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ORIGINAL PAPER

Gastrointestinal transport of Ca^{2+} and Mg^{2+} during the digestion of a single meal in the freshwater rainbow trout

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Abstract A diet containing an inert marker (ballotini beads, quantified by X-radiography) was used to quantify the transport of two essential minerals, Ca^{2+} and Mg^{2+} from the diet during the digestion and absorption of a single meal of commercial trout food (3% ration). Initially, net uptake of Ca^{2+} was observed in the stomach followed by subsequent Ca^{2+} fluxes along the intestine which were variable, but for the most part secretory. This indicated a net secretion of Ca^{2+} along the intestinal tract resulting in a net assimilation of dietary Ca^{2+} of 28%. Similar handling of Ca^{2+} and Mg^{2+} was observed along the gastrointestinal tract (GI), although net assimilation differed substantially between the cations, with Mg^{2+} assimilation being close to 60%, mostly a result of greater uptake by the stomach. The stomach displayed the highest net uptake rates for both cations (1.5 and 1.3 mmol kg^{-1} fish body mass for Ca^{2+} and Mg^{2+}, respectively), occurring within 2 h following ingestion of the meal. Substantial secretions of both Ca^{2+} and Mg^{2+} were observed in the anterior intestine, which were attributed to bile and other intestinal secretions, while fluxes in the mid and posterior intestine were small and variable. The overall patterns of Ca^{2+} and Mg^{2+} handling in the GI tract were similar to those observed for Na^+ and K^+ (but not Cl^-) in a previous study. Overall, these results emphasize the importance of dietary electrolytes in ionoregulatory homeostasis.

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Introduction

Active transport of Ca^{2+} at the gills allows freshwater fish to partake of a continuous supply of the electrolyte, as most freshwaters contain appreciable levels of Ca^{2+}. As numerous physiological processes depend on Ca^{2+} to occur, from skeletal formation and growth to reproduction and even neural activity, this constant availability of ambient Ca^{2+}, in theory, allows for relatively easy maintenance of homeostasis. While magnesium is likewise a vital element, experimental constraints have left the nature of branchial transport relatively unknown. As a result, evidence for active branchial uptake is scarce (Wendelaar Bonga et al. 1983; Hobe et al. 1984; Shearer and Asgard 1992), despite water concentrations frequently much lower than plasma values.

Branchial uptake of Ca^{2+} is not the only source of this element for fish, and contributions of the gills to total body Ca^{2+} uptake have been estimated at between 50 and 80 %, with the remainder coming from the diet (Lovelace and Podoliak 1952; Berg 1968; Simkiss 1974; Perry and Wood 1985). When combined with the relatively low water concentration of Mg^{2+} and scarce evidence for branchial uptake, freshwater fish appear to have a dietary requirement for both essential electrolytes. In fact, low dietary Ca^{2+} levels have resulted in marked skeletal abnormalities, including abnormal bone mineralization and spinal deformities, in addition to retarded growth and low feed efficiency (Andrews et al. 1973; Robinson et al.

1984, 1986, 1987; Takagi et al. 1989; Takagi and Yamada 1992; Scarpa and Gatlin 1993). The effects of a low-magnesium diet are slightly more varied, including reduced growth rate, higher mortality, hypomagnesemia and lower bone and muscle Mg^{2+} concentrations with higher Ca^{2+} and Na^{+} concentrations in both (Ogino and Chiou 1976; Ogino et al. 1978; Gatlin et al. 1982; Knox et al. 1981; Shim and Ng 1988). Conversely, too much dietary Ca^{2+} is also detrimental, as excess dietary Ca^{2+} has resulted in reduced growth in channel catfish (Andrews et al. 1973), and has been linked to an increase in mortality following stressors such as handling and experimentation (Scarpa and Gatlin 1993). Unfortunately, excess dietary magnesium has been relatively overlooked.

Recently, we have provided a quantitative description of the differential processing of Na^{+}, K^{+} and Cl^{-} as a single meal of commercial trout pellets passes through the digestive tract of freshwater rainbow trout (Bucking and Wood 2006b). This study revealed a strong overall net absorption of both Cl^{-} and K^{+}, but not Na^{+} from the meal, and a previously unsuspected role of the stomach in ion absorption. Therefore, the primary objective of the present study was to provide a quantitative description of the processing of two divalent ions (Ca^{2+} and Mg^{2+}) along the gastrointestinal (GI) tract of a freshwater rainbow trout during digestion. Analysis of the electrolyte concentrations at various time points up to 72 h after ingestion of a single meal were carried out for chyme in each section of the GI tract, as well as in blood plasma. This allowed for the investigation of the concentration gradients between chyme and blood plasma at each stage of digestion. Ballotini beads were employed as non-absorbable inert markers (McCarthy et al. 1993) to correct for the absorption of solid material and water from the chyme, which would otherwise create a bias affecting the perception of concentration changes, and hence absorption and secretion. The inert marker overcomes this problem, allowing the calculation of net absorptive or secretory fluxes in each segment over various time points. We have demonstrated that the ballotini beads move synchronously with a fluid phase maker, and used them to quantify water fluxes in these same experiments (Bucking and Wood 2006a).

Our overall hypothesis was that both ions would be strongly absorbed from the chyme on a net basis, based on the preceding review of the literature. Our results support our hypothesis, but surprisingly show that Mg^{2+} was absorbed more on a net basis than Ca^{2+}, indicating a differential handling of the two divalent ions. Large bi-directional fluxes of the two ions in various parts of the tract as well as an important role for the stomach in Ca^{2+} and Mg^{2+} absorption have also been identified.

Materials and methods

Diet preparation

Two diets were employed for the experiment. The first (referred to as the regular diet) consisted of repelleted commercial fish feed (Martin Mills, Ontario, Canada). The pelleted food was finely ground (Braun PowerMax Jug Blender; Gillette Company, Massachusetts, USA) and placed into a pasta maker (Popeil Automatic Pasta Maker; Ronco Inventions, California, USA) with 30% double distilled water (30% of ground food weight). This mixture was then extruded after thorough mixing (30 min) and hand-rolled to approximate the 5-point sized fish feed to which the fish had been previously accustomed, and air-dried for 2 days before storage at –20°C. The second diet (experimental diet) was prepared and stored as the first; however ballotini beads (Jencons Scientific, Pennsylvania, USA), composed of lead-glass for radiographic quantification, were incorporated during mixing at a 4% ground food weight ratio with the water. The ballotini beads (0.40–0.45 mm in diameter) did not appear to affect the palatability of the feed (Gregory and Wood 1998, 1999; Bucking and Wood 2006a, b), and tests revealed an even distribution of ballotini beads within the feed pellets. The feed contained 41% protein, 11% fat and 30% carbohydrates; the measured concentrations of Ca^{2+} and Mg^{2+} are given in the results. Tests determined that the water content of the food pellets approximately tripled (from 6.1 to 18.0%) during the brief period during which they were in contact with the tank water prior to ingestion, but there was no significant loss of Ca^{2+}, Mg^{2+}, or other ions.

Experimental animals and feeding schedule

Freshwater rainbow trout (*Oncorhynchus mykiss*) were obtained from Humber Springs Trout Farm (Orangeville, Ontario, Canada). The adult animals (300–400 g) of both genders were placed into holding tanks (500-l fiberglass tanks) that were supplied with flow-through dechlorinated Hamilton (Ontario, Canada) city tap water [Na^{+} = 0.6; Cl^{-} = 0.7; K^{+} = 0.05; Ca^{2+} = 0.5; Mg^{2+} = 0.1; titration alkalinity (to pH 4.0) = 1.9 mequiv l^{-1}; total hardness = 140 mg l^{-1} as $CaCO_3$; pH 8.0]. The animals were housed at a density of 30–35 fish per tank, and the water was temperature-controlled to approximate seasonal conditions (10–13°C).

Following a 2-week acclimation period to the lab facilities, a feeding schedule was implemented where the regular diet (described above) was fed at a 2% body weight ration every 48 h for 1 month. Feeding was then suspended for 1 week to allow for GI tract clearance before the fish were fed once to satiation with the experimental diet containing the ballotini beads.

Dissection and sampling of gastrointestinal tract

After the ballotini-labelled meal was fed to the fish, sampling took place at previously determined time points which fell between 0 and 72 h following feeding, which occurred immediately after 0 h. At least seven fish were individually selected at each time point and sacrificed by a sharp blow to the head. A terminal blood sample was taken by caudal puncture, and processed for plasma Ca^{2+} and Mg^{2+} assay as described by Bucking and Wood (2006a). An incision just below the lateral line was then made into the body wall, from anus to pectoral fins, to reveal the peritoneal cavity. Following retraction of the body wall, each compartment of the GI tract (the stomach, the caeca and anterior intestine, the mid intestine, and the posterior intestine) was then visually identified based on morphology. Each section was isolated with ligatures at both ends of the structure, followed by the removal of the entire GI tract via incisions at the esophagus and the rectum. The intact GI tract was then placed across an X-Ray film for visualization of the ballotini beads, and exposed at 50 kVp (kilovolts peak) for 5 s in a portable X-Ray machine (Faxitron X-Ray Corporation cabinet X-Ray system; Illinois, USA).

Following the X-Ray, each section was carefully emptied of its contents (chyme), which was subsequently vortexed until well mixed. A sub-sample of chyme was then collected and centrifuged (13,000*g*; 60 s), to obtain a fluid phase supernatant, which was removed and placed into liquid nitrogen for later analysis of ion content. The remaining non-centrifuged whole chyme and a sample of the experimental feed were then oven-dried (80°C) to a constant weight (48 h) to determine their dry mass and water content, while the supernatant was stored at –80°C. The whole chyme and food were then digested in sealed vials by adding five volumes of 1 N HNO_3 (Fisher, Pennsylvania, USA). The vials were placed in the oven at 80°C for 48 h, during which time they were vortexed twice. Following digestion, all samples (feed and whole chyme) were centrifuged to obtain a clear supernatant for analysis of Ca^{2+} and Mg^{2+} content.

Analytical techniques

A Varian 1275 Atomic Absorption Spectrophotometer (California, USA) was used to determine the concentrations of Ca^{2+} and Mg^{2+} in the plasma (μmol ml^{-1}) diet chyme (μmol g^{-1} wet weight) and fluid phase of the chyme (μmol ml^{-1}). Reference standards were used for the measurement of both ions studied (Fisher Scientific, Ontario, Canada). Beads were quantified in each GI tract section by placing the X-Ray of the GI tract on a fine grid, and manually counting the beads located in each grid section to ensure accuracy.

Calculations and statistical analysis

The relative ion concentration in the chyme (or food) were then referenced to the beads located in each:

$$\text{Relative ion concentration } (R_c) = I_c\left(\frac{M_w}{X_s}\right) \quad (1)$$

where "I_c" was the ion concentration (μmol g^{-1} wet mass) found in a chyme or food sample, "M_w" was the wet mass of the chyme sample (g) and "X_s" was the bead number in the chyme sample.

The apparent ion concentration (μmol ml^{-1}) of the secreted fluid added in the anterior intestine to the chyme entering from the stomach was calculated as the change in relative ion concentration (R_c; μmol $bead^{-1}$) between the stomach and anterior intestine divided by the corresponding change in relative water concentration (W_s; ml $bead^{-1}$) reported for these same experiments by Bucking and Wood (2006b):

$$\text{Fluid ion concentration } (I_f) = \frac{(R_c \text{ ant. int.} - R_c \text{ stomach})}{(W_s \text{ ant. int.} - W_s \text{ stomach})}. \quad (2)$$

Ion fluxes (mmol kg^{-1}) in various segments of the tract at different times were calculated according to:

$$\text{Ion flux } (F_I) = \frac{[(I_{s1} - I_{s2})/1,000 \times X_{s1}]}{M} \quad (3)$$

where "I_{s1}" was the relative concentration of each ion (μmol $bead^{-1}$) in the GI tract section of interest and "I_{s2}" was the relative concentration of each ion (μmol $bead^{-1}$) in the preceding section at the same time point, "X_{s1}" was the total number of beads in the section of interest, and M was the fish mass (kg). This calculation provided the amount of ion that was secreted or absorbed in section "x" when compared spatially to the preceding compartment of the GI tract

in relation to fish mass. For the stomach only, the ''preceding compartment'' at 2 h was the ingested food, thereafter the stomach itself was used at the previous time point.

Data have been reported as means ± SEM (N = number of fish), unless otherwise stated. The effect of location was tested using a repeated measures ANOVA with GI tract section as the main variable examined at each time point. The effect of time was tested using a one-way ANOVA with time as the main variable, and each GI tract section was examined individually. Significant effects ($P < 0.05$) were determined after applying a Tukey's HSD post hoc test. All statistical analyses were performed using SPSS (version 13).

Results

Calcium

The concentration of Ca^{2+} found in the prepared diet was 194.4 ± 3.0 (7) μmol g^{-1} original food weight. This provided an average dietary intake of 5.9 mmol Ca^{2+} kg^{-1} fish body mass in the single meal, as the food was ingested at a 3.06% body weight ration (Bucking and Wood 2006a, b). The concentration of Ca^{2+} in the chyme (μmol g^{-1} wet chyme weight; which incorporates both water and solid phases of the chyme) gradually decreased over time in the stomach, falling from 194.4 ± 3.0 (7) to 50.1 ± 8.6 (7) μmol g^{-1} wet chyme weight by 72 h, a decrease of 73% (Fig. 1a).

Chyme was first detected in the anterior and mid intestine at 8 h, and in the posterior intestine at 12 h. There was essentially no change over time in the Ca^{2+} chyme concentration found in the anterior intestine, which was maintained at 39.2 ± 3.9 (35) μmol g^{-1} wet chyme weight. While this value was initially lower than in the stomach, by 48 h no significant difference remained, due to falling stomach Ca^{2+} values (Fig. 1a). The mid intestine likewise maintained its Ca^{2+} chyme concentration for the duration of the experiment at 104.4 ± 4.7 (35) μmol g^{-1} wet chyme weight, approximately 2.5-fold higher than the anterior intestine (Fig. 1a). While Ca^{2+} concentrations in the posterior intestine were initially comparable to those in the mid-intestine, a transient peak was observed with chyme Ca^{2+} concentration increasing significantly at 24 h, only to subsequently return to initial values (Fig. 1a).

The fluid phase of the chyme in the stomach was found to contain increasing amounts of Ca^{2+} throughout the experiment, with the Ca^{2+} concentration increasing from 6.6 ± 0.8 μmol ml^{-1} (7) to peak at 46.6 ± 5.6 μmol ml^{-1} (7) at 48 h (Fig. 1b). Fluid phase Ca^{2+} concentrations throughout the intestine were much lower than in the stomach at all times, and a general decreasing trend along the intestinal tract sections was evident, which became significant by 24 h (Fig. 1b). Following an initial decrease, there was a gradual increase in Ca^{2+} concentration of the fluid phase of the chyme found in the anterior intestine, although these trends were not significant. However, this increase was significant in both the mid and posterior intestine, which displayed a 70 and 60% increase respectively (Fig. 1b). Additionally, the Ca^{2+} concentration observed in both the total chyme and the fluid phase (Fig. 1a, b) was higher than plasma values at almost every time point in every section. Plasma Ca^{2+} concentration exhibited a significant increase at 8 h to 2.34 ± 0.12 (7) μmol ml^{-1}, but remained otherwise unchanged (2.01 ± 0.1 (56), averaged over all time points except 8 h.

Referencing the Ca^{2+} concentrations to an inert marker revealed very different patterns. There was a general decreasing trend in the relative concentration of Ca^{2+} in the stomach chyme over 72 h, from 2.08 ± 0.18 to 1.04 ± 0.22 (7) μmol $bead^{-1}$, a 51% decline (Fig. 2). However, there was an increase in the relative concentration of Ca^{2+} in the anterior intestine upon the first appearance of chyme at 8 h, doubling in value from that in the stomach (Fig. 2). With the exception of 12 h, the mid intestine was similar to the anterior intestine, and both decreased by 70% by 72 h (Fig. 2). In contrast, the posterior intestine displayed no temporal trends, remaining at 1.37 ± 0.28 (28) μmol $bead^{-1}$ (Fig. 2). Thus, by comparing the relative Ca^{2+} concentration in the originally ingested food and that was finally present in the posterior intestine, the net absorption efficiency for Ca^{2+} was 28%.

Magnesium

The experimental diet contained a concentration 108.6 ± 0.9 (7) μmol Mg^{2+} g^{-1} original food weight, which corresponded to a dietary load in each fish of approximately 3.3 mmol kg^{-1} fish body mass. The stomach displayed a similar decline in Mg^{2+} concentration in the total chyme as for Ca^{2+} (Fig. 1a) with Mg^{2+} concentrations decreasing by 93% from ingested values to 7.7 ± 1.8 (7) μmol g^{-1} wet chyme weight by 72 h (Fig. 3a). Initially (8h) Mg^{2+} concentrations in the anterior intestine were comparable to those in the stomach, but thereafter considerably exceeded stomach values as the latter continued to decline. There was also a small but significant 12% decrease over time in the concentration of Mg^{2+} (μmol g^{-1} wet weight) found

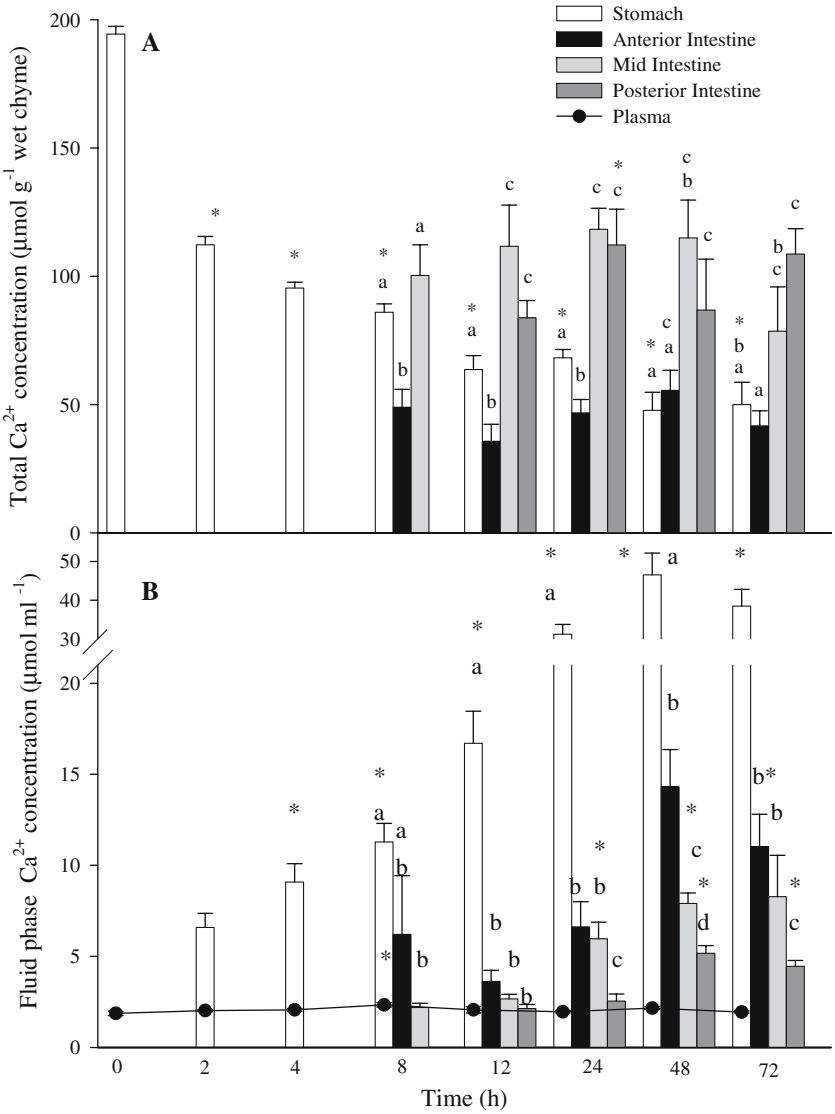

Fig. 1 **a** Temporal and spatial changes in the concentration of Ca^{2+} in the total chyme (µmol g^{-1} wet chyme weight) following feeding (immediately after 0 h). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section or 0 h values for plasma). *Bars* that share letters demonstrate no significant differences between GI tract sections within a time point. **b** Changes in the concentration of Ca^{2+} in the fluid phase isolated from total chyme (µmol ml^{-1}) following feeding (immediately following 0 h). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section or 0 h values for plasma). *Bars* that share letters demonstrate no significant differences between GI tract sections within a time point. Simultaneous measurements of plasma Ca^{2+} concentrations in the same fish at each time have been included as a point of reference (data from Bucking and Wood 2006a)

in the chyme in the anterior intestine (Fig. 3). The Mg^{2+} concentration in the total chyme of the mid intestine was consistently higher than in the anterior intestine, but the decrease in Mg^{2+} concentration in the mid intestine (40% by 72 h) was larger than the decrease seen in the anterior intestine (Fig. 3a). A transient increase was seen at 24 h in the concentration of Mg^{2+} in the chyme of the posterior intestine, followed by a decrease to below initial values (from 50.6 ± 3.8 to 37.9 ± 2.9 by 72 h; Fig. 3a).

The fluid phase of the chyme displayed Mg^{2+} concentration patterns quite unlike those seen with Ca^{2+} with no significant changes from 2 h through 24 h, with a mean concentration of 34.5 ± 3.1 (35) µmol ml^{-1}. There was a delayed (until 48 h) decrease in Mg^{2+} fluid phase values in the stomach, decreasing from 34.2 ± 07 (7) to 8.3 ± 1.9 (7) µmol ml^{-1} by 72 h (Fig. 3b). Transitory peaks appeared in all three intestinal segments; however the size and duration of the peak tended to increase along the intestinal tract. The Mg^{2+} concentration in the fluid phase initially decreased between adjacent sections of the GI tract; however after 48 h this pattern was reversed, and increased between sections (Fig. 3). As with Ca^{2+} (Fig. 1a, b), all Mg^{2+} concentrations, in both the fluid phase and total chyme were substantially higher than measured plasma values at all time points (Fig. 3a, b). There was once again significant increase in plasma $Mg^{2\pm}$ concentration at 8 h (to 0.97 ± 0.04 µmol ml^{-1}), however it remained unchanged for all other time points (0.81 ± 0.05 (56) µmol ml^{-1}). Concentrations of Mg^{2+} in the fluid phase of the intestine were greater than Ca^{2+} concentrations (cf. Fig. 1b), but less than in the total chyme.

Despite the differences in fluid phase patterns between Mg^{2+} and Ca^{2+}, the relative concentration of Mg^{2+} in the stomach chyme referenced to the inert

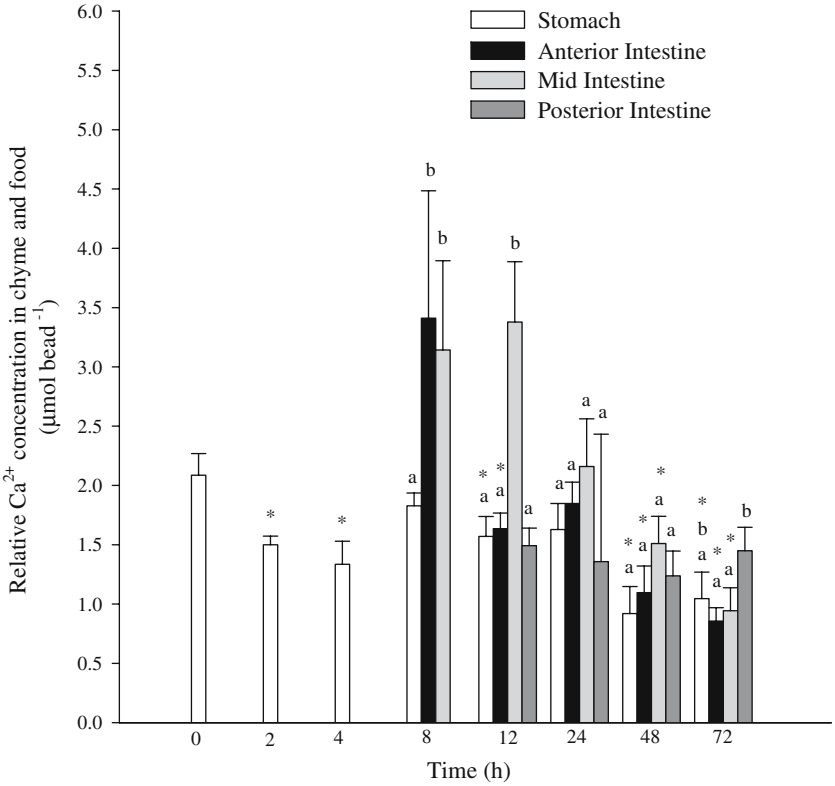

Fig. 2 Changes in the relative concentration of Ca^{2+} (µmol $bead^{-1}$) following feeding (immediately after 0 h). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section or 0 h values for plasma). *Bars* that share letters demonstrate no significant differences between GI tract sections within a time point

marker (Fig. 4) displayed a qualitatively similar pattern to that seen in the relative concentration of Ca^{2+} (Fig. 2). However by 72 h, the relative concentration of Mg^{2+} had decreased by 90% (Fig. 4) whereas for Ca^{2+} the decrease was only 51%. There was once again a large increase in the concentration of Mg^{2+} in the anterior intestinal chyme, and at 2.5-fold, slightly larger than the relative increase seen with Ca^{2+}. With the exception of the 12-h time point, all three intestinal segments displayed similar relative concentrations of Mg^{2+} in their respective chyme contents, and all decreased by 72 h, falling by over 70% (Fig. 4). By comparison of the relative Mg^{2+} concentration in the originally ingested food with that present at 72 h in the posterior intestine, the net absorption efficiency for Mg^{2+} was 60%.

Discussion

The spatial and temporal handling of the two divalent cations, Ca^{2+} and Mg^{2+}, from the ingested diet occurred in a qualitatively similar pattern along the GI tract. The stomach appeared to be an important site of absorption of both minerals, although only about 50% of the ingested Ca^{2+} was absorbed by the stomach (Fig. 2), while over 90% of ingested Mg^{2+} was absorbed (Fig. 4). The calculated fluxes (Fig. 5a, b) showed that the absorption of both Ca^{2+} and Mg^{2+} in the stomach reached approximate peaks of 1.25 and 1 mmol kg^{-1}, respectively within the first 2 h following ingestion; however thereafter, the fluxes were variable. To our knowledge, there have been no previous measurements of Ca^{2+} or Mg^{2+} fluxes in the stomach of teleosts. However, Mg^{2+} uptake from the forestomach of ruminants has not only been previously observed, but was also the main route of dietary Mg^{2+} absorption (Tomas and Potter 1976; Leonhard-Marek et al. 1998).

Notably, the absorption of Mg^{2+} from the chyme in the stomach of the rainbow trout may pose some problems as it has been used as a non-absorbed reference in previous studies of the marine fish GI tract (e.g. Parmalee and Renfro 1983). However, differences may exist between freshwater and marine species that may negate this problem. Baldisserotto et al. (2004) likewise observed a decrease between the Ca^{2+} concentration of the stomach fluid and the intestinal fluid, and postulated that absorption of Ca^{2+} may have occurred, although the results were not conclusive. They also observed a surge in plasma Ca^{2+} concentrations shortly after a meal, previously noted in Bucking and Wood (2006a), which also suggests rapid absorption of dietary Ca^{2+} in the stomach in the first few hours after ingestion. A fluid shift due to large secretion of fluid into the GI tract during the process of digestion (Bucking and Wood 2006a), could account for a por-

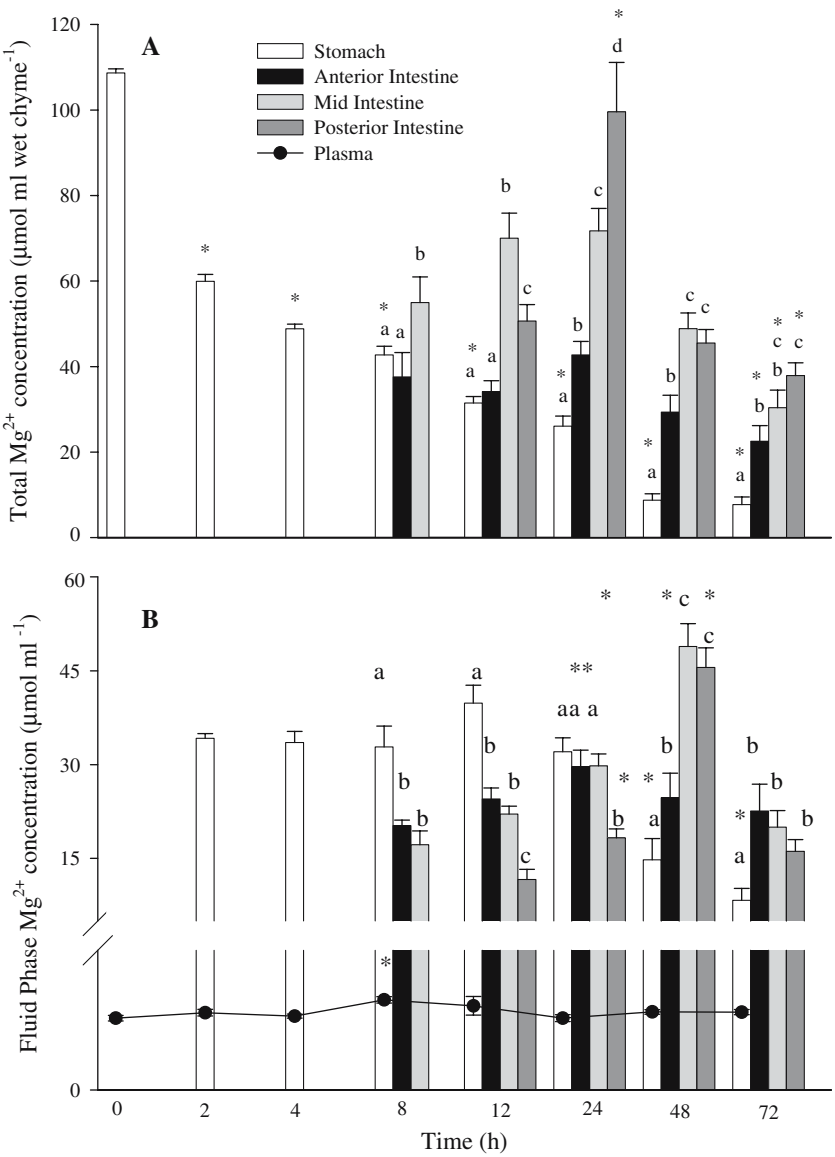

Fig. 3 **a** Temporal and spatial changes in the concentration of Mg^{2+} in the total chyme (μmol g^{-1} wet chyme weight) following feeding (immediately following 0 h). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section). *Bars* that share letters demonstrate no significant differences between GI tract sections within a time point. **b** Changes in the concentration of Mg^{2+} in the fluid phase isolated from total chyme (μmol ml^{-1}) following feeding (immediately following 0 h). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section). *Bars* that share letters demonstrate no significant differences between GI tract sections within a time point. Simultaneous measurements of plasma Mg^{2+} concentrations in the same fish at each time have been included as a point of reference (data from Bucking and Wood 2006a)

tion of the increase in plasma Ca^{2+} concentration. However increases in plasma ions were variable, with only three ions (Na^+, Ca^{2+} and Mg^{2+}) being affected and their respective peaks occurring at different times (Bucking and Wood 2006a), suggesting this is not the case.

The calculated fluxes along the intestinal tract for both cations were variable, however both Ca^{2+} and Mg^{2+} exhibited net secretion in the anterior intestine (Fig. 5a, b), as also seen with Na^+ and Cl^- (and in contradiction to K^+; Bucking and Wood 2006b). Using the fluid secretion measurements reported in Bucking and Wood (2006b) the large increase in Ca^{2+} and Mg^{2+} as chyme entered the anterior intestine amounted to calculated concentrations of 16.47 ± 10.02 (7) and 17.08 ± 8.82 (7) μmol ml^{-1} in the secreted fluid, respectively. These values were much higher than the concentrations recorded by direct measurements of bile in the rainbow trout (Grosell et al. 2000), although due to the high variability, not significantly different. If the secretion of fluid and other electrolytes into the anterior intestine was indeed a combination of bile, pancreatic and intestinal secretions (Bucking and Wood 2006a), the large Ca^{2+} and Mg^{2+} secretion could be due to the binding of Ca^{2+} and especially Mg^{2+} to enzymes and other ligands that are abundant in bile and pancreatic fluids. If water was absorbed across the intestinal epithelium as discussed by Bucking and Wood (2006a), these ions would remain behind.

A vital element, Mg^{2+} is an essential component of over 300 enzymes in the mammalian body (Ebel and Gunther 1980; Heaton 1990; Black and Cowan 1995).

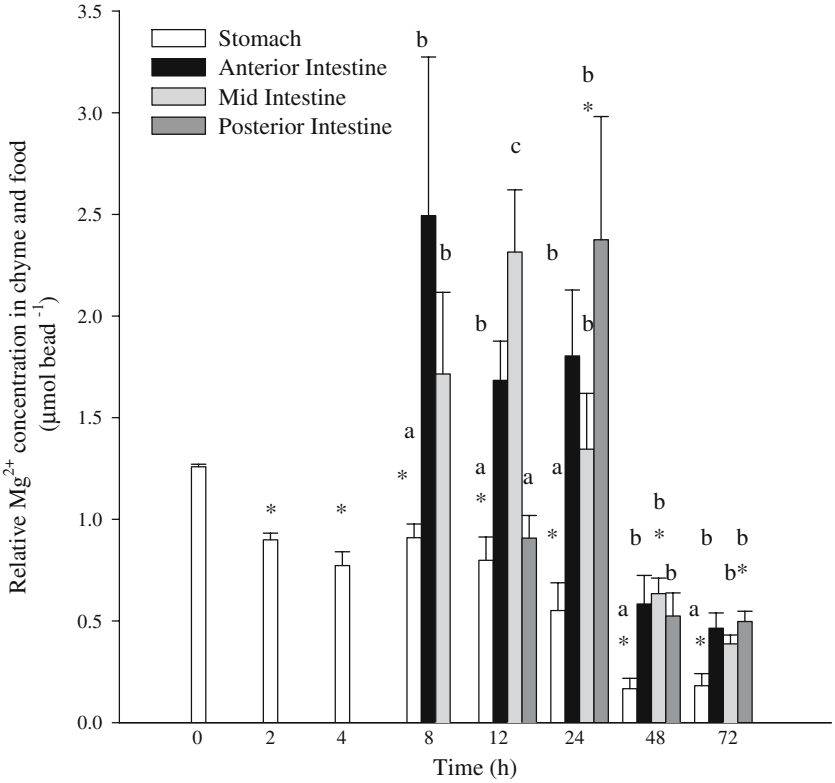

Fig. 4 Changes in the relative concentration of Mg^{2+} (µmol $bead^{-1}$) following feeding (0 h immediately preceded feeding). Values are means ± SEM ($N = 7$). *Asterisk* indicates a significant difference from initial values (defined by the first appearance within that section). *Bar*s that share letters demonstrate no significant differences between GI tract sections within a time point

Many enzymes that require phosphate compounds, such as ATPases, kinases and phosphatases which are abundant in pancreatic fluids, also require Mg^{2+} for activation (Gunther 1977; Schweigel and Martens 2000). In addition to regulating $Na^+/K^+/Cl^-$ and K^+/Cl^- symport activity and numerous membrane channels (Flatman 1993; Stanfield et al. 1994), Mg^{2+} is also believed to be involved in controlling ATP-dependent ion pumps (Bijvelds et al. 1998). It therefore seems surprising that mammals do not appear to actively control Mg^{2+} uptake from the diet (Schweigel and Martens 2000). In fact, absorption of Mg^{2+} from the diet proceeds in a linear fashion with intake (Hardwick et al. 1990), suggesting that GI uptake may be mostly a passive, diffusive process. However in fish, Van der Velden et al. (1992) observed an increase in prolactin activity preceding the appearance of hypomagnesemia symptoms in the Mozambique tilapia, suggesting a possible role for prolactin in the response to low Mg^{2+} levels.

According to the calculated fluxes, Mg^{2+} was absorbed to a small extent along the mid and posterior intestines, although the results are somewhat variable (Fig. 5). Gastrointestinal transport of Mg^{2+} is only superficially understood. As Mg^{2+} is lipophilic, in order to cross the enterocyte membrane a channel or transporter would most likely be utilized (Schwiegel and Martens 2000), and a Mg^{2+}/H^+ antiport mechanism has been suggested for mammals (Scharrer and Lutz 1990; Leonhard et al. 1991; Leonhard-Marek et al. 1998). Additionally, the concentration of Mg^{2+} in the fluid phase of the GI tract (10–50 µmol ml^{-1}) was higher than that found intracellularly (free $Mg^{2+} \leq 1$ mmol l^{-1}, total $Mg^{2+} \leq 5$–20 mmol l^{-1}; Fig. 3b), a fact that may aid in the passive import of Mg^{2+} into the cell (Ross 1962; Hardwick et al. 1990). Additionally, a large negative potential difference (PD) that is maintained in enterocytes (–50–70 mV; e.g. Groot et al. 1983; Halm et al. 1985; Bijvelds et al. 2001), would also aid in the passive entry of Mg^{2+} into the cell (Bijvelds et al. 2001). However, basolateral extrusion may hence require active transport.

Mg^{2+} efflux is often coupled with Na^+ influx and may occur via a Mg^{2+}/Na^+ exchanger as in a number of different cell types (e.g. DiPolo and Beauge 1988; Xu and Willis 1994), or it may be coupled to the transport of other ions that are accumulated in the enterocyte via Na^+-dependent mechanisms (Bijvelds et al. 1998). However, an electrically neutral Cl^-/Mg^{2+} cotransporter has been suggested in the freshwater tilapia enterocyte in the absence of Na^+ antiport activity, suggesting that the intestinal epithelium possesses a distinctive transport process (Bijvelds et al. 1996).

On the other hand, Ca^{2+} appeared to be slightly secreted along the mid and posterior intestinal tract, however the results were variable and close to 0 in

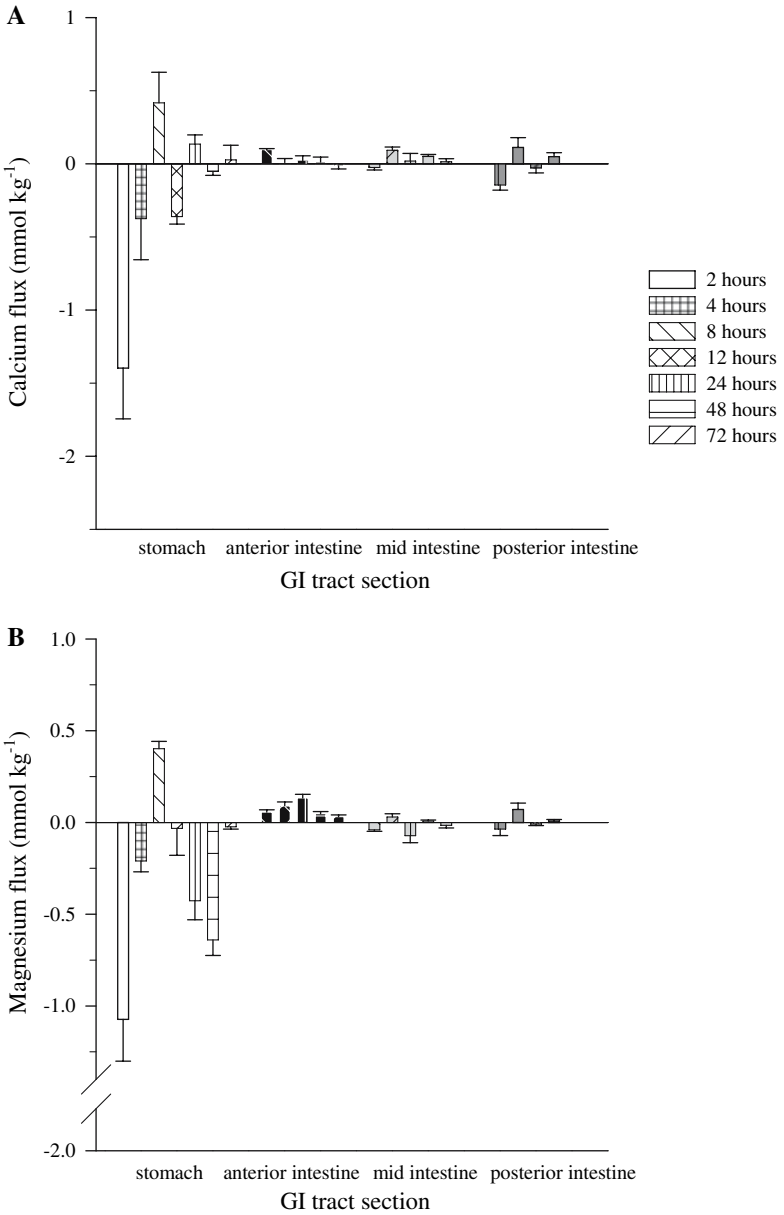

Fig. 5 Calculated ion fluxes (mmol kg^{-1}) along the gastrointestinal tract of the rainbow trout during digestion of a single meal **a** Ca^{2+}, and **b** Mg^{2+}. Feeding occurred immediately after 0 h. Positive values indicate net secretion, while negative values indicate net absorption

some cases (Fig. 5a). In mammals, it was once thought that Ca^{2+} and Mg^{2+} compete for a single apical transporter (Karbach and Rummel 1990), although it is now thought that the two cations possess individual transport pathways (Schweigel and Martens 2000; Hoenderop et al. 2005). Transport of Ca^{2+}along the piscine intestinal tract is believed to be passive in nature across the apical membrane (Flick and Verbost 1994), and evidence for a Na^{+}-dependent active basolateral transporter has been previously shown (Flik et al. 1990; reviewed by Flik et al. 1993). Although it is still not clear how Ca^{2+} is transported out of the enterocyte, a Ca^{2+}/Na^{+} exchanger has been suggested (Flick et al. 1990).

The average uptake rate of Mg^{2+} from the stomach was calculated to be 25.7 μmol kg^{-1} h^{-1} over the entire 72-h time period, a value much higher than measured branchial uptake rates for the mineral (1 μmol kg^{-1} h^{-1} for carp, Van der Velden et al. 1992; 2 μmol kg^{-1} h^{-1} for tilapia, Van der Velden et al. 1992; Bijvelds et al. 1996). Subsequent secretions along the intestinal tract however reduced the net absorption from the diet and resulted in a net assimilation of close to 60% of the Mg^{2+} found in the feed. Flik et al. (1993) observed that the dietary uptake of Mg^{2+} provided at least 80% of the required Mg^{2+} in tilapia. Low dietary Mg^{2+} content has not been exclusively shown to increase branchial uptake, although Shearer and Asgard (1992) found

that the dietary requirement of rainbow trout decreased when sufficient Mg^{2+} was available in the water. As such, body contents of Mg^{2+} in fish can exceed the dietary intake (Shearer 1989; Dabrowska et al. 1991; Bijvelds et al. 1996), indicating additional sources of Mg^{2+}, presumably the surrounding water. These additional sources are insufficient to compensate for a low dietary intake (Bijvelds et al. 1996), possibly because such a large proportion of the dietary Mg^{2+} content is assimilated.

The average rate of net uptake of Ca^{2+} from the diet by the stomach was 21.4 μmol kg^{-1} h^{-1}, considerably lower than total whole body uptake from the water measured previously in freshwater rainbow trout (50–60 μmol kg^{-1} h^{-1}; Perry and Wood 1985). Perry and Wood (1985) also showed that cutaneous uptake of Ca^{2+} from the surrounding water accounted for up to half of the total whole body Ca^{2+} uptake, the other half occurring at the gills at a measured rate of 24–30 μmol kg^{-1} h^{-1}. When ingested values are compared with excreted values at 72 h (Figs. 2, 4), approximately 28% of ingested Ca^{2+} was assimilated by the GI tract of the rainbow trout (intestinal secretions following gastric absorption reduced the net assimilation of Ca^{2+} from the diet). Surprisingly, ambient water Ca^{2+} concentration appears to have little effect on the dietary requirement of Ca^{2+} in various fish species ranging from blue tilapia (*Oreochromis aurea*) to goldfish (*Carassius auratus*) to the red sea bream (*Chrysophrys major*) (Sakamoto and Yone 1978; Yamane et al. 1982; Robinson et al. 1984, 86, 87; Scarpa and Gatlin 1993). This might be explainable by the low assimilation of Ca^{2+} from the diet under normal conditions, leaving a reserve of Ca^{2+} to absorb from the diet, should environmental Ca^{2+} concentrations fall. Interestingly, low dietary Ca^{2+} has been reported to increase branchial uptake rates of Ca^{2+} in the goldfish (Ichii and Mugiya 1983), whereas high dietary Ca^{2+} has been shown to decrease branchial uptake rates in rainbow trout (Baldisserotto et al. 2004).

Overall, GI handling of dietary Ca^{2+} and Mg^{2+} in freshwater rainbow trout was similar to that of Na^+ and K^+ (Bucking and Wood 2006b), in that a surprising role for the stomach in the absorption of dietary ions was revealed. However, chyme-plasma concentration gradients were in favor of Ca^{2+} and Mg^{2+} absorption (like K^+ but unlike Na^+; Bucking and Wood 2006b), indicating that absorption may be diffusional in nature. Handling of both cations by the GI tract was qualitatively similar, however Mg^{2+} was assimilated from the diet to a greater extent, possibly a reflection of lower environmental availability. Regardless, the diet was a significant source of both Ca^{2+} and Mg^{2+}, indicating a role of the diet in piscine ionoregulation.

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