe (Fig. 1C) under anesthesia.

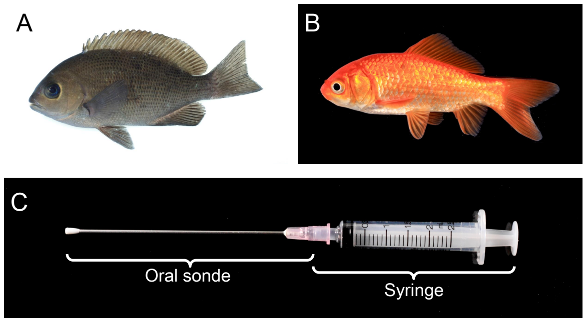

Fig. 1. Experimental fish (A: nibbler fish; B: goldfish) and equipment for the injection of calcium consomme solution (C).

Blood samples were collected from the caudal vessels of each individual with heparinized syringes at 3, 9, and 24 h after injection from nibbler fish (control and experimental) and goldfish (control and experimental) under anesthesia. The blood was collected into a 1.5 mL tube. Thereafter, the blood was centrifuged at 15,000 rpm for 3 min. The separated plasma was immediately frozen and kept at –80 °C until use. The plasma total calcium levels (mg/dL) of fish were determined using an assay kit (Calcium E, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) with a microplate reader (CORONA ELECTRIC Co., Ltd., Hitachinaka, Ibaraki, Japan) as described by Suzuki *et al.* (2011) and Sato *et al.* (2017).

Experiment II: Influence of the oral administration of different calcium solutions on plasma calcium levels in nibbler fish:

To confirm the above results in nibbler fish, consomme solutions of different calcium concentrations (0.1 and 1 M) were orally injected into nibbler fish. Nibbler fish were divided into three groups. Two groups were anesthetized with 0.03% of tricaine (Tokyo Chemical Industry Co., Ltd.) and administered a 0.1M (first group) and 1M calcium (second group) consomme solution directly into the digestive tract through the oral cavity by an oral sonde with a syringe (Fig. 1C), as described in experiment I. The third group (control group) was given only the consomme solution in the same way.

Blood samples were collected from the caudal vessels of each individual with heparinized syringes at 3 h after injection under anesthesia. The blood was collected into a 1.5 mL tube. Thereafter, the blood was centrifuged at 15,000 rpm for 3 min. The separated plasma was immediately frozen and kept at –80 °C until use. The plasma total calcium levels (mg/dL) in nibbler fish were determined using an assay kit (Calcium E, FUJIFILM Wako Pure Chemical Corporation) with a microplate reader (CORONA ELECTRIC Co., Ltd.) as described by Suzuki *et al.* (2011) and Sato *et al.* (2017).

Statistical analysis

All results are expressed as the means ± SE. The statistical significance between the control and experimental groups was assessed by student *t*-test. In all cases, the selected significance level was $p < 0.05$.

Results

Experiment I: Comparison of the oral administration of a high-calcium solution on the plasma calcium levels of marine and freshwater teleosts:

In nibbler fish (marine teleost), plasma calcium levels tended to increase at 3 h after the injection of a high-calcium consomme solution, although there was no significant difference between experimental and control groups (Fig. 2A). At 9 h (Fig. 3A) and 24 h (Fig. 4A) after injection, plasma calcium levels in nibbler fish did not change as compared with those in control fish.

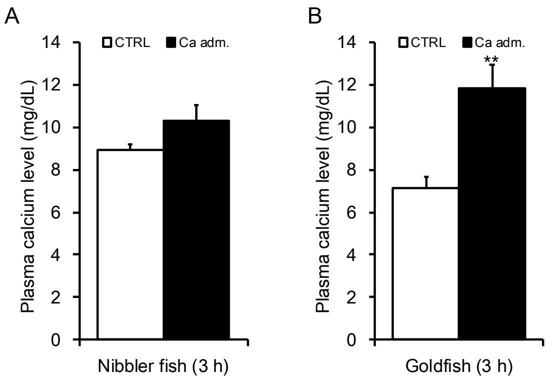

Fig. 2. Comparison of the oral administration of a calcium (0.6 M) consomme solution on the plasma calcium levels of nibbler fish (A) and goldfish (B). At 3 hours after administration, plasma calcium concentrations of nibbler fish and goldfish were measured. Each n = 8. **: $p< 0.01$. CTRL: control group; Ca Adm.: calcium administration group.

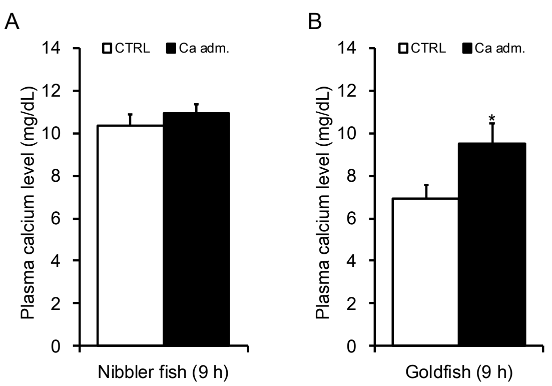

Fig. 3. Comparison of the oral administration of a calcium (0.6 M) consomme solution on the plasma calcium levels of nibbler fish (A) and goldfish (B). At 9 hours after administration, plasma calcium concentrations of nibbler fish and goldfish were measured. Each n = 8. *: $p< 0.05$. CTRL: control group; Ca Adm.: calcium administration group.

In goldfish (freshwater teleost), plasma calcium levels increased significantly at 3 h (Figure 2B) and 9 h (Fig. 3B) after the administration of a high-calcium consomme solution as compared with control goldfish injected with only a consomme solution. At 24 h after injection, plasma calcium concentration in goldfish decreased to the control levels (Fig. 4B). There was no significant difference between experimental and control groups at 24 h after injection (Fig. 4B).

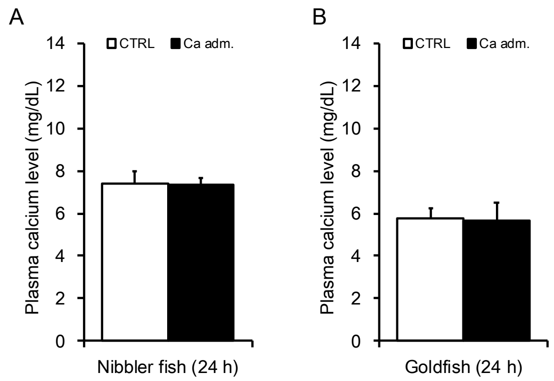

Fig. 4. Comparison of the oral administration of a calcium (0.6 M) consomme solution on the plasma calcium levels of nibbler fish (A) and goldfish (B). At 24 hours after administration, plasma calcium concentrations of nibbler fish and goldfish were measured. Each n = 8. There was no significant difference between control and experimental groups in these fish. CTRL: control group; Ca Adm.: calcium administration group.

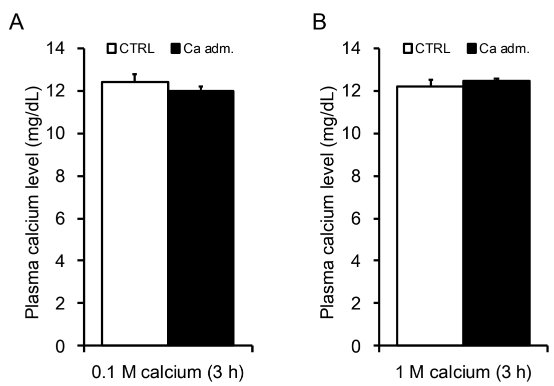

Fig. 5. Effect of the oral administration of calcium (A: 0.1 M; B: 1 M) consomme solution on plasma calcium levels in nibbler fish. At 3 hours after administration, plasma calcium concentrations were measured in nibbler fish. Each n = 3. There was no significant difference between control and experimental groups in nibbler fish. CTRL: control group; Ca Adm.: calcium administration group.

Experiment II: Influence of the oral administration of different calcium solutions on plasma calcium levels in nibbler fish:

To confirm the results of experiment I in nibbler fish, consomme solutions of different calcium concentrations (0.1 and 1 M) were orally injected into nibbler fish. At the most effective time-course (3 h after injection; Fig. 2 B) in goldfish, the plasma calcium in the injected nibbler fish was measured. In the ingested calcium concentrations (0.1 and 1 M), plasma calcium levels in nibbler fish were not changed as compared with those of control nibbler fish (Fig. 5).

Discussion

In the present study, we examined the effects of the oral administration of a high-calcium consomme solution on plasma calcium levels in nibbler fish (marine teleost) and compared them with those in goldfish (freshwater teleost). We found that, in nibbler fish, plasma calcium levels did not change significantly after the injection of a high-calcium (0.6 M) consomme solution, although plasma calcium concentrations in goldfish increased significantly at 3 and 9 h after the administration of a high-calcium (0.6 M) consomme solution, as compared with those in control goldfish. To confirm the above results in nibbler fish, consomme solutions of different calcium concentrations (0.1 and 1 M) were orally injected into nibbler fish. At the most effective time course (3 h after injection) in goldfish, the plasma calcium in the injected nibbler fish was measured. The ingested calcium (0.1 and 1 M) consomme solutions did not influence plasma calcium levels in nibbler fish as compared with those in control nibbler fish. We have previously reported on the effects of the oral administration of a high-calcium consomme solution (0.25 M) into freshwater teleosts such as goldfish (*Carassius auratus*) (Sasayama *et al.*, 1996) and eels (*Anguilla japonica*) (Suzuki *et al.*, 1999) on plasma calcium levels. As a result, plasma calcium concentrations increased significantly in both goldfish (Sasayama *et al.*, 1996) and eels (Suzuki *et al.*, 1999), as shown by our results (Figs. 2B, 3B). These facts, together with our obtained data, strongly suggest that the regulation of orally administered calcium seems to be different between seawater- and freshwater-adapted teleosts.

It has been known that marine teleosts excrete insoluble white feces, which is often referred to as calcium precipitates (Hickman, 1968; Walsh *et al.*, 1991; Mekuchi *et al.*, 2010; Whittamore *et al.*, 2010; Mekuchi *et al.*, 2013). The evacuation of ingested excess calcium by calcium precipitates was closely associated with seawater adaptation (Mekuchi *et al.*, 2010). Insoluble calcium precipitates were formed and excreted through the digestive tract in seawater-adapted eels (*Anguilla japonica*) but not in freshwater-adapted eels (Mekuchi *et al.*, 2010). In addition, Mekuchi *et al.* (2013) reported that calcium precipitates in the intestine of eels (*Anguilla japonica*) were formed by a chemical reaction of Mg^{2+} and Ca^{2+} derived from ingested seawater with bicarbonate (HCO_3^-) produced by the pancreas. In the European flounder (*Platichthys flesus*), furthermore, increasing the intestinal luminal Ca^{2+} concentration (10, 40, and 90 mM) caused a large elevation in intestinal HCO_3^- production in a dose-dependent manner (Cooper *et al.*, 2010). In nibbler fish, therefore, it is possible that orally ingested Ca^{2+} binds to HCO_3^- and then forms insoluble calcium precipitates.

These facts, together with our obtained results, suggest that the regulation of orally administered calcium is different between seawater- and freshwater-adapted teleosts. Marine teleosts, which live in a high-calcium environment (around 40 mg/dL) (Björnsson and Nilsson, 1985; Bentley, 1998), may have a specific mechanism to suppress the extra-calcium obtained by food ingestion. In the intestine and pancreas of eels (*Anguilla* *japonica*), the mRNA expression of bicarbonate transporters, such as solute carrier family 26, significantly increased when eels transferred from freshwater to seawater (Mekuchi *et al.*, 2013). To elucidate the regulation of orally administered calcium between seawater- and freshwater-adapted teleosts, we are planning to examine the expression levels of bicarbonate transporters after the injection of a high-calcium consomme solution in both freshwater- and seawater-adapted eels.

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