Am J Physiol Regul Integr Comp Physiol 291: R1764–R1772, 2006.
First published August 10, 2006; doi:10.1152/ajpregu.00224.2006.

Gastrointestinal processing of Na^+, Cl^-, and K^+ during digestion: implications for homeostatic balance in freshwater rainbow trout

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Submitted 30 March 2006; accepted in final form 1 August 2006

Bucking, Carol, and Chris M. Wood. Gastrointestinal processing of Na^+, Cl^-, and K^+ during digestion: implications for homeostatic balance in freshwater rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 291: R1764–R1772, 2006. First published August 10, 2006; doi:10.1152/ajpregu.00224.2006.—The role of the gastrointestinal tract in maintaining ionic homeostasis during digestion, as well as the relative contribution of the diet for providing electrolytes, has been generally overlooked in many aquatic species. An experimental diet that contained an inert reference marker (lead-glass beads) was used to quantify the net transport of Na^+, K^+, and Cl^- during the digestion and absorption of a single meal (3% ration) by freshwater rainbow trout (*Oncorhynchus mykiss*). Secretion of Cl^- into the stomach peaked at 8 and 12 h following feeding at a rate of 1.1 $mmol \cdot kg^{-1} \cdot h^{-1}$, corresponding to a theoretical pH of 0.6 in the secreted fluid (i.e., 240 mmol/l HCl). The majority (~90%) of dietary Na^+ and K^+ was absorbed in the stomach, whereas subsequent large fluxes of Na^+ and Cl^- into the anterior intestine corresponded to a large flux of water previously observed. The estimated concentration of Na^+ in fluids secreted into the anterior intestine was ~155 mmol/l, equivalent to reported hepatic bile values, whereas the estimated concentration of Cl^- (~285 mmol/l) suggested seepage of HCl acid from the stomach in advance of the chyme front. Net absorption of K^+ in the stomach occurred following the cessation of Cl^- secretion, providing indirect evidence of K^+ involvement with HCl acid production. Overall, 80–90% of the K^+ and Cl^- contents of the meal were absorbed on a net basis, whereas net Na^+ absorption was negligible. Chyme-to-plasma ion concentration gradients were often opposed to the direction of ion transport, especially for Na^+ and Cl^-.

Ballotini beads; inert markers; ionoregulation; *Oncorhynchus mykiss*

TO MEET THE CHALLENGE OF LIVING in a hypoosmotic environment, freshwater rainbow trout (*Oncorhynchus mykiss*) typically exhibit a compensating active branchial influx of electrolytes (14, 38, 47), as well as a large renal water output. Associated losses of ions to the environment via urinary excretion are minimized by high renal reabsorptive capacities for electrolytes, resulting in the production of large amounts of very dilute urine (55). Most studies have examined branchial and renal fluxes of electrolytes and water in the absence of feeding, and typically, the role of the gastrointestinal (GI) tract in homeostasis has been overlooked in freshwater fish. One notable exception is the study of Smith et al. (46) who surveyed natural diets of salmonids and estimated that NaCl uptake from food may potentially exceed uptake from the water across the gills. Indeed, they suggested that dietary salt uptake may temporarily exceed the requirements to maintain homeostasis and that a need for excretion of excess Na^+ may occur, with gills as the main effectors, as later shown by Smith et al. (47).

Potentially, drinking associated with feeding (32) could create water gain and salt loss due to the osmotic differences that exist with the surrounding environment (44). On the other hand, recent measurements of high osmolality of partially digested food, or chyme (3), suggest that exactly the opposite might occur (i.e., salt gain and water loss by the extracellular fluid of the fish) during digestion of a meal, and indeed, these results suggest a loss of endogenous water. Earlier, Wood (55) speculated that ingestion of a fish meal could create dietary electrolyte loads up to 10 times higher than baseline renal excretion rates, if all electrolytes were absorbed. Dietary uptake of ions has been found to be especially important in maintaining internal electrolyte homeostasis during chronic low environmental pH exposure, a circumstance which specifically decreases branchial Na^+ and Cl^- uptake (8, 9, 11). Pyle et al. (43) and Kamunde et al. (28, 29) have presented evidence that dietary NaCl is very important in mitigating the pathological effects of sublethal Cu exposure.

Overall, under nonfeeding conditions, the marine teleost intestine exhibits a net absorption of Na^+ and Cl^- and secretion of K^+ and HCO_3^- (10, 49) under symmetrical conditions in vitro. The absorption of electrolytes by the intestine is believed to originate with a basolateral Na^+-K^+-ATPase transporter, or sodium pump, that creates an inwardly directed Na^+ electrochemical gradient, generating movement of Na^+ and Cl^- into the enterocyte (34) through a variety of apical transporters, one of which is the Na^+-K^+-$2Cl^-$ transporter (e.g., 40). Both are subsequently secreted into the serosal fluid, Na^+ by the sodium pump, and Cl^- by a possible combination of three transporters: Cl^- channel, K^+-Cl^- symporter, and Cl^-/HCO_3^- antiporter (10, 35, 49). New evidence from Grosell et al. (22) suggests a role for a basolateral H^+ extrusion, either via a Na^+/H^+ exchanger or an H^+ pump, responsible for energizing Cl^-/HCO_3^- exchange. In contrast, K^+ may enter the cell via the apical Na^+-K^+-$2Cl^-$ cotransporter, but is believed to exit the enterocyte mainly via diffusion, possibly back into the intestinal lumen (17, 40). However, these models are based largely on seawater teleosts, under nonfeeding conditions and in vitro preparations, and evidence exists that in vivo these models require modification. For example, the concentration of K^+ in the intestinal fluid of marine teleosts (under nonfeeding conditions) is lower than seawater values (e.g., 23), indicating that absorption might be occurring in vivo (e.g., 44), in contrast to the secretion seen in vitro (10, 49). Additionally, little is known about ion transport via the GI tract of freshwater teleosts under feeding conditions in vivo.

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Recent findings of large biphasic fluxes of water in various sections of the tract during digestion of a meal (3) suggest that coinciding fluxes of Na^+, Cl^-, and K^+ might occur. Therefore, the primary objective of the present study was to provide a quantitative description of the processing of three monovalent ions (Na^+, K^+, and Cl^-) along the GI tract of a freshwater rainbow trout during digestion. Analysis of the electrolyte concentrations found in the chyme at various time points up to 72 h after ingestion of a single meal were carried out in each section of the GI tract, allowing investigation of the concentration gradients between chyme and blood plasma at each stage of digestion. Ballotini beads were employed as nonabsorbable inert markers (37) 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 (3).

Our overall hypotheses were that biphasic fluxes of Na^+, K^+, and Cl^- would accompany the previously observed water fluxes and that all three ions would be strongly absorbed from the chyme on a net basis, reflecting the ionorgeulatory need of an animal living in an ion-poor environment. Our results support the first hypothesis but, surprisingly, show that only two of the three monovalent ions are strongly absorbed on a net basis. An important role for the stomach in ion absorption is also identified.

MATERIALS AND METHODS

Experimental animals. Adult freshwater rainbow trout (*Oncorhynchus mykiss*) originating from Humber Springs Trout Farm (Orangeville, ON, Canada), were held in 500-liter fiberglass tanks supplied with flow-through dechlorinated Hamilton (ON, 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; total hardness = 140 mg/l as $CaCO_3$; pH 8.0]. The water was temperature controlled to approximate seasonal conditions (10–13°C). The animals, ranging in mass from 300 to 400 g, were allowed a 2-wk acclimation period before experimentation was begun. All procedures were in accordance with approved McMaster University animal care protocols.

Diet preparation and feeding schedule. Following the 2-wk acclimation period, a feeding schedule was implemented where repelleted commercial fish feed (Martin Mills) was fed at a 2% body wt ration every 48 h for 1 mo. Feeding was then suspended for 1 wk to allow for GI tract clearance before the fish were fed to satiation with the same repelleted commercial fish feed, but now also containing Ballotini beads (Jencons Scientific). The repelleting of both diets consisted of grinding commercial fish feed pellets (crude protein = 41%; carbohydrates = 30%; fat = 11%) into a fine mince (Braun PowerMax Jug Blender; Gillette), which was then transferred into a pasta maker (Popeil Automatic Pasta Maker; Ronco Inventions) with double-distilled water at a ratio of 2:1 (powder/water). Ballotini beads (4% dry feed wt) were incorporated into one of the minces. These two mixtures were then extruded and hand-rolled to approximate 5 point-sized fish feed, to which the fish were previously accustomed. The repelleted diets were air-dried for 2 days and stored at −20°C until use. The Ballotini beads (0.40–0.45 mm in diameter) were composed of lead-glass for radiographic quantification, and their addition did not appear to affect the palatability of the feed, since both diets were readily consumed (20, 21). The measured total concentrations of Na^+, K^+, and Cl^- in the feed are given in RESULTS. Tests demonstrated that the water content of the feed pellets approximately tripled during the brief period in which they were in contact with the tank water prior to ingestion (from 6.1 to 18.0%), but there was no loss of Na^+, K^+, or Cl^- (3).

Sampling of GI tract. After the diet containing Ballotini beads was provided, at least seven fish were killed at various time points by cephalic concussion. [Initial trials with chemical anesthesia (MS-222) proved unsuccessful as it induced vomiting in some fish]. A terminal blood sample was taken by caudal puncture and processed for plasma Na^+, K^+, and Cl^- measurements, as described by Bucking and Wood (3). A lateral incision was made into the body wall to reveal the peritoneal cavity, and each compartment of the GI tract (the stomach, the anterior intestine, including the ceca, the midintestine, and the posterior intestine) was then visually identified and isolated by ligating at both ends of the structure with sutures, followed by the removal of the entire GI tract via sections at the esophagus and the rectum. The intact GI tract was subsequently exposed at 50 kVp (kilovolts peak) for 5 s in a portable X-ray machine (Faxitron X-ray cabinet X-ray system). Following the X-ray, each section was emptied of its contents (i.e., chyme). A subsample of chyme was centrifuged (13,000 *g*, 60 s), and the fluid phase removed, placed into liquid nitrogen, and then stored at −80°C for later analysis of ion content. The remaining whole chyme was dried (at 80°C) to a constant weight (48 h) to determine the dry mass and water content of the original chyme. The chyme was then digested (5 volumes of 1 N HNO_3; Fisher Scientific) and placed back in the oven for 48 h, during which time it was vortexed twice. Following digestion, samples were centrifuged (13,000 *g*, 60 s), and the extracted supernatant was analyzed for ion content. The experimental feed (7 samples taken from the feed containing the Ballotini beads) was also digested, and the supernatant was extracted in the same manner as for the chyme.

Analysis of gut contents and calculations. Ion concentrations in the digested feed, whole chyme (μmol/g wet wt), fluid phase (μmol/ml), and blood plasma (μmol/ml) were determined by using either a Varian 1275 Atomic Absorption Spectrophotometer (Na^+ and K^+) or a chloridometer (CMT 10 Chloride Titrator; Radiometer, Copenhagen, Denmark; Cl^-). Reference standards were used for the measurement of all ions studied [Fisher Scientific and Radiometer (Copenhagen, Denmark)]. Quantification of beads in each GI tract section occurred via manual counts of the beads observed in the X-ray of the GI tract, which was placed on a fine grid to ensure accuracy.

Ion concentrations in the chyme (or food) were then referenced to the beads located in each:

$$\text{Relative ion concentration (}\mu\text{mol/bead)} = \frac{[X]\ \text{chyme} \cdot \text{dry chyme sample weight}}{\text{bead number in chyme sample}} \quad (1)$$

where [*X*] was the concentration of the ion of interest in the chyme (μmol/g dry wt).

The apparent ion concentration (μmol/ml) 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 ([*X*]; μmol/bead) between the stomach and ceca divided by the corresponding change in relative water concentration ([*Y*]; ml/bead) as reported for these same experiments by Bucking and Wood (3)

$$\text{Fluid ion concentration (}\mu\text{mol/ml)} = \frac{([X]\ \text{chyme ant. int.} - [X]\ \text{chyme stomach})}{([Y]\ \text{chyme ant. int.} - [Y]\ \text{chyme stomach})} \quad (2)$$

Ion fluxes (mmol/kg) in various segments of the tract at different times were calculated according to

$$\text{Ion flux (mmol/kg)} = \left(\frac{(Z_a \cdot ([X]_a - [X]_b)/1{,}000)}{M}\right) \quad (3)$$

where a is the compartment of interest and b is the preceding compartment at the same time point, Z is the total bead number in the specified GI tract section, $[X]$ is the relative concentration of the ion (μmol/bead) in the specified GI tract section, and M is fish weight in kilograms. The "preceding compartment" for the stomach at 2 h was the ingested food and, thereafter, the stomach itself at the previous time point. The preceding compartment for the midintestine was the anterior intestine, and the preceding compartment for the posterior intestine was the midintestine.

Statistical analysis. Data have been reported as means ± SE (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 honestly significant difference post hoc test. All statistical analyses were performed using SPSS (version 13).

RESULTS

Sodium. Feeding to satiation resulted in an average ration of 3.06 ± 0.20% body wt (21), based on original food weight (6% water content) and fish wet weight, measured by bead counts in the stomach and ceca up to 8 h (i.e., in the absence of defecation). As the measured concentration of Na^+ found in the prepared diet was 215.6 ± 5.1 μmol/g original food wt (7), a 3% ration corresponds to an average Na^+ intake of ~6.4 mmol/kg fish wt. The Na^+ concentration found in the stomach chyme decreased from that in the original food by ~95% over 72 h, from 215.6 ± 5.1 to 13.4 ± 3.3 μmol/g wet chyme wt (7; Fig. 1*A*). The concentration of Na^+ in the fluid phase sampled from the stomach also decreased over time, falling by 90%, from 140.7 ± 3.0 (7) in the first chyme sample at 2 h to 14.0 ± 3.5 μmol/ml (7) at 72 h, between 22 and 90% lower than plasma Na^+ values (μmol/ml) at all time points (Fig. 1*B*).

There was a large threefold increase in the chyme Na^+ concentration at 8 h in the anterior intestine, its time of first appearance in that segment. There was also a tendency for chyme Na^+ concentrations to increase further between adjacent segments, which was significant at several time points (Fig. 1*A*). The Na^+ concentration in the fluid phase found in the intestine displayed very similar temporal and spatial trends compared with total chyme (Fig. 1, *A* and *B*), and the Na^+ concentration seen in both was between 100 and 212 μmol/ml or μmol/g wet chyme wt at all time points. Throughout the intestine, the Na^+ concentration in the fluid phase was slightly but consistently lower than that found in the total chyme, and while the fluid phase initially contained lower Na^+ concentrations than plasma, by 48 h the Na^+ concentration was equal or greater (Fig. 1*B*).

Referencing the Na^+ concentrations to the inert marker revealed more dramatic changes in Na^+ dynamics. Approximately 90% of the original Na^+ found in the ingested food (μmol/bead) was absorbed by the stomach, with the relative Na^+ concentration consistently falling over 72 h from 2.17 ± 0.18 to 0.27 ± 0.22 μmol/bead (7; Fig. 2), similar to the pattern seen in absolute concentrations (Fig. 1). Again, there was a large appearance of Na^+ in the anterior intestine at 8 h with the arrival of chyme (Fig. 2). However, the increase in the

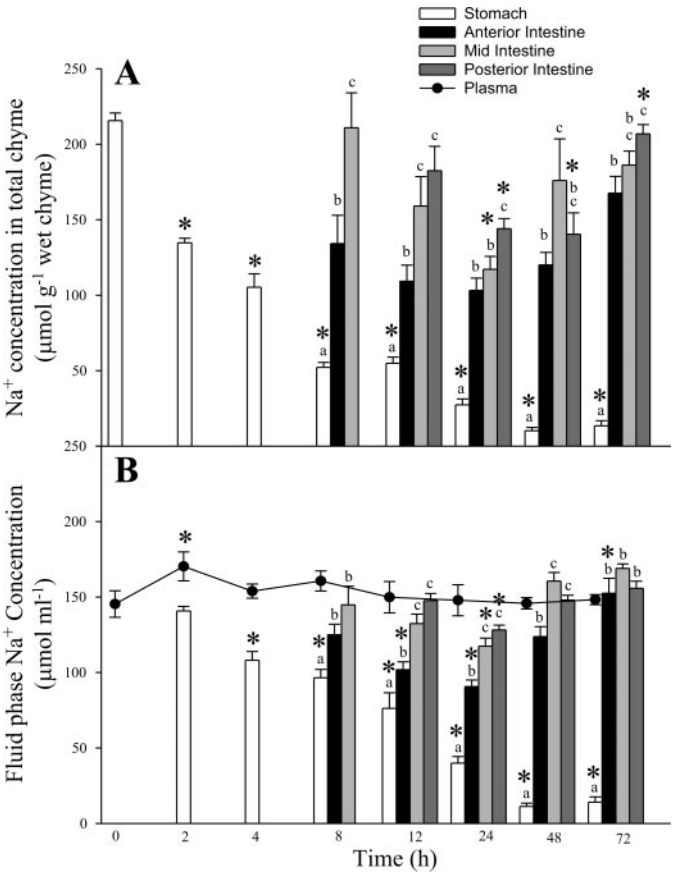

Fig. 1. *A*: temporal and spatial changes in the concentration of Na^+ in the total chyme (μmol/g wet chyme wt) following feeding (immediately following 0 h). Values are means ± SE (n = 7). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between gastrointestinal (GI) tract sections within a time point. *B*: changes in the concentration of Na^+ in the fluid phase (μmol/ml) isolated from total chyme following feeding (immediately following 0 h). Values are means ± SE (n = 7). *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 Na^+ concentrations in the same fish at each time have been included as a point of reference (data from Ref. 3).

relative concentration of Na^+ in the chyme was ninefold, almost triple the increase seen in the simple concentration data of Fig. 1*A*. In contrast to the impression given by the wet chyme Na^+ concentration data, slightly more than 50% of the Na^+ found in the anterior intestine was subsequently absorbed over the next 64 h (Fig. 2).

Chyme found in the midintestine contained a similar amount of Na^+ (i.e., not significantly different, with the exception of the 24-h time period) to that found in the anterior intestine and, likewise, decreased by 50% by 72 h (Fig. 2). The posterior intestine exhibited a transient decrease at 48 h followed by a recovery to initial values. By 72 h, the relative concentration of Na^+ in the chyme in the posterior intestine was comparable to that found in the originally ingested feed. Overall, these measurements indicate substantial net Na^+ absorption in the stomach despite concentrations lower than plasma values, followed by large scale net Na^+ secretion in the anterior intestine, and compensating Na^+ absorption in the remainder of the tract. Overall, Na^+ absorption from the food was negligible (−9%).

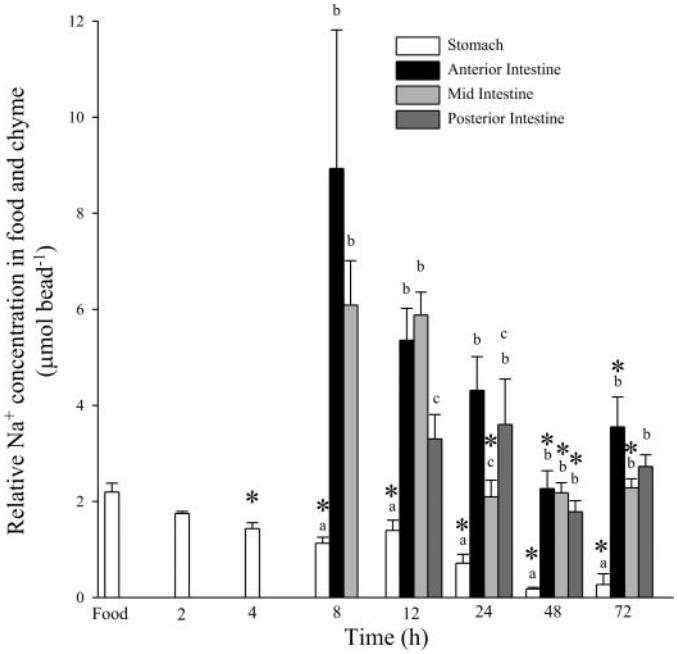

Fig. 2. Changes in the relative concentration of Na^+ (μmol/bead) following feeding (immediately after 0 h). Values are means ± SE (n = 7). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between GI tract sections within a time point.

Chloride. The measured concentration of Cl^- in the prepared diet was 186.5 ± 15.8 μmol/g original food wt (7), resulting in a total intake of Cl^- from the meal around 5.2 mmol/kg fish wt, slightly less than that of Na^+. In marked contrast to Na^+, the Cl^- concentration in the stomach chyme was maintained at about 185 μmol/g wet chyme wt for the duration of the experiment, while that in the fluid phase remained at a similar value (about 190 μmol/ml), excluding 4, 48, and 72 h when the fluid phase concentration appeared to increase slightly (Fig. 3, *A* and *B*). The Cl^- concentration in the fluid phase of the stomach chyme was also consistently higher than plasma values (130 μmol/ml; Fig. 3*B*). There was, again, a significant increase in concentration in total chyme at 8 h in the anterior intestine, although at twofold somewhat less on a relative basis than the threefold increase seen in Na^+ (cf., Fig. 1*A*). However, the Cl^- concentration found along the intestine decreased to become similar in all three sections at 48 h, and by 72 h had become 75% lower than that found in the original food (Fig. 3*A*).

The fluid phase found along the intestinal tract displayed very different spatial and temporal trends when contrasted with total chyme (Fig. 3, *A* and *B*). The intestinal fluid also contained much lower Cl^- concentrations than the stomach and plasma but otherwise displayed no obvious spatial trends within intestinal segments. However, there was a temporal trend observed in all three intestinal segments with the fluid phase values reaching a minimum of 23.19 ± 1.45 μmol/ml (21) at 24 h with increases thereafter (Fig. 3*B*). The concentration of Cl^- in the fluid phase also displayed a very different pattern when compared with the Na^+ concentration (Fig. 1*B*), as the fluid phase of chyme contained much more Cl^- than Na^+ in the stomach, but much more Na^+ than Cl^- in the intestine. The stomach chyme also displayed a decrease in the concentration of Na^+ in the fluid phase, whereas the Cl^- concentration was maintained (Fig. 1*B* vs. Fig. 3*B*). Both Na^+ and Cl^- demonstrated a transient decrease at 24 h in the fluid phase along the intestinal tract (Figs. 1*B* and 3*B*).

The relative Cl^- concentration (μmol/bead; Fig. 4) exhibited a very different pattern than that seen with the actual Cl^- concentration (μmol/g wet chyme wt; Fig. 3*A*) and was also very different from that for Na^+ (cf., Fig. 2). In contrast to the constancy in the actual Cl^- concentration, there was a marked increase in the relative concentration of Cl^- in the stomach, increasing from 1.87 ± 0.19 μmol/bead (7) found in the food to 6.41 ± 1.87 μmol/bead (7) at 24 h followed by a return to Cl^- levels found in the food by 48 h (Fig. 4). There was again (as with Na^+; Fig. 2) a large increase in the relative concentration of Cl^- when the chyme first appeared in the anterior intestine at 8 h, increasing from the stomach by eightfold (Fig. 4), markedly greater than the twofold rise in actual concentration of Cl^- (Fig. 3*A*). This large peak traveled along the intestinal tract over time, appearing in the midintestine at 12 h and the posterior intestine at 24 h. Aside from this, the relative

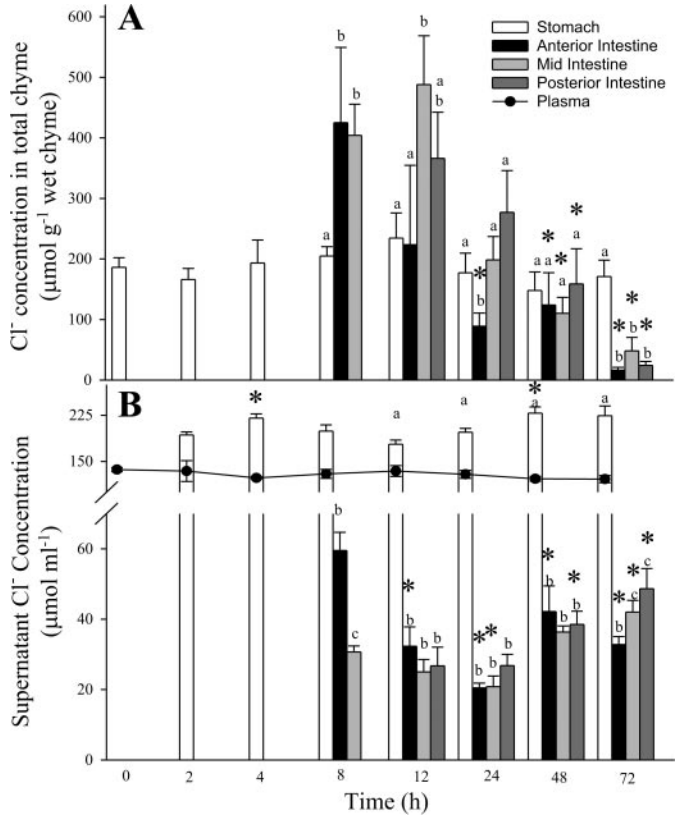

Fig. 3. *A*: temporal and spatial changes in the concentration of Cl^- in the total chyme (μmol/g wet chyme wt) following feeding (immediately after 0 h). Values are means ± SE (n = 7). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between GI tract sections within a time point. *B*: changes in thee concentrations of Cl^- (μmol/ml) in the fluid phase isolated from total chyme following feeding (immediately following 0 h). Values are means ± SE (n = 7). *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 Cl^- concentrations in the same fish at each time have been included as a point of reference (data from Ref. 3).

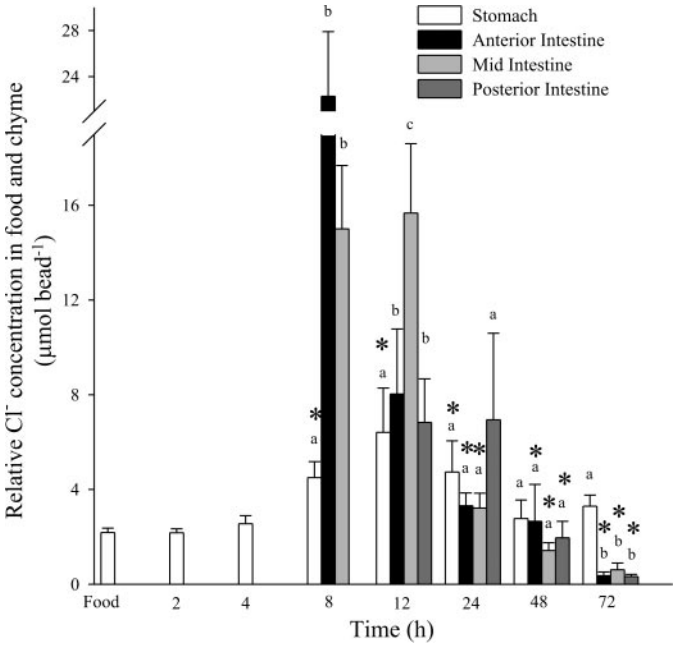

Fig. 4. Changes in the relative concentration of Cl^- (μmol/ bead) following feeding (immediately after 0 h). Values are means ± SE (n = 7). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between GI tract sections within a time point.

Cl^- concentration seen along the intestine decreased in all sections to eventually become similar at 24 h, followed by simultaneous decreases in all three sections to become 0.36 ± 0.09 μmol/bead (21), 81% less than the originally ingested relative Cl^- concentration [1.87 ± 0.19 μmol/bead (7)] in the food (Fig. 4).

Thus the overall pattern in the stomach was one of initial Cl^- secretion (despite lumen fluid values higher than plasma values) followed by later absorption together with a similar biphasic effect in the anterior intestine. There was a net absorption of Cl^- in the lower intestinal tract, again despite concentration gradients between the intestinal fluid and plasma. Overall, Cl^- absorption from the food was greater than 80%, very different from Na^+.

Potassium. The measured concentration of K^+ in the prepared diet was 96.5 ± 1.8 μmol/g original food wt (7). The amount of K^+ ingested in the food was roughly half that of Na^+ and Cl^-, around 3 mmol/kg fish wt. The K^+ concentration found in the total wet chyme of the stomach (μmol/g wet chyme wt) displayed a decrease from an initial value of ~95 mmol/l in the food, and by 48 h all GI tract segments contained similar K^+ concentrations (5–10 mmol/l). These concentrations were ~89% less than in the ingested food and were essentially maintained until 72 h (Fig. 5*A*). Notably, in contrast to Na^+ and Cl^- (Figs. 1*A* and 3*A*), there was no increase but rather a marked decrease in K^+ concentration when chyme first appeared in the anterior intestine at 8 h (Fig. 5*A*). The fluid phase extracted from the total chyme displayed similar temporal trends as seen in the total chyme (Fig. 5*A*), with the stomach fluid phase initially at about 55 μmol/ml at 2 h, decreasing over time to eventually become similar to the fluid phase found along the intestinal tract, which ranged between 4 and 12 μmol/ml (Fig. 5*B*). The fluid phase in the stomach (at all time points) and the intestine (with the exception of 24 and 48 h) contained significantly more K^+ than the plasma (3 μmol/ml; Fig. 5*B*).

Again, referencing these concentration changes to the inert marker revealed rather different patterns. Unlike Na^+ and Cl^-, the relative concentration of potassium in the stomach chyme did not begin to fall from food levels [1.12 ± 0.01 μmol/bead (7)] until 24 h following ingestion. However, thereafter it decreased by over 90% over the subsequent 48 h to 0.11 ± 0.04 μmol/bead (7; Fig. 6). There were no obvious spatial trends along the length of the alimentary tract, with variable changes occurring in all sections. However, there was an overall significant decrease in all sections, with the exception of the posterior intestine, which was maintained at about 0.1–0.2 μmol/bead up to 72 h following ingestion (Fig. 6). Notably there was a lack of secretion of K^+ into the anterior intestine at 8 h (Fig. 6) compared with both Na^+ (Fig. 2) and Cl^- (Fig. 4).

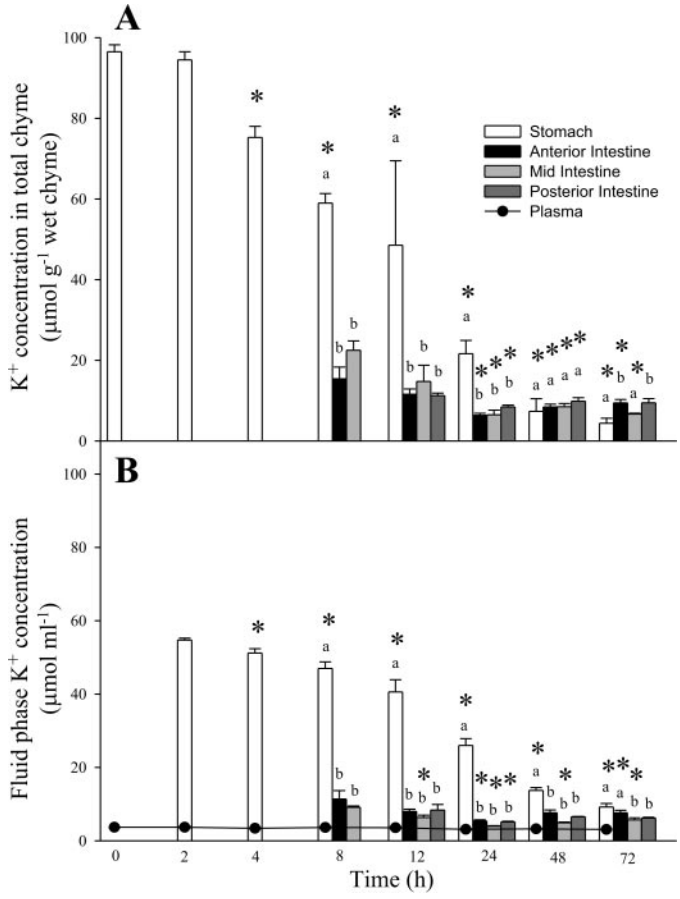

Fig. 5. *A*: temporal and spatial changes in the concentration of K^+ in the total chyme (μmol/g wet chyme wt) following feeding (immediately following 0 h). Values are means ± SE (n = 7). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between GI tract sections within a time point. *B*: changes in the fluid phase (μmol/ml) concentration of K^+ isolated from total chyme following feeding (immediately following 0 h). Values are means ± SE (n = 7). *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 K^+ concentrations in the same fish at each time have been included as a point of reference (data from Ref 3).

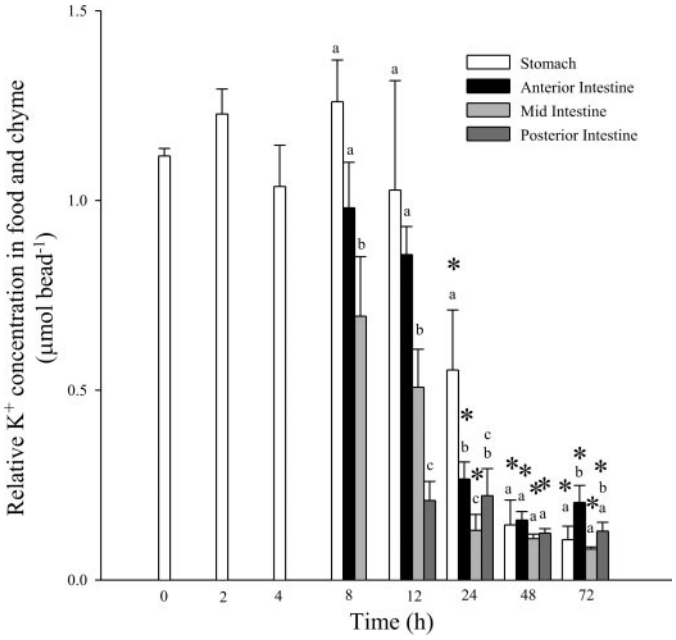

Fig. 6. Changes in the relative concentration of K^+ (μmol/bead) following feeding (immediately after 0 h). Values are means ± SE ($n = 7$). *Significant difference from initial values (defined by the first appearance within that section). Bars that share letters demonstrate no significant differences between GI tract sections within a time point.

The overall pattern was, therefore, one of initial stability and later strong net absorption of K^+ in the stomach, with additional K^+ absorption in the intestine, such that net K^+ absorption from the food was close to 90%, again very different from Na^+. With the exception of two time points in the midintestine, the fluid phase of the GI tract contents contained a higher concentration of K^+ than in the plasma.

DISCUSSION

Examination of the relative concentrations of the ions studied revealed that the stomach was a site of both absorption (Na^+ and K^+) as well as secretion (Cl^-). In fact, a majority of the Na^+ and K^+ ingested in the food was absorbed by 72 h in the stomach (Figs. 2 and 6). In contrast, the anterior intestine was a site of initial massive Na^+ and Cl^- secretions (Figs. 2 and 4), although the concentration of K^+ remained more or less unchanged from that in the stomach (Fig. 6). Fluxes of each ion in each section of the GI tract were calculated by *Eq. 3*. This procedure provides the magnitude of the flux, but only for the stomach can this be converted to a true rate by dividing by time, because of the nature of the calculation (see MATERIALS AND METHODS). Therefore, in Fig. 7, the fluxes of these three ions, at various times and in different parts of the tract, are displayed as absolute values rather than rates.

The flux rate of Cl^- into the stomach lumen (Fig. 7*B*) at 8 and 12 h (the peak of Cl^- secretion) was about 1.1 $mmol \cdot kg^{-1} \cdot h^{-1}$. This rate is two- to threefold higher than the rate of Cl^- influx seen at the gill (50, 51, 56) even above reabsorption rates at the kidney (e.g., 4, 56) and also against the concentration gradient of Cl^- from the plasma to the stomach lumen (Fig. 3*B*). In mammals, feeding triggers the production of HCl acid created via equimolar secretion of H^+ and Cl^- ions across the apical membrane of parietal cells into the lumen of the stomach. Although HCl acid formation in rainbow trout has not been extensively studied, its formation is believed to be highly conserved across all vertebrate species (31). The major difference between mammals and all other vertebrates appears to be the evolution of separate cells for the production of pepsinogen (chief cells) and HCl (parietal cells) in the former, while nonmammalian vertebrates possess only oxynticopeptic cells for the production of both (24, 45, 51). If the Cl^- flux into the stomach is used to approximate the flux of H^+, the resulting acid secretion (1.1 $mmol \cdot kg^{-1} \cdot h^{-1}$) is approximately 10-fold higher than basal acid secretion rates observed in the *Gadus morhua* (cod; 27), and fourfold higher than observed following stimulation (via histamine infusion; 26) of the stomach in the same species (36).

The production of HCl acid in the stomach of the rainbow trout appeared to peak at 12 h (Figs. 4 and 7*B*), and corresponded with the cessation of proposed fluid secretion reported in these same experiments by Bucking and Wood (3). The estimated concentration of Cl^- in the fluid secreted into the stomach between 2 and 12 h was about 240 mmol/l, a value similar to that seen in mammalian stomachs (16). If accompanied by equimolar H^+, the fluid would have a pH of 0.6. A previous study by Dabrowski et al. (7) showed a similar peak in stomach Cl^- concentration between 10 and 20 h following ingestion of a meal in the rainbow trout. The production of HCl acid involves the transport of plasma Cl^- into the stomach lumen, a process which has been implicated in the production of hypochloremia. However, this was not observed in this study (Fig. 3*B*); a possible explanation may be found within the principles of piscine acid-base regulation.

In the currently accepted model of HCl acid production in the vertebrate stomach (reviewed in Ref. 42), the Cl^- obtained from the plasma at the basolateral membrane is exchanged for intracellular HCO_3^- left behind by the apical secretion of H^+. The secretion of HCO_3^- into the plasma results in the well-known alkaline tide, a metabolic alkalosis in the systemic bloodstream. This phenomenon has been observed in mammals, reptiles, and amphibians (52) and recently in elasmobranchs (57), although it has not been documented in freshwater teleosts. If this occurred in the freshwater trout, the probable response would be an enhanced uptake of Cl^- in exchange for HCO_3^- at the gill so as to restore acid-base homeostasis (reviewed in Ref. 19). Such an exchange may have masked any losses of plasma Cl^- during the formation of HCl acid and would explain the lack of hypochloremia seen during the experiment (Fig. 3*B*). Clearly, Cl^- influx rate measurements at the gills immediately after feeding, as performed by Smith et al. (47) for Na^+, as well as blood acid-base measurements would be helpful in further understanding the responses to feeding in future studies.

The observed net K^+ absorption in the stomach of the rainbow trout from 12 h onward (Fig. 7*C*) corresponded with the probable reduction of HCl acid production after this time point, while up to that point the relative K^+ concentration had been maintained (Fig. 6). This may be explained by the fact that the secretion of H^+ ions (or most likely H_3O^+ ions) into the stomach lumen is produced by an apical H^+-K^+-ATPase, which uses the hydrolysis of ATP to drive the exchange of luminal K^+ for cytoplasmic H^+. The K^+ is subsequently recycled back into the stomach lumen to continue acid produc-

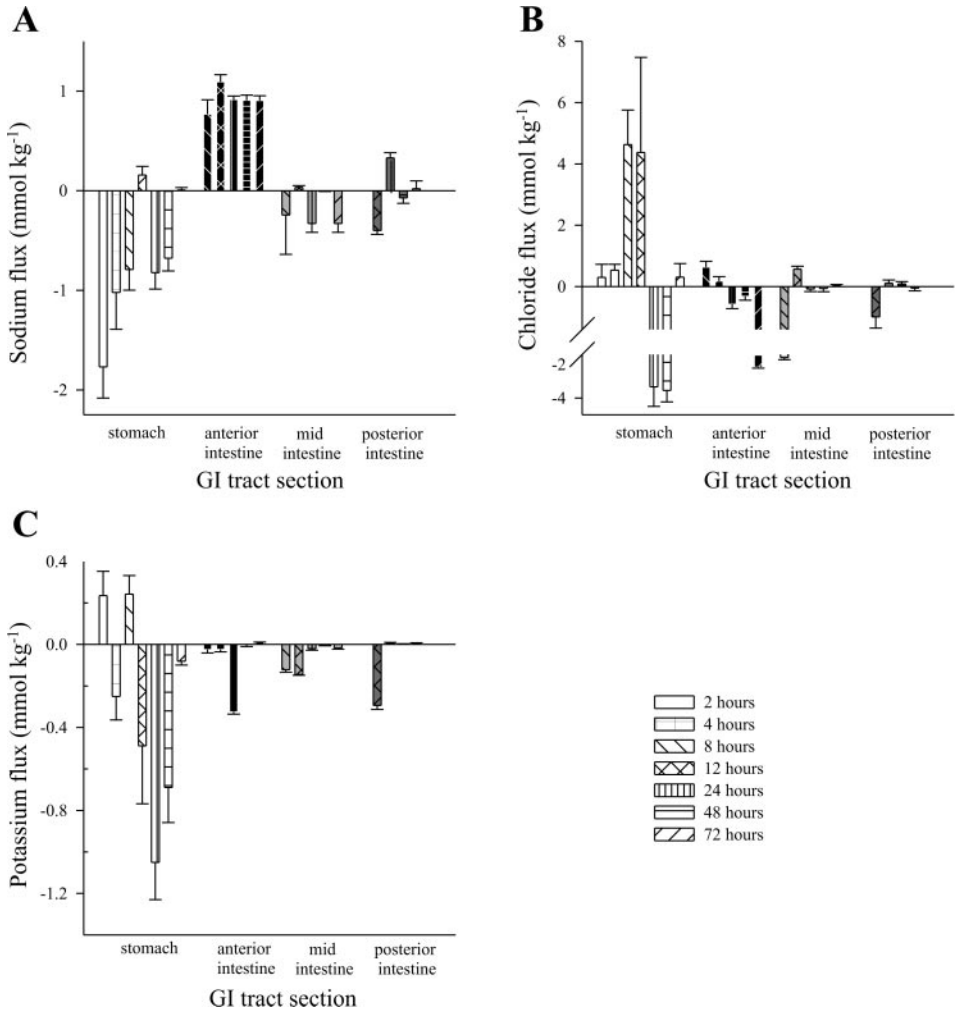

Fig. 7. Calculated ion fluxes (mmol/kg) along the GI tract of the rainbow trout during digestion of a single meal: sodium flux (*A*), chloride flux (*B*), and potassium flux (*C*). Feeding occurred immediately after 0 h. Positive values reflect net secretion, whereas negative values reflect net absorption.

tion (16). There is evidence of a putative H^+-K^+-ATPase in the stomachs of both elasmobranchs and teleosts (6, 12, 18, 48).

The stomach was the location of marked absorption of Na^+ and K^+ from the diet (Figs. 2, 6, and 7, *A* and *C*), although in the case of Na^+, this was against the concentration gradient from the chyme to the plasma (Fig. 1*B*). However, Smith et al. (47) also observed absorption of dietary Na^+ in the stomach of the freshwater rainbow trout; in fact, 65% of the dietary load was absorbed by 7 h, very similar to the results seen here. The stomach epithelium of a rainbow trout consists mainly of columnar cells (2, 15, 30), cells that are often specialized for transport (58). Recently, the stomach has also been shown as a site of iron (5), Ca^{2+} (1), and Cu (41) absorption in freshwater rainbow trout. The estimated uptake rates of Na^+ (Fig. 7*A*) were initially (0–2 h) about 0.8 mmol·kg^{-1}·h^{-1}, exceeding those found in the gills (e.g., 43, 47, 50, 56, 47). However, they fell dramatically over the next 2 h to 0.3 mmol·kg^{-1}·h^{-1}.

The flux rate of potassium out of the stomach lumen between 12 and 48 h was ~0.03–0.1 mmol·kg^{-1}·h^{-1} (Fig. 7*C*). This is comparable with the branchial influx rate observed in unfed rainbow trout (0.07 mmol·kg^{-1}·h^{-1}; 13) and higher than the renal reabsorption rates (0.01 mmol·kg^{-1}·h^{-1}; 56); however, no hyperkalemia was observed (Fig. 5*B*). In fact, despite large secretions of Cl^- into the stomach and almost complete absorption of dietary Na^+, relatively few perturbations in blood plasma constituents were observed, with the exception of the rise in plasma Na^+ levels at 2 h (Fig. 1*B*). This suggests that ion handling had been altered at one or more of the additional sites of electrolyte regulation; the gills, the kidney, and, in the case of K^+, the extracellular/intracellular interface, as K^+ is located mainly in the intracellular compartment. Indeed, Smith et al. (47) likewise observed little change in plasma Na^+ levels following feeding and suggested that observed increases in electrolyte efflux and/or decreases in electrolyte influx rates were critical in maintaining optimal concentrations.

Another important secretion into the GI tract is bile. Bucking and Wood (3) postulated that the majority of fluid secreted into the anterior intestine at 8 h (~3.5 ml/kg) was mainly a result of gall bladder bile secretions [~2 ml/kg in rainbow trout (24)]. Secretion of bile may also explain, at least in part, the coinciding large secretions of Na^+ and Cl^- (Figs. 2 and 4). The concentration of Na^+ in the fluid secreted into the anterior intestine was calculated as 155.1 ± 22.7 μmol/ml (7), which is roughly equivalent to the observed concentration of Na^+ found in the hepatic bile of the rainbow trout but half that seen in gall bladder bile released to the intestine (24). This may reflect absorption of ions occurring in the anterior intestine that, due to the nature of this study, cannot be seen against the large background of net secretion. In contrast, the calculated concentration of Cl^- in the secreted fluid was 287 ± 57 μmol/ml

(7), roughly twofold higher than hepatic bile and over four times higher than that found in gall bladder bile (24).

A possible explanation could be the additional seepage of HCl acid from the stomach (earlier estimated at 240 mmol/l) in advance of the chyme front. Support is found in the noticeable traveling peak in the relative concentration of Cl^- along the intestinal tract (Fig. 4), as well as a slight movement of liquid (tracked with PEG-4000) from the stomach before the movement of chyme (tracked with Ballotini beads) (3). This could possibly aid in activating transport processes and GI secretions in anticipation of receiving chyme. Interestingly, there was no significant change in the relative K^+ concentration when chyme moved from the stomach to the anterior intestine, the site and time of the proposed secretion of bile (Fig. 6). This is in accordance with the fact that bile contains only a relatively low concentration of K^+, ~8 mmol/l (Bucking C and Wood CM, unpublished observation).

There was only slight and variable Na^+ absorption by the midintestine (Fig. 7*A*), the site of the majority of net water absorption, although there certainly could have been Na^+ and water absorption present in the anterior intestine that was simply not visible due to large volumes of secreted bile (3). In contrast, Cl^- was clearly absorbed by the anterior intestine, especially during later time points (Figs. 4*A* and 7*B*). The Na^+ absorbed by the intestine appears to be almost entirely endogenous in nature, secreted with bile and other intestinal secretions into the anterior intestine, while the Cl^- and K^+ that was absorbed could be either endogenous or exogenous, in light of the proposed Cl^- section and K^+ recycling that occurs in the stomach. In contrast to both Na^+ (Fig. 1*B*) and Cl^- (Fig. 3*B*), the K^+ absorption along the intestinal tract was down the concentration gradient observed from the chyme to the plasma (Fig. 5*B*).

While the prepared diet contained significantly less water than a natural diet (18 vs. 80%), the ion content was approximately equal. It has been suggested that the stomach is responsible for liquefying the ingested food to approximate natural prey water contents, potentially creating an avenue for endogenous water loss as a result (3). This would then render the commercial diets similar to natural prey.

When comparing ingested relative values (μmol/bead) to excreted values, i.e., food at 0 h to posterior intestine at 72 h, 85% of dietary Cl^- and 89% of dietary K^+ was absorbed on a "net" basis by the GI tract (Figs. 4 and 6), the former in spite of the marked secretion of Cl^- in the stomach and the anterior intestine. This represents net absorption rates of 64 and 37 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for Cl^- and K^+, respectively. Considering that the branchial net flux of Cl^- and K^+ is minimal in freshwater fish [although slightly positive (e.g., 56)], this represents a substantial influx of ions while feeding. In contrast, there was a slight net secretion of ~9% of the Na^+ found in the food (not significantly different from 0%), indicating that dietary Na^+ is not utilized on a net basis by the fish under normal conditions. However, under stressful conditions, dietary Na^+ may be assimilated to a greater extent, as dietary Na^+ has been shown to prevent physiological consequences of decreased Na^+ uptake at the gills under various conditions, such as environmental acid and Cu exposure (9, 11, 28, 29, 43).

The fact that almost the entire dietary load of K^+ was absorbed may relate to the low concentration of K^+ in freshwater, with values $< 10\%$ of Na^+ in the acclimation water of the present study. Cl^- absorption may have occurred because of its involvement in the alkalinization of the intestinal tract contents via a Cl^-/HCO_3^- exchanger, a transporter known to be found in the intestinal tract of marine teleosts (reviewed in Ref. 54). The same process may occur in freshwater rainbow trout due to the high pH of the intestinal lumen contents, which likely results from the linked secretion of endogenous HCO_3^- (44). Clearly, the amount of electrolyte absorbed from the diet may depend on many factors from the ion status of the fish at the time of feeding (46) to environmental conditions.

ACKNOWLEDGMENTS

This study was supported by a Natural Sciences and Engineering Research Council Discovery Grant (to C. M. Wood) who is also supported by the Canada Research Chair Program.

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