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Effects of dietary NaCl on the in vivo apparent absorption of dietary nutrients determined in rainbow trout (*Oncorhynchus mykiss*)

M. Nakajima, S. Sugiura *

School of Environmental Sciences, University of Shiga Prefecture, Hassaka 2500, Hikone, Shiga 522-8533, Japan

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ABSTRACT

Rainbow trout (mean body weight 52.3 g) were reared in freshwater and fed a semi-purified low-sodium (Na) diet (Na content 0.027%) or NaCl-supplemented diets (Na content 0.35% and 1.75%). The chyme of the proximal and distal intestines of fish were collected by dissection. The diets and chyme were analyzed, and apparent digestibility (net absorption) and indigestibility of dietary nutrients was estimated based on yttrium as a reference. Na concentration of the intestinal chyme, both proximal and distal regions, was similar regardless of dietary Na levels. A major Na secretion into the proximal lumen was noted in fish fed the low-Na diet. The net phosphorus (P) absorption was already ~90% at the proximal intestine, but dietary Na had no detectable effect on net P absorption, both in the proximal and distal intestine. The Ca content was more than two times higher in the distal than the proximal chyme. Dietary Na had no effect on chyme Ca content, but tended to increase Mg content in the proximal and distal chyme. There was no apparent effect of dietary Na on net Fe and Zn absorption in the proximal and distal intestine. Dietary Na decreased net absorption of Mn. Dietary Na tended to increase dry matter and organic carbon digestibilities in the distal intestine. Digestibility of crude protein (CP) was increased by dietary Na in the proximal, but not distal, region. These results collectively indicate that dietary Na may slightly increase digestibilities of macronutrients in rainbow trout reared in freshwater.

Statement of relevance

Sodium (Na) is an important ion for dietary nutrient absorption in the intestine. However, the effect of dietary Na on an in vivo nutrient absorption efficiency has been little studied in fish. Hence, we evaluated the effect of dietary NaCl on nutrient digestibility (absorption) in rainbow trout reared in freshwater.

1. Introduction

Traditionally, fish meal has been used as the main ingredient for commercial fish feeds. However, due to the soaring price of fish meal in recent decades, use of fish meal in aquafeeds is becoming increasingly difficult (Hardy, 2010). Replacing fish meal with more sustainable ingredients such as plant or agricultural by-products therefore has been recognized as a priority research subject (Kaushik and Seiliez, 2010; NRC, 2011). Fish meal is high in sodium (Na) content, but plant ingredients are mostly low in Na. Hence, replacing fish meal with plant sources results in a diet low or deficient in Na content.

Na is an essential ion for the active (carrier-mediated) transepithelial absorption of various dietary nutrients in the intestine (Bakke et al., 2011; Ferraris, 2001). The essentiality of Na for the apical uptake of nutrients has been demonstrated by numerous in vitro assays. Na also plays an important role in paracellular diffusion of nutrients. An in vitro assay using Caco-2 cell monolayers demonstrated that NaCl and other substances increase luminal osmolality, leading to increased paracellular absorption of various solutes by solvent drag (Shimizu, 2010). More recently, NaCl is shown to have an effect of increasing tight junction (TJ) permeability by multiple mechanisms (Lerner and Matthias, 2015). Paracellular diffusion is quantitatively more important than transcellular transport even for macromolecules such as glucose (Lerner and Matthias, 2015). These mammalian findings illuminate earlier observations in seawater adapted euryhaline fishes that imbibe plenty of seawater and have increased TJ permeability (Ferraris and Diamond, 1997). NaCl therefore increases intestinal nutrient absorption via both transcellular and paracellular pathways. In animals, the essentiality of Na has also been confirmed by in vivo experiments (e.g., Csaky, 1963; Debnam, 1982, or more recently, Chamorro et al., 2007a, 2007b; Sklan and Noy, 2000; Yin et al., 2008). In fish, however, little is known regarding the effect of dietary Na on intestinal nutrient absorption, not to mention the optimum dietary Na level to maximize nutrient absorption.

* Corresponding author.
E-mail address: sugiura@ses.usp.ac.jp (S. Sugiura).

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Dietary Na requirement in fish has been studied extensively based on fish growth, feed efficiency, and other response indicators (NRC, 2011). However, the dietary requirement is still uncertain or unestablished for even major aquaculture species. This is because fish absorb Na efficiently via the gills (Kumai and Perry, 2012), which supplies most or all of the Na required for fish. However, if the Na content is low in both water and diets, the Na deficiency may well ensue. This could lower nutrient absorption or digestibility.

Conversely, an excess dietary Na may create a strong downhill electrochemical gradient across the brush border membrane of enterocytes, which may drive Na-dependent transport of dietary nutrients. An excess Na may also increase paracellular permeability as mentioned above. In humans, an excess intake of Na is physiologically detrimental (IOM, 2005), however, fish are known to excrete excess Na efficiently by the gills, via the passive paracellular route and other mechanisms (Smith et al., 1995). This can be a fish-specific advantage to induce Na-driven nutrient absorption in the intestine. Fish in seawater drink plenty of seawater that can supply plenty of Na in the gut lumen. It has been shown that several euryhaline fishes grow better in saline water than in freshwater. The better growth is generally attributed to the decreased energy consumption for osmoregulation. However, the salinity effect appears to be more complex, involving multiple factors, such as better feed utilization or nutrient absorption (Boeuf and Payan, 2001; Ferraris and Diamond, 1997; Zydlewski and Wilkie, 2013).

Based on these evidence and reasoning, the present authors hypothesized that dietary Na is important in intestinal nutrient absorption and digestibility. Hence, the present study was conducted in order to examine the effects of dietary Na on intestinal nutrient absorption using diets of three different Na levels: low Na (deficient level), normal Na (requirement level), and high Na (over-fortification). The effects were evaluated based on in vivo apparent digestibility (net absorption) of crude protein (CP), dry matter, organic matter (total carbon), phosphorus (P), and selected minerals using rainbow trout.

2. Materials and methods

2.1. Fish and rearing condition

A group of rainbow trout *Oncorhynchus mykiss* with a mean body weight of 52.3 g were randomly stocked into each of 9 plastic tanks (60-l size) at 16–17 fish per tank. Three tanks were assigned to each test diet. Fish were reared in a flow-through system: each tank was continuously supplied with sand-filtered lake water at 0.2 L min^{-1}, and continuously aerated using an air-stone. Water temperature during the feeding trials ranged from 14.6 to 17.8 °C. Water quality conditions (influent) during feeding trial were as follows: pH, 7.2 to 7.6; COD, 0.0 to 2.0 mg L^{-1}; PO_4^{3-}-P, <0.01 mg L^{-1}; NH_4^+-N, <0.01 mg L^{-1}; and DO, 9.6 to 11.0 mg L^{-1}. The elemental concentrations of rearing water (influent) was as follows: Na, 3.58 mg L^{-1}; Ca, 6.38 mg L^{-1}; Mg, 1.08 mg L^{-1}; Fe, 0.22 mg L^{-1}; Mn, 10.6 μg L^{-1}; and Zn, 3.76 μg L^{-1} (means of $n = 3$ samples). A 14:10 h (light/dark) photoperiod was applied during the feeding trial.

2.2. Diets

The basal diet (Table 1) was designed to contain the minimal amount of Na, and relatively high amounts of protein and digestible carbohydrates, both of which require Na for their transcellular absorption. The basal diet (Na−: 0.027% Na, dry basis) was low in Na, but otherwise contained all the essential nutrients at levels higher than the dietary requirements (NRC, 2011). Yttrium (Y) was included in the basal diet as a non-digestible inert marker to determine digestibility or absorption of dietary nutrients (Sugiura et al., 1998). A normal Na diet (Na+: 0.35% Na) and a high Na diet (Na++: 1.75% Na) were made by supplementing NaCl to the basal diet. To make diets, all the ingredients and water were mixed thoroughly using a Hobart mixer, and the dough was divided into equal thirds. Two of them were added with appropriate amounts of NaCl and mixed thoroughly. The dough was cold-extruded through a meat chopper, and the noodle-like strands were crushed by hands to make pellets (~4 mm diameter, ~5 mm length). The pelleted diets (containing ~17% water) were sieved (to remove fine), placed in hermetically sealed plastic bags, and stored at −20 °C until use. The analytical composition of the diets (Table 2) indicate that the CP content (%, dry basis) were 43.1 (Na−), 42.3 (Na+), and 40.7 (Na++). The P content were 0.81 (Na−), 0.74 (Na+), and 0.71 (Na++). The other components analyzed were also slightly highest in the Na− diet, and slightly lowest in the Na++ diet, as the supplemental Na replaced the whole diet.

Table 1
Ingredient composition of the basal low Na diet.

Ingredients	g kg^{-1} diet
Casein	360
Wheat gluten	100
Corn oil	80
Squid oil	75
Dextrin	200
CMC-NH_4	20
α-cellulose	80
Vitamin mix[a]	15
Mineral mix (—Na)[b]	45
Amino acid mix[c]	25

To make diets, the ingredient mixture was added with an appropriate amount of water, which supplied the following trace minerals (mg kg^{-1} diet): $FeSO_4 \cdot 7H_2O$ 350, $ZnSO_4 \cdot 7H_2O$ 150, $MnSO_4 \cdot 5H_2O$ 90, $CuSO_4 \cdot 5H_2O$ 12, KI 2, Na_2SeO_3 1, $CoCl_2 \cdot 6H_2O$ 2.

[a] Vitamin mix supplied the following (mg kg^{-1} diet): thiamine mononitrate 20, riboflavin 30, niacin 80, calcium pantothenate 50, pyridoxine hydrochloride 20, folic acid 10, vitamin B12 0.05, d-biotin 0.5, choline chloride 2000, myo-inositol 500, ascorbic acid 2000, cod liver oil 5000, alpha-tocopherol acetate 500, menadione sodium bisulfite 30, dextrin (carrier) 4759.5.

[b] Mineral mix (—Na) supplied the following (g kg^{-1} diet): KCl 10, $MgSO_4 \cdot 7H_2O$ 10, KH_2PO_4 20, Y_2O_3 (marker) 5.

[c] Amino acid mix supplied the following (g kg^{-1} diet): DL-Met 5, L-Arg-HCl 1, L-His 0.5, L-Lys-HCl 3, Gly 5, L-Thr 0.5, DL-Ala 5, Betaine 2, Taurine 3.

2.3. Feeding and fecal sampling

Fish were hand-fed twice daily at 0800 and 1800 h to apparent satiation with respective test diets for 10 consecutive days. Six hours after the last feeding (0800 h), all fish were lightly anesthetized with MS-222 (100 mg L^{-1}), the spinal cords were severed, and were

Table 2
Analytical composition of the test diets (per dry matter basis).

	Na− diet	Na+ diet	Na++ diet	Req.[a]
CP (%)	43.1	42.3	40.7	38
Total C (%)	48.5	48.3	46.8	–
Ash (%)	3.4	4.2	8.0	–
Minerals				
Na (%)	0.027	0.346	1.751	NR
Ca (%)	0.051	0.050	0.049	NR
P (%)	0.809	0.744	0.708	0.70
Mg (%)	0.101	0.100	0.096	0.05
Y (%)	0.378	0.375	0.355	NR
Fe (mg kg^{-1})	135	130	152	30–60
Zn (mg kg^{-1})	52	52	49	15
Mn (mg kg^{-1})	21	21	17	12

Each value represents the mean of 3 samples.
Na− (basal diet), Na+ (basal +NaCl 15 g kg^{-1}), Na++ (basal +NaCl 50 g kg^{-1}).
CP (crude protein), Total C (total carbon).

[a] Dietary requirements (available or digestible nutrient basis; data from NRC, 2011).
NR: not required under practical conditions. Dashed line indicates no data available.